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Confirming Vertical Fetal Infection with COVID-19: Neonatal and Pathology Criteria for Early

Onset and Transplacental Transmission of SARS-CoV-2 from Infected Pregnant Mothers

David A. Schwartz, MD, MS Hyg; Denise Morotti, BS; Babak Beigi, MD; Fereshteh Moshfegh, MD;

Nazanin Zafaranloo, MD; Luisa Patanè, MD

Department of Pathology. Medical College of Georgia, Augusta University, Augusta, GA, USA (Dr.

Schwartz); Pathology Unit, ASST Papa Giovanni XXIII and Medical Genetics Laboratory, ASST Papa

Giovanni XXIII, Bergamo, Italy (Ms. Morotti); Department of Neonatology, Tehran University of Medical

Sciences and Universal Scientific and Educational Network, Tehran, Iran (Dr. Beigi); Department of

Pediatrics, Iran University of Medical Sciences, Tehran, Iran (Dr. Moshfegh); Department of Pediatrics,

Iran University of Medical Sciences, Tehran, Iran (Dr. Zafaranloo); Obstetrics and Gynecology

Department, ASST Papa Giovanni XXIII, Bergamo, Italy (Dr. Patanè)

Corresponding author:

David A. Schwartz, MD

Department of Pathology

Medical College of Georgia

1950 Grace Arbor Court

Atlanta, GA 30329, USA

Email for correspondence: davidalanschwartz@gmail.com

ABSTRACT

Increasing numbers of pregnant women with COVID-19 are being reported around from the world. The majority of neonates delivered to pregnant women infected with the new coronavirus SARS-CoV-2 have been negative for the virus, but a small number have tested positive for infection. It is important to determine whether vertical transmission of COVID-19 occurs and the mechanisms for its development. Based on a number of clinical and laboratory findings it has been suggested that transplacental transmission may be occurring, but a method to confirm this is necessary. This communication analyzes and evaluates the covariables that have been discussed as potential indicators of vertical and, specifically, intrauterine transmission including the timing of onset of neonatal illness, neonatal viral test positivity, neonatal antibody testing for IgG and IgM, and viral analysis of swabs of whole specimens of placental tissue. None of these methods can provide confirmatory evidence that infection developed prior to labor and delivery, or that transplacental transmission occurred. This commentary proposes that diagnosis of early-onset neonatal COVID-19 infection should be limited to neonates with positive RT-PCR testing for SARS-CoV-2 within the initial 72 hours of life. It also proposes 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 such nucleic acid methods such as in situ hybridization. Evaluating placentas from neonates with COVID-19 using these methods will be instrumental in determining the potential role and prevalence of transplacental transmission of the coronavirus.

INTRODUCTION

Following the introduction of a new virus into the human population one of the most important questions that typically arises is what its effect will be on pregnant women, and will it be transmissible to the developing fetus. This has been true with many emerging viral agents including hemorrhagic fever agents such as the filoviruses (Ebola and Marburg viruses) and arenaviruses (Lassa virus), retroviruses (human immunodeficiency virus (HIV)), hepeviruses (hepatitis E virus) and most recently, flaviviruses (Zika virus). All of these emerging viral infections were shown to be capable of intrauterine maternal-fetal transmission, often and in some cases typically, with disastrous results. In addition, two pathogenic coronaviruses that were first identified in last 20 years that also caused this concern – the severe acute respiratory syndrome coronavirus (SARS-CoV) causing severe acute respiratory syndrome (SARS), and Middle East respiratory syndrome coronavirus (MERS-CoV) causing the Middle East respiratory syndrome (MERS). Although these two coronaviruses resulted in maternal morbidity and mortality as well as perinatal deaths, there were fortunately no confirmed cases of intrauterine transmission identified.[1,2]

In December 2019 a new coronavirus was identified in Wuhan, Hubei Province, China – it was the 7th pathogenic member of the Family *Coronavirdae* to be reported to cause human disease. This new virus, termed severe acute respiratory syndrome coronavirus-2 or SARS-CoV-2 and producing a disease termed coronavirus disease 2019 (COVID-19), quickly spread from Wuhan throughout China, then to neighboring countries and ultimately throughout the world, and was declared to be a pandemic by the World Health Organization (WHO) on March 11, 2020. One of the major questions that arose early in the pandemic and has continued to be of significance is the ability of the virus to be transmitted from the mother to her infant, termed vertical transmission. In particular, it is important to understand whether SARS-CoV-2 is being transmitted *in utero* via the transplacental route. This Commentary examines

clinical, laboratory and pathology methods for determining whether transplacental maternal-fetal transmission of COVID-19 is occurring, and how this process can be evaluated and ultimately confirmed.

MECHANISMS OF VERTICAL TRANSMISSION

Viruses can be vertically transmitted from mother to infant through 3 different mechanisms – intrauterine, intrapartum and postpartum routes.[3] Intrauterine viral transmission can occur via two major mechanisms - the hematogenous route and ascending route. The hematogenous route is characteristic of most mother-to-fetus transmissible viral agents. In this mechanism, the virus is circulating in the maternal blood stream during pregnancy, enters the placenta via maternal blood perfusing the placenta through the uterine arterioles, and crosses the maternal-placental interface to reach the fetal vessels in the chorionic villus tree to be transmitted through the umbilical blood vessels to the fetus.[4,5] Hematogenous vertical transmission occurs with such viruses as rubella, cytomegalovirus, parvovirus, Zika virus and Ebola virus, and requires that the virus be present in the maternal blood – viremia. The ascending route of intrauterine fetal infection occurs when microorganisms present in the lower genital tract ascend the cervicovaginal tract to reach the pregnant uterine cavity, from where they breach the placental membranes and infect the amniotic fluid. This mechanism almost always is the result of a bacterial infection.[3]

Intrapartum transmission occurs around the time of labor and delivery when the fetus passes through an infected birth canal during vaginal delivery. This type of vertical transmission can occur with herpes simplex virus (HSV)[6,7] – intrapartum transmission causes 85% of vertical HSV infections and is the basis for performing cesarean section delivery in infected mothers. Human papillomavirus (HPV) and human immunodeficiency virus (HIV) can also be transmitted to the infant through intrapartum exposure during labor and delivery.[8,9]

Postpartum vertical transmission of viruses develops following delivery – it can occur through contaminative transmission of a virus from an infected mother via respiratory secretions and fomites, skin-to-skin-contact, and breast milk. Respiratory viruses may be transmitted by this mechanism, as well those viral agents present in breast milk including HIV,[10] cytomegalovirus (CMV),[11] Ebola virus[12] and others.

The large majority of infants born to pregnant women with COVID-19 have been uninfected in China, the United States, Europe and all other regions where the pandemic is occurring.[13-19] The low rate of neonatal infection could be expected based on data of vertical transmission existing with other RNA respiratory viruses including the coronaviruses SARS-CoV and MERS-CoV,[1] as well as influenza, respiratory syncytial virus (RSV), parainfluenza and human metapneumovirus.[1,2] The factors inherent in the observed inhibition of respiratory RNA viruses to undergo intrauterine vertical transmission result from both viral and host factors, and involve the ability of the virus to penetrate the maternal-fetal interface including the placenta, avoiding the innate immune system, and tropism of the virus to host cells.[2]

Among the reports of neonates who have had positive tests for COVID-19, some were asymptomatic,[20-23] as well as neonates having symptomatic illness.[22,24] Among symptomatic infants testing positive for COVID-19, there has been variation in the onset of illness, ranging from within 1 or 2 hours after delivery, to within the first 24 to 72 hours of life, and up to many days to weeks following birth.[13,23-25] Some of these cases have been considered to be suspicious for vertical transmission, but the mechanisms and timing for the neonate's acquiring the infection have remained unknown.

DETERMINING TRANSPLACENTAL TRANSMISSION FROM THE ONSET OF NEONATAL ILLNESS

There is no agreed upon definition of early-onset neonatal COVID-19 infection. For bacterial infections such as Group B streptococcus and *Escherichia coli*, early-onset neonatal sepsis (EOS) is based

upon timing of onset of either bacteremia or bacterial meningitis, which in term infants is less than 7 days of life. In preterm infants in the neonatal intensive care unit (NICU), EOS is defined as occurring in the first 72 hours of life, and is generally believed to be the result of bacterial agents transmitted vertically from mother to infant before or during delivery.[26,27] The majority (80 to 90%) of EOS bacterial infections clinically present in the initial 24 to 48 hours following delivery.[28]

Zeng et al. reported that among 33 neonates whose mothers had COVID-19 infection in Wuhan, China, there were 3 delivered by cesarean section who developed fever and pneumonia and tested positive for the virus on the 2nd day of life.[22] Although it might appear reasonable to correlate the early onset of symptoms with vertical SARS-CoV-2 infection and even intrauterine maternal-fetal transmission in these cases and others, there are problems with this assumption that would make it speculative for several reasons.[17] These include 1) symptoms such as neonatal respiratory distress or pneumonia are not specific for COVID-19 – they can have multiple causes and, because preterm delivery is present in between 37 and 63 percent of infected neonates it may have a non-infectious etiology that is related to prematurity, or is caused by infection by other agents; 3) even in those cases where neonatal pneumonia has radiographic features consistent with COVID-19 pulmonary disease, its presence cannot be construed to result specifically from transplacental viral transmission; 3) the incubation period of COVID-19 following an initial fetal or perinatal exposure to the virus is unknown; and 4) the exact mechanism of transmission of SARS-CoV-2 – transplacental versus intrapartum – cannot be reliably distinguished by either the presence or absence of neonatal symptoms in a neonate with a positive RT-PCR test for SARS-CoV-2.[29-33] Thus, the onset of symptoms shortly after birth in a neonate with early positive testing for COVID-19 may be suggestive of vertical transmission, but does not confirm a transplacental mechanism.

Determining Transplacental Transmission From Timing Of Neonatal Viral Testing By RT-PCR

The current "gold standard" for diagnosis of COVID-19 in neonates is by reverse transcription-polymerase chain reaction (RT-PCR) analysis of specimens from nasopharyngeal (NP) swabs and, in lesser numbers of infants, from swabs of the oropharynx (OP). However, many factors can affect the sensitivity (ability to detect a positive case) and specificity (ability to determine a negative case) of these tests. Reverse transcription-polymerase chain reaction analysis has many advantages for testing among children and adults, but its efficacy for neonatal diagnosis has not been established. Both analytical as well as external factors can have a detrimental effect on the accuracy of RT-PCR results. Preanalytical factors are the major source of errors in laboratory testing and include problems due to the specimen source, sampling methods, the timing of sampling, sample storage and transport, and presence of interfering substances.[34] The performance of diagnostic kits has been reported in some cases to be suboptimal.[34] In adult patients, false negative results have been demonstrated to be another problem with RT-PCR.[35,36]

The Centers for Disease Control and Prevention (CDC) recommends using (NP) swabs for molecular testing because in most patients because the major mechanisms of transmission of COVID-19 is via the respiratory route and the nasopharynx appears to have the highest concentration of virus.[37] However, in neonates, this has not been definitively established. In fact, it remains unknown as to when the virus first appears in the nasopharynx of neonates who are infected with SARS-CoV-2. The mechanism(s) of nasopharyngeal colonization by SARS-CoV-2 in cases of potential intrauterine or intrapartum fetal infection also have not been determined, especially in cases where the fetus may become infected hematogenously via transplacental infection.

There has been significant variation in the timing of SARS-CoV-2 positivity for NP and OP specimens taken from neonates. In some cases, specimens taken shortly following birth were initially negative, to subsequently become positive days or even weeks later.[24,38,39] In other cases, initial testing was positive in the late neonatal period.[24,40] Early positive testing for COVID-19 of neonates from

infected mothers has also been reported[17,22] – in some cases with positive results obtained from specimens taken immediately or within a few hours after birth.[24,39] In those cases of early test positivity, the neonates were delivered by cesarean section, and as a result the possibility of intrauterine vertical transmission remains high. However, it cannot be confirmed based upon the information available.

In a review of 179 newborn infants delivered to women with COVID-19 during the 3rd trimester, SARS-CoV-2 was detected from NP swabs in 6 infants – 1 at 16 hours, 2 at 36 hours and 3 at 48 hours following delivery – with the authors stating that the timing of transmission could not be determined in these cases.[41]

We propose that a neonate having a positive test for SARS-CoV-2 in the initial 72 hours of life be considered to have early-onset COVID-19 infection - it is this group of neonates that are most suspicious for having acquired COVID-19 from vertical transmission prior to or around the period of delivery. The likelihood is even greater when the initial testing is positive sooner - either immediately or within hours after birth. To address this, we also propose the term very early-onset COVID-19 infection for infants with test positivity within the initial 24 hours of life. However, testing positive for SARS-CoV-2 within these time frames do not determine whether infection occurred via the transplacental route.

DETERMINING TRANSPLACENTAL TRANSMISSION FROM NEONATAL ANTIBODY ANALYSIS

Among all 5 classes of antibodies, IgG is the only antibody class with the capability to cross the placenta from the maternal to the fetal circulation, providing passive immunity to the developing fetus and neonate. As a result, the occurrence of elevated levels of IgG in the newborn circulation following delivery is not useful for diagnosing the acute onset of neonatal infection. Because IgM does not cross the placenta, the concept of using postpartum elevated levels of this antibody class to suggest the possibility of intrauterine infections in neonates was initially introduced in the 1960's.[42,43] Currently, the majority of congenital infections are not diagnosed using neonatal IgM levels for several reasons. These

include the propensity for IgM assays to have reliability problems including both false-positive and false-negative results as well as cross-reactivity and testing challenges, as well as technological advances that have been made in the molecular diagnosis of infectious diseases that have very high levels of sensitivity and specificity.[44,45]

There have been several neonates delivered from SARS-CoV-2-infected mothers who have had elevated levels of specific IgG and IgM antibodies detected shortly after birth. In one case a newborn was found to have developed IgM and IgG specific antibodies to SARS-CoV-2 together with elevated cytokines when tested 2 hours following delivery, but multiple nasopharyngeal swabs from the infant were negative for the virus.[46] In another report, 2 neonates delivered to mothers with COVID-19 were found to have levels of both IgG and IgM that were higher than normal – both infants had NP swabs that tested negative for COVID-19 by RT-PCR.[22] These cases have demonstrated that using elevated IgM levels to diagnose neonatal COVID-19, or to surmise that intrauterine infection has occurred, is not highly reliable.

DETERMINING TRANSPLACENTAL TRANSMISSION FROM VIRAL ANALYSIS OF SWABS OR HOMOGENIZED WHOLE SAMPLES OF PLACENTA

The placenta is a unique heterogenous organ - it is composed of cells from two genetically distinct individuals. It is also distinctive in possessing a dual blood supply from two individuals - the maternal circulation entering the placenta through numerous uterine spiral arterioles and circulating within the intervillous space, and the fetal circulation entering the placenta through the umbilical arteries and circulating throughout all levels of the chorionic villous tree. These two circulations are in close contact with one another, but remain separated by a highly specialized trophoblastic barrier. In addition, both maternal and fetal cells are present at the maternal-fetal interface. As a result, removing a sample of placental tissue using a swab, needle or excisional biopsy contains both maternal and fetal

cells including maternal red and white blood cells in the intervillous space. Following homogenization of

the tissue prior to nucleic acid extraction, the maternal and fetal cells become mixed. A positive PCR test

for an infectious agent on a homogenized sample of placental tissue or a swab of its contents establishes

that the agent is present in the sample, but cannot determine precisely localize the agent to being in the

maternal component, fetal component, or both. This is illustrated in a recent communication in which

swabs and biopsies were taken from the placenta of a stillborn infant delivered to a mother with COVID-

19.[47] These specimens were diagnosed using RT-PCR as being positive for SARS-CoV-2; however,

because the samples included both cells of maternal and fetal origin it was not possible to confirm

whether the coronavirus was in cells from the mother or fetus. Interestingly, despite the placental

specimens testing positive for COVID-19, amniotic fluid, maternal vaginal swabs and all specimens from

the fetal autopsy including swabs from the mouth, axillae, meconium, and fetal blood obtained within

minutes of birth were negative for SARS-CoV-2 by RT-PCR testing. In another report, the amnionic

surface of the placenta was swabbed and found to be positive for SARS-CoV-2 following delivery to an

asymptomatic mother with COVID-19.[48] The neonate was asymptomatic and uninfected. Thus, this form

of placental sampling and PCR analysis cannot reliably be used for confirmation of transplacental viral

transmission.

DETERMINING TRANSPLACENTAL TRANSMISSION FROM ANATOMIC LOCALIZATION OF VIRAL SIGNAL

IN PLACENTAL TISSUE SECTIONS USING IN SITU PCR OR IMMUNOHISTOCHEMISTRY

Pathology of the placenta, the largest of fetal organs, has been instrumental in understanding

the mechanisms of transmission of many different infectious agents from pregnant women to the fetus.

Techniques such as immunohistochemistry using antibodies to viral antigens and nucleic acid techniques

such as in situ hybridization and RNAscope that detect target RNA molecules within intact cells have the

advantage of identifying virus within specific cell types in defined anatomic compartments of the

placenta.[49,50] As a result, these techniques can definitively localize a virus to such fetal cells as the

syncytiotrophoblast, Hofbauer cells (fetal-derived villous stromal macrophages), extravillous trophoblast, and chorionic villous endothelial cells. These methods have been successfully used in past epidemics to identify and confirm transplacental maternal-fetal viral infection. In cases where both mother and neonate are found to be infected, the placental finding of virus in such chorionic villus cell types as syncytiotrophoblast using immunohistochemistry for Ebola virus (Figure 1) or in Hofbauer cells using an RNAscope in situ hybridization methodology for Zika virus (Figure 2) can confirm transplacental transmission of the pathogen.

The use of in situ hybridization was recently used to identify and localize SARS-CoV-2 in the placentas from 2 infected neonates from Italy. In a study of 22 pregnant women with COVID-19 at an Italian hospital, 2 neonates were found to have positive NP swabs for SARS-CoV-2.[39] Placentas from both infants demonstrated chronic intervillositis, which was accompanied by the presence of CD-68-positive macrophages both in the intervillous and the villous space. The placental tissues were evaluated using in situ hybridization with RNAscope technology, a method that enables the detection of the SARS-CoV-2 spike protein mRNA by using the V-nCoV2019-S probe. In both placentas this methodology demonstrated the presence of the coronavirus in the syncytiotrophoblast, indicating the presence of SARS-CoV-2 on the fetal side of the placenta (Figure 3), and proving that intrauterine infection of fetal cells within the placenta had occurred in the intrauterine environment and prior to delivery. This was the first demonstration of SARS-CoV-2 in chorionic villus tissue of the placenta of infected neonates, establishing intrauterine fetal exposure and infection with the coronavirus.

This communication proposes that the identification of virus in chorionic villous tissue of the placenta, using either in situ nucleic acid hybridization methods as was performed by Patanè and colleagues[39], or by immunohistochemistry using virus-specific antibodies to detect viral antigen, provides prima facie evidence of intrauterine fetal infection in the appropriate clinical setting.

CONCLUSIONS

There have been approximately 217 neonates delivered to pregnant women with COVID-19

reported in the literature.[51] More recently there were 19 additional infected neonates reported from 10

hospital in Iran, among whom there were infants with early and late positive testing for the virus.[24] The

relative risks and proportions of neonatal infection made by intrauterine transmission of SARS-CoV-2

from either transplacental or ascending infection, acquiring infection during labor and delivery, or

following delivery from the mother, other individuals or the environment remain unknown. This

knowledge is of critical importance - it can guide management of pregnant mothers with COVID-19,

delivery, and postpartum care of the neonate in order to minimize the risk of neonatal infection. In

addition, identifying mechanisms of neonatal COVID-19 infection will be useful in determining such

factors as the need for cesarean section, neonatal isolation in intensive care, guidelines for neonatal

resuscitation, safety of rooming-in, avoidance of skin-to-skin contact and breast feeding, and

postpartum contact between the neonate, family members and other individuals. Until these data are

established, there will continue to be variation in the care of pregnant women with COVID-19 and their

neonates. Institutions, hospitals, professional organizations, and the public health community have

attempted to use the limited data available to provide recommendations for clinical care that reduces

risk of neonatal infection within the contexts of local social and clinical settings.[50]

In determining whether intrauterine vertical transmission of SARS-CoV-2 has occurred there are

multiple findings that are suggestive – these include positive RT-PCR testing of a neonate for the virus at

or shortly after birth, early onset of symptoms, and elevated levels of specific IgM antibodies following

delivery. However, confirmation of intrauterine transplacental transmission of COVID-19 should be

reserved for those neonates who have demonstrable identification of viral antigen or RNA in fetal-

derived placental cells following microscopic pathology testing of tissue sections. These methods include

using immunohistochemistry for identification of viral antigen, or detection of viral RNA using in situ

hybridization or RNAscope methods. It is clear that many, if not most, pathology laboratories will not

have the capability of performing these types of tests on placentas. However, it is suggested that

whenever feasible, placentas be retained from those neonates suspected of having early-onset COVID-

19 and, when necessary, have formalin-fixed tissues sent to appropriate pathology laboratories for

special testing and evaluation of transplacental infection.

Figure legends:

Figure 1. The placenta from a woman having Ebola virus disease during pregnancy. Both she and her newborn infant died from the infection. Immunohistochemical analysis demonstrates Ebola virus antigen in trophoblast. Immunohistochemistry using rabbit polyclonal antisera against Ebola virus, Sudan virus, and Reston virus, and Ebola virus hyperimmune mouse ascitic fluid, original magnification x100. This case and the methodology for immunohistochemical staining was described by the Centers for Disease Control and Prevention in Muehlenbachs et al. [50]

Figure 2. Zika virus RNA (red arrow) is positive by RNAscope in a stromal cell, presumably a Hofbauer cell, in the chorionic villus of the placenta of a preterm infant with congenital Zika virus infection and microcephaly. In situ hybridization for Zika virus, magnification x100.

Figure 3. Brown dots represent positive signals from the SARS-CoV-2 spike protein mRNA in this tissue section from a neonate with SARS-CoV-2 infection following delivery from a mother with COVID-19. The pattern of RNAscope assay positivity confirms coronavirus infection of the syncytiotrophoblast. This case and the methodology for RNAscope staining was described in Reference 39. In situ hybridization for SARS-CoV-2, original magnification x100.

References

1. Schwartz DA, Graham AL. Potential maternal and infant outcomes from coronavirus 2019-nCoV (SARS-CoV-2) infecting pregnant women: Lessons from SARS, MERS, and other human coronavirus infections. *Viruses*. 2020;12(2):194. doi: 10.3390/v12020194.

2. Schwartz DA, Dhaliwal A. Infections in pregnancy with COVID-19 and other respiratory RNA virus diseases are rarely, if ever, transmitted to the fetus: Experiences with coronaviruses, HPIV, hMPV RSV, and influenza [published online ahead of print, 2020 Apr 27]. *Arch Pathol Lab Med*. 2020;10.5858/arpa.2020-0211-SA. doi:10.5858/arpa.2020-0211-SA.

3. Schwartz DA. The Pathology of Pregnancy. In: Strayer DS and Saffitz JE, eds. *Rubin's Pathology. Clinicopathologic Foundations of Medicine*. 8th edition. Philadelphia: Wolters Kluwer; 2020. Pages 555-581.

4. Nahmias AJ, Panigel M, Schwartz DA. Hematogenous infections of the placenta - an interdisciplinary and evolutionary perspective. *Placenta.* 1994;15(1):107-136. doi: https://doi.org/10.1016/S0143-4004(05)80339-X

5. Nahmias AJ, Panigel M, Schwartz DA. The eight most frequent blood-borne infectious agents affecting the placenta and fetus. A synoptic review. *Trophoblast Res.* 1994;8:193-213. doi: http://dx.doi.org/10.1016/S0143-4004(05)80344-3.

6. Bhatta AK, Keyal U, Liu Y, Gellen E. Vertical transmission of herpes simplex virus: an update. *J Dtsch Dermatol Ges*. 2018;16(6):685-692. doi:10.1111/ddg.13529.

7. Schwartz DA, Caldwell E. Herpes simplex virus infection of the placenta. The role of molecular pathology in the diagnosis of viral infection of placental-associated tissues. *Arch Pathol Lab Med*. 1991;115(11):1141-1144.

8. Trottier H, Mayrand MH, Coutlée F, et al. Human papillomavirus (HPV) perinatal transmission and risk of HPV persistence among children: Design, methods and preliminary results of the HERITAGE study. *Papillomavirus Res*. 2016;2:145-152. doi:10.1016/j.pvr.2016.07.001

9. Moore DL, Allen UD. HIV in pregnancy: Identification of intrapartum and perinatal HIV exposures. *Paediatr Child Health*. 2019;24(1):42-49. doi:10.1093/pch/pxy181

10. Liang K, Gui X, Zhang YZ, Zhuang K, Meyers K, Ho DD. A case series of 104 women infected with HIV-1 via blood transfusion postnatally: high rate of HIV-1 transmission to infants through breast-feeding. *J Infect Dis*. 2009;200(5):682-686. doi:10.1086/605123

11. Stiehm ER, Keller MA. Breast Milk Transmission of Viral Disease. In: Woodward B, Draper HH, Eds. Advances in Nutritional Research. Immunological Properties of Milk; 2001, Volume 10. Pgs. 105-122. New York: Springer. ISBN : 978-1-4613-5182-5

12. Sissoko D, Keïta M, Diallo B, et al. Ebola virus persistence in breast milk after no reported illness: A likely source of virus transmission from mother to child. *Clin Infect Dis*. 2017;64(4):513-516. doi:10.1093/cid/ciw793.

13. Zaigham M, Andersson O. Maternal and perinatal outcomes with COVID-19: A systematic review of 108 pregnancies [published online ahead of print, 2020 Apr 7]. *Acta Obstet Gynecol Scand*. 2020;10.1111/aogs.13867. doi:10.1111/aogs.13867

14. Schwartz DA. 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*. 2020; 144(7): 799-805. doi:10.5858/arpa.2020-0901-SA.

15. Perlman J, Oxford C, Chang C, Salvatore C, Di Pace J. Delivery room preparedness and early neonatal outcomes during COVID19 pandemic in New York City [published online ahead of print, 2020 May 14]. *Pediatrics*. 2020;e20201567. doi:10.1542/peds.2020-1567

16. Juan J, Gil MM, Rong Z, Zhang Y, Yang H, Poon LC. Effects of coronavirus disease 2019 (COVID-19) on maternal, perinatal and neonatal outcomes: a systematic review [published online ahead of print, 2020 May 19]. *Ultrasound Obstet Gynecol*. 2020;10.1002/uog.22088.

17. Schwartz DA. Vertical transmission of severe acute respiratory syndrome coronavirus 2 from the mother to the infant [published online ahead of print July 20, 2020]. *JAMA Pediatr*. 2020. doi:10.1001/jamapediatrics.2020.2135

18. Yang Z, Wang M, Zhu Z, Liu Y. Coronavirus disease 2019 (COVID-19) and pregnancy: a systematic review [published online ahead of print, 2020 Apr 30]. *J Matern Fetal Neonatal Med*. 2020;1-4. doi:10.1080/14767058.2020.1759541

19. Mullins E, Evans D, Viner RM, O'Brien P, Morris E. Coronavirus in pregnancy and delivery: rapid review. *Ultrasound Obstet Gynecol*. 2020;55(5):586-592. doi:10.1002/uog.22014.

20. Yu N, Li W, Kang Q, et al. Clinical features and obstetric and neonatal outcomes of pregnant patients with COVID-19 in Wuhan, China: a retrospective, single-centre, descriptive study. *Lancet Infect Dis*. 2020;20(5):559-564. doi:10.1016/S1473-3099(20)30176-6

21. Wang S, Guo L, Zhang J, et al. A case report of neonatal COVID-19 infection in China [published online ahead of print, 2020 Mar 12]. *Clin Infect Dis*. 2020;ciaa225. doi:10.1093/cid/ciaa225.

22. Zeng L, Xia S, Yuan W, et al. Neonatal early-onset infection with SARS-CoV-2 in 33 neonates born to mothers with COVID-19 in Wuhan, China [published online ahead of print, 2020 Mar 26]. *JAMA Pediatr*. 2020;e200878. doi:10.1001/jamapediatrics.2020.0878.

23. Alzamora MC, Paredes T, Caceres D, Webb CM, Valdez LM, La Rosa M. Severe COVID-19 during pregnancy and possible vertical transmission [published online ahead of print, 2020 Apr 18]. *Am J Perinatol*. 2020;10.1055/s-0040-1710050. doi:10.1055/s-0040-1710050.

24. Schwartz DA, Mohagheghi P, Beigi B, Zafaranloo N, Moshfegh F, Yazdani A. Spectrum of neonatal COVID-19 in Iran: 19 infants with SARS-CoV-2 perinatal infections with varying test results, clinical findings and outcomes. *J Matern Fetal Neonatal Med.* 2020. In Press. doi: 10.1080/14767058.2020.1797672.

25. Gordon M, Kagalwala T, Rezk K, Rawlingson C, Ahmed MI, Guleri A. Rapid systematic review of neonatal COVID-19 including a case of presumed vertical transmission. *BMJ Paediatrics Open* 2020;**4:**e000718. doi: 10.1136/bmjpo-2020-000718.

26. Lin CY, Hsu CH, Huang FY, et al. The changing face of early-onset neonatal sepsis after the implementation of a maternal group B Streptococcus screening and intrapartum prophylaxis policy--a study in one medical center. *Pediatr Neonatol*. 2011;52(2):78-84. doi:10.1016/j.pedneo.2011.02.001.

27. Simonsen KA, Anderson-Berry AL, Delair SF, Davies HD. Early-onset neonatal sepsis. *Clin Microbiol Rev*. 2014;27(1):21-47. doi:10.1128/CMR.00031-13.

28. Polin RA, Committee on Fetus and Newborn. Management of neonates with suspected or proven early-onset bacterial sepsis. *Pediatrics*. 2012;129(5):1006-1015. doi:10.1542/peds.2012-0541

29. Zimmermann P, Curtis N. COVID-19 in children, pregnancy and neonates. *Ped Infect Dis J.* 2020, 39(6):469-477. doi: 10.1097/INF.0000000000002700.

30. Zhu H, Wang L, Fang C, et al. Clinical analysis of 10 neonates born to mothers with 2019-nCoV pneumonia. *Transl Pediatr*. 2020;9(1):51-60. doi:10.21037/tp.2020.02.06.

31. Yang P, Wang X, Liu P, et al. Clinical characteristics and risk assessment of newborns born to mothers with COVID-19. *J Clin Virol*. 2020;127:104356. doi:10.1016/j.jcv.2020.104356.

32. Peng Z, Wang J, Mo Y, et al. Unlikely SARS-CoV-2 vertical transmission from mother to child: A case report. *J Infect Public Health*. 2020;13(5):818-820. doi:10.1016/j.jiph.2020.04.004

33. Schwartz DA. The effects of pregnancy on women with COVID-19: Maternal and infant outcomes. *Clin Infect Dis*. ciaa559. DOI: https://doi.org/10.1093/cid/ciaa559. Published online 11 May 2020.

34.Younes N, Al-Sadeq DW, Al-Jighefee H, et al. Challenges in laboratory diagnosis of the novel coronavirus SARS-CoV-2. *Viruses*. 2020;12(6):E582. Published 2020 May 26. doi:10.3390/v12060582.

35. Kucirka LM, Lauer SA, Laeyendecker O, Boon D, Lessler J. Variation in false-negative rate of reverse transcriptase polymerase chain reaction-based SARS-CoV-2 tests by time since exposure [published online ahead of print, 2020 May 13]. *Ann Intern Med*. 2020;M20-1495. doi:10.7326/M20-1495.

36. Li D, Wang D, Dong J, Wang N, Huang H, Xu H, Xia C.   False-negative results of real-time reverse-transcriptase polymerase chain reaction for severe acute respiratory syndrome coronavirus 2: Role of deep-learning-based CT diagnosis and insights from two cases. *Korean J Radiol*. 2020 Apr;21(4):505-508.   https://doi.org/10.3348/kjr.2020.0146

37. Centers for Disease Control and Prevention. Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19. May 22, 2020. https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html Accessed.

38. Buonsenso D, Costa S, Sanguinetti M, et al. Neonatal late onset infection with severe acute respiratory syndrome coronavirus 2 [published online ahead of print, 2020 May 2]. *Am J Perinatol*. 2020;10.1055/s-0040-1710541. doi:10.1055/s-0040-1710541

39. Patanè L, Morotti D, Giunta MR, et al. Vertical transmission of COVID-19: SARS-CoV-2 RNA on the fetal side of the placenta in pregnancies with COVID-19 positive mothers and neonates at birth [published online ahead of print, 2020 May 18]. *Am J Obstet Gynecol MFM*. 2020;100145. doi:10.1016/j.ajogmf.2020.100145.

40. Coronado Munoz A, Nawaratne U, McMann D, Ellsworth M, Meliones J, Boukas K. Late-onset neonatal sepsis in a patient with COVID-19. *N Engl J Med.* 2020;382: e49. doi: 10.1056/NEJMc2010614.

41. Egloff C, Vauloup-Fellous C, Picone O, Mandelbrot L, Roques P. Evidence and possible mechanisms of rare maternal-fetal transmission of SARS-CoV-2. *J Clin Virol*. 2020;128:104447. doi:10.1016/j.jcv.2020.104447.

42. Alford CA, Schaefer J, Blankenship WJ, Straumfiord JV, Cassady G. A correlative, immunologic, microbiologic and clinical approach to the diagnosis of acute and chronic infections in newborn infants. *N Engl J Med*. 1967;277(9):437-449. doi:10.1056/NEJM196708312770901

43. Haider SA. Serum IgM in diagnosis of infection in the newborn. *Arch Dis Child*. 1972;47(253):382-393. doi:10.1136/adc.47.253.382.

44. Ford-Jones EL. An approach to the diagnosis of congenital infections. *Paediatr Child Health*. 1999;4(2):109-112. doi:10.1093/pch/4.2.109.

45. Kimberlin DW, Stagno S. Can SARS-CoV-2 infection be acquired in utero? More definitive evidence is needed. *JAMA.* 2020;323(18):1788–1789. doi:10.1001/jama.2020.4868.

46. Dong L, Tian J, He S, et al. Possible vertical transmission of SARS-CoV-2 from an infected mother to her newborn. *JAMA.* 2020. doi:10.1001/jama.2020.4621.

47. Baud D, Greub G, Favre G, Gengler C, Jaton K, Dubruc E, et al. Second-trimester miscarriage in a pregnant woman with SARS-CoV-2 infection [published online ahead of print, 2020 Apr 30]. *JAMA*. 2020;323(21):2198-2200. doi:10.1001/jama.2020.7233.

48. Ferraiolo A, Barra F, Kratochwila C, et al. Report of positive placental swabs for SARS-CoV-2 in an asymptomatic pregnant woman with COVID-19. *Medicina*. 2000. *56*(6):306;

doi: https://doi.org/10.3390/medicina56060306

49. Schwartz DA. Viral infection, proliferation, and hyperplasia of Hofbauer cells and absence of inflammation characterize the placental pathology of fetuses with congenital Zika virus infection. *Arch Gynecol Obstet*. 2017;295(6):1361–1368. doi:10.1007/s00404-017-4361-5.

50. Muehlenbachs A, de la Rosa Vazquez O, Bausch DG, et al. Ebola virus disease in pregnancy: Clinical, Histopathologic, and immunohistochemical findings. *J Infect Dis*. 2017;215(1):64–9. doi:

https://doi.org/10.1093/infdis/jiw206

51. Gupta M, Zupancic JAF, Pursley DM. Caring for newborns born to mothers with COVID-19: More questions than answers*. Pediatrics*. 2020;e2020001842; doi: https://doi.org/10.1542/peds.2020-001842.