The COVID-19 Ontario Pregnancy Event (COPE) Network

Study Title: Assessing the mother-to-infant transmission capabilities of COVID-19 infection among pregnant women in Ontario, Canada

Study Design: A multi-centre, prospective cohort study

Date and Version No: 15 November 2024, Version 11.0

Sponsor/Coordinating Centre: Ottawa Hospital Research Institute (OHRI)

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LIST OF ABBREVIATIONS

BORN	Better Outcomes Registry and Network
CHEO	Children's Hospital of Eastern Ontario
CoV	Coronaviruses
COVID-19	Coronavirus Disease 2019
CTO	Clinical Trials Ontario
ddPCR	Droplet digital PCR
eCRF	Electronic Case Report Form
EDTA	Ethylenediaminetetraacetic acid
H1N1	Influenza A virus subtype H1N1
HCP	Healthcare professional
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
MERS	Middle East Respiratory Syndrome
MRN	Medical Record Number
NSO	Newborn Screening Ontario
OHRI	Ottawa Hospital Research Institute
PCR	Polymerase Chain Reaction
PHIPA	Personal Health Information Privacy Act
REB	Research Ethics Board
RNA	Ribonucleic acid
RT-qPCR or RT-PCR	Real-time Fluorescent Quantitative PCR
SARS	Severe Acute Respiratory Syndrome
SARS-CoV-2	Severe Acute Respiratory Syndrome coronavirus 2
SCC	Study Coordinating Centre
SOP	Study Standard Operating Procedures
TCPS 2	Tri-Council Policy Statement 2
The COPE Network	The COVID-19 Ontario Pregnancy Event (COPE) Network
TOH	The Ottawa Hospital
VTM	Viral Transport Media

1. BACKGROUND AND RATIONALE

In response to the rapid global spread of cases and deaths, the World Health Organization (WHO) characterized the novel Coronavirus Disease 2019 (COVID19) outbreak as a global pandemic on March 11, 2020.[1] Since the initial detection in Wuhan, China in December 2019, the number of COVID-19 cases has reached alarming rates and continues to rapidly increase. As of April 14th, 2020 there have been almost 1,844,863 confirmed cases and 117,021 deaths globally.[2] Currently there are no antivirals available and therapeutic treatments are limited; numerous clinical research investigations commencing imminently.

1.1 Transmission, clinical presentation and diagnosis of COVID-19

The name of virus responsible for the COVID-19 outbreak, Severe Acute Respiratory Syndrome (SARS) coronavirus 2 (SARS-CoV-2), was chosen based on its genetic similarities with the coronavirus that caused the SARS outbreak in 2003.[3] The exact origin of the virus is currently unknown.[4] Existing literature supports that the novel β-Coronavirus (termed SARS-CoV-2) belongs to the family Coronaviridae, from the order Nidovirales, which are all enveloped, non-segmented positive-sense RNA viruses which may cause illness in humans and animals.[5,6] The Coronaviridae family of viruses include causative viruses such as Severe Acute Respiratory Syndrome (SARS) and Middle Eastern Respiratory Syndrome (MERS).[7] Much like influenza and other respiratory viruses, SARS-CoV-2 is primarily transmitted person-to-person through close inhalation of respiratory droplets formed during a cough, sneeze or exhale.[8] People without the disease can also acquire the infection by touching surfaces contaminated by SARS-CoV-2, defined as fomite transmission, then touching mucous membranes found in the face such as the eyes, nose, or mouth or by faecal transmission.[9] Clinical manifestations among adults include fever, cough, myalgia, headache and shortness of breath.[10] Severe cases can result in life threatening pneumonia and acute respiratory distress syndrome.[11] The incubation period is estimated to be between 1 and 14 days, with 97.5% of cases developing symptoms within 10.5 days (95% CI: 7.3-15.3) of infection.[12] Due to the small number of cases, it is not yet certain whether pregnancy and childbirth aggravate clinical presentation of COVID-19.

1.2 Implications of COVID-19 infection for pregnant women

Currently, there are limited data on the clinical characteristics of COVID-19 infection in pregnant women, including any adverse impacts on the developing fetus.[8] Three early case-series reports from China reported a total of 31 pregnancies affected by COVID-19. One of the case series included nine pregnant women in Wuhan, all hospitalized with COVID-19 pneumonia during the third trimester of pregnancy. Clinical manifestations were similar to those reported among non-pregnant adults and included fever, cough, myalgia, malaise and sore throat. No women required mechanical ventilation, there were no maternal deaths and newborn Apgar scores at 1 and 5 minutes were high.[13] Another case series included 13 COVID-19-infected pregnant women admitted to hospital in other areas of China. The study reported that three of the women were discharged from hospital with an ongoing pregnancy, while the other 10 women were delivered by cesarean, five urgently for fetal indications (three for fetal distress, one for premature rupture of membranes and one stillbirth).[14] The third Chinese case series included 10 live born infants exposed to maternal COVID-19 infection while *in utero* and reported six preterm and two small-for-gestational-age infants. One infant born at 34 weeks' gestation developed complications and died nine days after birth. Other clinical complications in the newborns were also reported, including prolonged hospitalization and respiratory distress.[15]

More recently, Breslin et al. reported a case series which included 43 confirmed COVID-19 pregnant women that presented to an affiliated pair of New York City hospitals between March 13 and March 27, 2020.[16] Disease severity among these pregnant women were similar to previous reports in non-pregnant individuals: 86% mild, 9.3% severe and 4.7% critical. None of the neonates were tested positive for COVID-19 through PCR testing of nasopharyngeal swab samples. Although cases were initially identified through routine testing of symptomatic patients, New York City later initiated universal testing of all obstetrical admissions on March 22nd which additionally allowed for the capture of asymptomatic cases. Among the 43 cases reported, an unexpectedly large proportion of patients were initially asymptomatic (n=14) and presented a milder course of disease. Considering 12 of these asymptomatic positive cases were identified through this new universal testing strategy, the authors advocate for universal testing of all pregnant women upon admission for delivery.[16] Although reports of COVID-

19 cases among newborns and young infants have been rare thus far, healthcare providers need to be extra vigilant when assessing newborn babies delivered by infected mothers, as early identification and isolation are imperative to virus control.

1.3 Susceptibility to and severity of COVID-19 in pregnancy

It is well-established that certain infections disproportionately impact pregnant women leading to increased rates of hospitalization and complications, likely due to physiological, physical, and immunological adaptations to pregnancy.[17] Based on the lack of information currently available, we are unable to fully understand the risks and susceptibility of SARS-CoV-2 infection during pregnancy. Di Mascio et al. recently conducted a systematic review reporting pregnancy and perinatal outcomes of CoV spectrum infections during pregnancy.[18] They found a total of 19 case series studies with 79 pregnancies affected by CoV infections: 41 pregnancies in the review (51.9%) were affected by COVID-19, 26 (32.9%) by SARS and 12 (15.2%) by MERS. Among the six studies reporting information on COVID-19 infection during pregnancy, preterm birth (<37 weeks' gestation) was the most commonly reported adverse pregnancy outcome, occurring in 41.1% (95% CI: 25.6-57.6) of cases. These COVID-19 infected pregnant women also had higher rates of miscarriage, pre-eclampsia, and perinatal death, compared to the general population. Whether these complications are a result of COVID-19 infection requires further investigation.

1.4 The effects of previous outbreaks on pregnant women

Coronaviruses (CoV) are a family of single-stranded RNA viruses, named for the appearance of crown-like proteins that project on the viral envelope.[8] Apart from SARS-CoV-2, two other notable human strains of coronaviruses include SARS-CoV and Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV). The WHO reported 774 and 858 deaths associated with SAS-CoV[19] and MERS-CoV[20], respectively. Within a short time interval, the novel SARS-CoV-2 coronavirus has led to more deaths than these two previous strains combined.[21]
Evidence of poor maternal and perinatal outcomes from several other outbreaks of viral respiratory illness over the past 20 years (i.e., 2009 A/H1N1 influenza pandemic,[22–25] SARS[26,27] and MERS[8]) indicate a need for enhanced surveillance of pregnant women during this current pandemic to determine whether COVID-19 poses any threat to maternal, fetal or infant heath.
Although SARS and MERS are not identical to SARS-CoV-2, evidence on the effects of these viruses during pregnancy can help to guide obstetrical care and inform control measures.

1.5 Testing for the presence of SARS-CoV-2

According to the WHO, real-time fluorescent quantitative PCR (RT-qPCR or RT-PCR) is currently recognized as the gold standard for clinical detection of SARS-CoV-2.[28] Still, RT-PCR is limited by the ability to detect the virus in the sample, and is especially problematic in cases with low viral load in the infected patient's throat,[29] resulting in a false negative rate currently reported to be between 30 to 33%.[30,31] Numerous factors may influence RT-PCR testing results including sample collection and transportation, RNA extraction and degradation in storage, and effective functioning of the kit.[32] Despite these limitations of RT-PCR, especially among low viral load specimens, all maternal and neonatal case reports published thus far have only tested for the presence of SARS-CoV-2 using RT-PCR.
Droplet digital PCR (ddPCR), a biotechnological refinement of PCR, has shown improved sensitivity and accuracy for SARS-CoV-2 detection, especially for tests involving low viral load samples.[28,33] Further, COVID-19 testing using ddPCR has demonstrated lower rates of false negatives and positives, compared to RT-PCR testing.[34] Thus, ddPCR can be used in combination with RT-PCR to improve detection and robustness of estimates.

1.6 Mother-to-infant transmission

A crucial question that remains unanswered concerns whether SARS-CoV-2 can be transmitted by the infected pregnant mother to the infant during pregnancy, delivery or post-partum. Mother-to-infant transmission after maternal primary infection can occur during several time periods: intrauterine life via trans-placental viral transfer, delivery via ingestion or aspiration of vaginal secretions and postpartum through breastfeeding.[35] Due

to the scarce evidence currently available, the mother-to-child transmission capabilities of SARS-CoV-2 are not yet fully understood.

The small number of studies published thus far have not detected any evidence of vertical transmission from mother to fetus/infant[13–15,36,37]; however, several newborns have tested positive for SARS-CoV-2 with case studies purporting the potential for vertical transmission.[38–41] Further assessment is warranted as nearly all of the published findings included women infected during the third trimester of pregnancy and delivered by cesarean section.[42]

The placenta is a superior example of a pregnancy diary and typically undergoes morphological changes in response to environmental stimuli. A case report of one COVID-19 positive pregnancy found that all collected biological specimens, including a placenta sample, tested negative for SARS-CoV-2.[43] Another review of three placentas found no morphological changes related to infection, though all of the women in this case series contracted the virus late in the third trimester and long after the placenta had undergone the bulk of its development.[13] Furthermore, the role of the SARS-CoV-2 in various placental lesions (e.g., preeclampsia, abnormal placentation, intrauterine growth restriction [IUGR], and disruption of the chorionic membrane) has yet to be explored.

In addition to the consideration of vertical transmission, mothers may pass SARS-CoV-2 onto their infants via breastmilk or combination feeding, similar to women infected with HIV-1. High levels of the HIV-1 virus in the mother increases the RNA viral load in maternal plasma and breastmilk which increases the rate of postnatal transmission by nearly 30%.[44] The potential for neonatal transmission also includes the impact of aerosol production during the breastfeeding process which warrants additional evaluation. As a result of the unclear and conflicting findings, we cannot rule out the potential for SARS-CoV-2 to permeate through infected breastmilk via consumption or inhalation to infect a nursing infant.

In view of these uncertainties, we propose to undertake a study of COVID-19-infected pregnant women admitted to hospitals for birth across Ontario.

2. AIM & STUDY OBJECTIVE

The overall aim of this research is to generate clinical evidence regarding the impact of COVID-19 infection during pregnancy on maternal, fetal and infant health.

Our objective will be to assess the mother-to-infant transmission potential of SARS-CoV-2 among pregnant women delivering at hospitals across Ontario with confirmed or suspected COVID-19.

3. METHODOLOGY

3.1 Study Design

In order to assess the mother-to-infant and potential vertical transmission of SARS-CoV-2 infection in pregnant women, maternal and neonatal biological samples will be prospectively collected from women with confirmed or suspected COVID-19 at participating hospitals across Ontario. Samples will be tested for the coronavirus serology and viral load.

3.2 Study Setting

The COVID-19 Ontario Pregnancy Event (COPE) Network is a provincial collaboration that was established in response to the pandemic, and currently consists of 12 participating hospitals across Ontario. This network, consisting of physicians and scientists from various institutions across the province, will help unite the resources

and expertise necessary to answer critical research questions related to SARS-CoV-2 infection during pregnancy. The current list of participating sites can be found in **Section 6.1**.

3.3 Study Population

The study population will consist of pregnant women with confirmed COVID-19 at any point during pregnancy or suspected COVID-19 at time of delivery (as identified at local hospital) , who will be delivering at a participating hospital within Ontario (current list of participating sites can be found in **Section 6.1**).

3.4 Recruitment

In this global emergency of COVID-19, information regarding the effects of infection during pregnancy are urgently required to help inform obstetrical recommendations, guidelines and practice. Given the low number of cases reported thus far, any pregnant woman admitted to a participating hospital with either suspected or confirmed COVID-19 infection, at any point during pregnancy, will be included in the study.

To facilitate rapid generation of evidence, ensure complete capture of cases, and prevent unnecessary burden on healthcare professionals, we will use an alteration to the consent procedures. This study poses minimal risk to the participants, and given the current circumstances of a global pandemic, and the additional precautions to manage this population, an alternation to the consent requirements are justified to ensure that this research can be carried out.[45] Using a verbal consent script, the healthcare provider will explain the research study and collection of samples to the participant. Verbal consent will be obtained at a pre-natal visit (in-person, virtual, or telephone), at the time of admission or prior to delivery. If the healthcare provider is unable to obtain verbal consent prior to delivery, sample collection will proceed and consent will be obtained prior to breastmilk and/or neonatal nasopharyngeal swab collection. At this time, consent for use of any previously collected samples will also be sought.

3.5 Study Outcomes

Outcomes for the study objective will be ascertained through the collection and testing of biological samples from the mother and/or infant. Specifically we will:

1) Assess maternal nasopharyngeal or oropharyngeal swab, vaginal mucosa, ano-rectal swab, amniotic fluid, placenta (including subamniotic swab), breastmilk, cord blood and neonatal nasopharyngeal swab for RNA particles of coronavirus, by ddPCR.
2) Assess maternal serum for anti-coronavirus antibodies, by immunoassay.
3) Examine the impact of coronavirus on the neonate with respect to serology and viral load, in addition to placenta pathology findings and ddPCR.
4) Assess vertical transmission and the effect of coronavirus through placental pathology examination using placental pathology synoptic report (**Appendix 2**).

3.6 Data Collection

3.6.1 *Timing of Sample Collection*

Upon admission to labour and delivery, and prior to delivery wherever possible, the following maternal sample types will be collected: nasopharyngeal or oropharyngeal swab, peripheral blood, ano-rectal swab and vaginal mucosa collected through a vaginal swab. At delivery, wherenever possible, we will collect an amniotic fluid sample through direct syringe aspiration. Should the maternal biological samples not be able to be collected prior to or during delivery, samples may be collected post-delivery, prior to discharge.

Immediately following delivery, we will collect cord blood and placenta samples (including submaniotic swab). Prior to discharge from the hospital, we will collect a neonatal nasopharyngeal swab sample (approximately 12-24 hours post-birth) and a breastmilk sample (nearest to first lactation as possible). The timing of sample

collection is summarized in **Table 2** presented below. Pathologists at each participating site will be requested to send the local placenta pathology report and slides (or scanned slides if slides are not feasible), collected as per standard of care.

[redacted]

Table 2. Timing of biological sample collection

Biological sample	Timing of sample collection			
	Prior to delivery	At delivery	Post delivery	[redacted]
Maternal peripheral blood	X			[redacted]
Maternal nasopharyngeal or oropharyngeal swab	X			[redacted]
Maternal vaginal mucosa	X (swab prior to rupture of membranes, if possible)			
Maternal ano-rectal swab	X			
Amniotic fluid		X		
Cord blood		X (from blood gases)		
Placenta (includes subamniotic swab)			X (ex vivo)	
Neonatal nasopharyngeal swab			X (12-24 hours post-birth)	[redacted]
[redacted]			[redacted]	[redacted]
Breastmilk sample			X	[redacted]

[redacted]

3.6.2 *Sample Collection Process and Storage*

In order to reduce sample degradation and ascertain consistency between sites, samples will be collected and stored as per the Lab Manual following requirements for working with biohazardous materials and Transportation of Dangerous Goods (TDG) while abiding by local institutional obligations. We will collect date of diagnosis for participants. Should the date of diagnosis not be available, we will use date of COVID-19 hospital admission as the estimated date of viral onset. Details of sample collection (e.g. date, time, methodology) will be documented on the Sample Collection Information Form. The Form will be used to review duration of infection where appropriate, and relationship to gestational age. **Figure 1** presents the general study workflow related to all samples variants. Detailed sample collection procedures are outlined in the Lab Manual which is provided to each participating site.

Samples collected at each participating site will be affixed with a patient label containing the patient name and medical record number [MRN]. At least one patient label will be shipped alongside samples to identify patient source of the samples. This will avoid the possibility of errors, and will also reduce the workload of the healthcare staff collecting the samples during a pandemic urgent situation. Further, the patient identifiers on these samples (patient name and MRN) will be used to link the clinical data to the BORN Ontario Birth Registry for supplemental pregnancy and demographic information upon participant consent.

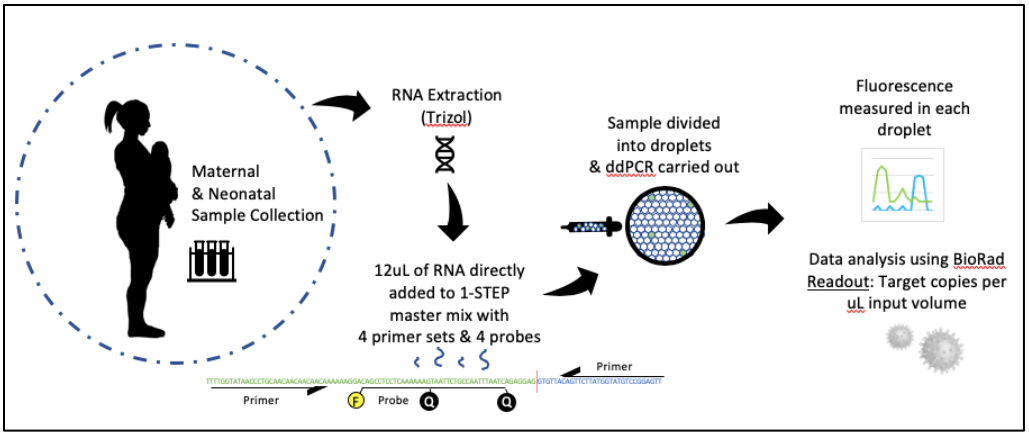


Figure 1: Study Workflow

3.6.3 *Shipment of Samples*

All specimens will be transported as UN3373 Biological Substance Category B with appropriate packaging, labelling and documentation.

Samples will be received by the Study Coordinating Centre (SCC), the Ottawa Hospital Research Institute (OHRI). The patient identifiers will be removed and a COPE Network unique study ID will be affixed and the study ID and participant information will be retained in a master log stored on the secure network at the OHRI. The SCC andauthorized personnel at participating sites will have access to their master file through the hospital shared drive, which is stored on the SCC hospital shared drive. Once the samples are received and assigned a study ID, the samples will then be distributed to specific laboratories within Ottawa for analysis (see **Table 3** for list of laboratories within Ottawa).

Table 3. Laboratories receiving shipped samples

Institution	Name of laboratory	Responsible personnel
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

3.6.4 *Data Linkage with the BORN Ontario Birth Registry*

The Better Outcomes Registry and Network (BORN) Ontario birth registry is a province-wide database, held and administered at the Children's Hospital of Eastern Ontario (CHEO). The database captures all hospital births >500 grams or >20 weeks' gestation in the province. The routine data collection includes information on maternal demographics and health behaviours, pre-existing health problems, obstetric complications and birth outcomes. Data are collected from medical records, clinical forms, and patient interview when a woman is admitted to hospital to give birth.

In response to the urgent public health crisis, BORN Ontario recently approved the collection of data for any cases of COVID-19 infected pregnant individuals admitted to Ontario hospitals between March 1, 2020 and March 1, 2021 (or until the pandemic is declared over and 40 weeks pass to allow collection from newly pregnant individuals who will continue their pregnancy) for the registry purpose of facilitating and improving care. This

includes information from Ontario hospitals on COVID-19 laboratory results, illness, and outcomes. In order to support the analysis and minimize hospital staff burden, we will utilize the data collected as part of this BORN Ontario COVID-19 initiative to provide clinical and demographic information on our participants. Please refer to **Appendix 1** for a list of the data elements (COVID-19 Case Report Form) that will be collected through the study through the data linkage with BORN Ontario.

BORN Ontario has policies for the secure transfer and retention of personal health information in accordance with Ontario's health privacy legislation, the Personal Health Information Protection Act (PHIPA). The data and personal health information (PHI) collected by BORN is securely held, and all analyses will be performed within the BORN secure network environment. The data will be accessible only to the BORN Ontario Analyst who links and assembles the files, and to the study staff at the OHRI who will be responsible for conducting the analyses.

COVID-19 data collection in BORN Ontario is currently optional for hospitals in the province. To ensure data completeness, some sites may complete the Case Report Form which will capture the same data that is collected in BORN Ontario and additional laboratory or clinical variables from routine clinical tests performed for COVID-19 suspected or positive patients Data in the CRF will be sent to the SCC securely and confidentially using a restricted-access database in SharePoint.

Data from medical charts and sample analysis will be sent to BORN Ontario for linkage with the BORN Ontario data, including both the COVID-19 data and routinely collected perinatal and infant health data, upon participant consent.

[redacted]

[redacted]

3.9 COPE Network Site-Specific Placental Control Group

Evidence has shown that placentas not complicated by SARS-CoV-2 may also display pathological changes, thus highlighting the importance of including a sufficient number and variety of controls that would allow valid comparisons to be made. Subgroup analyses based on mode of delivery (cesarean section vs. vaginal) as well as maternal comorbidities (including but not limited to: preeclampsia, chronic hypertension, gestational and chronic diabetes, IUGR) will necessitate having sufficient controls with these characteristics.

In addition to the control participants recruited at TOH, COPE Network Sites with capacity to collaborate will be asked to send slides and pathology reports of placentas that, to the best of their knowledge, were not complicated by SARS-CoV-2. The following clinical indications may warrant placental pathology examination and would provide valuable control comparisons:

- Multiple births (both dichorionic and monochorionic)
- Preterm birth (<34 weeks of gestation)
- Placenta previa
- Elective Cesarean section delivery
- Clinical suspicion of abruption
- Compromised clinical condition of neonate (intensive care admission, cord pH<7.0, Apgar score of less than 5 at 5 minutes, ventilatory assistance, hematocrit less than 25%, hydrops fetalis, congenital anomalies)
- Fetal congenital anomalies
- Maternal cancer history

Placentas that were sent for pathology examination for reasons listed above, between April 2020 to July 2021 may be included in the 'pandemic' control group, and placentas sent to pathology prior to March 2019 (i.e., a year prior to the beginning of the pandemic) will be included in the 'pre-pandemic' control group for the purpose of comparing COVID-19 placental pathology to background pathology during both time points.

4. STATISTICAL METHODS

4.1 Sample Size

Due to the novel nature of this study, the anticipated sample size it not yet known and therefore a typical power calculation cannot be performed. As of March 2nd 2021, Ontario has a total of 303, 763 confirmed COVID-19 cases reported.[50] Considering a total of approximately 13 major Ontario hospital sites will be involved in this study, our sample is anticipated to range anywhere from 150 to upwards of 200 participants.

4.2 Analysis Plan

4.2.1 *Droplet Digital PCR*

We will follow manufacturer instructions of the Bio-Rad QX200 Droplet Digital PCR System. We will employ a multiplex strategy that will co-amplify the viral RNA coding for the N gene in the FAM channel using US CDC primers and probe (N1) and Hong Kong primers and probe (N).[46,47] The Hex channel will contain primers and probe for the viral RNA coding for the E gene (E-Sarbeco) designed by the WHO[48] and an internal RT and amplication control, RNAse P. This custom multiplex primer probe approach will promote high sensitivity in FAM with confirmatory specificity in Hex.[49] In brief, the 20 uL reaction mixture comprises 5 uL of Supermix, 2 uL of reverse transcriptase, 1 uL of 300 mM DTT from One-Step RT-ddPCR Advanced Kit for Probes (Bio-Rad), 1 uL of mixture of primers and probe and 11 uL of RNA template. Each reaction mix was converted to droplets using the QX200 Droplet Generator (Bio-Rad,), transferred to a 96-well plate (Bio-Rad), heat sealed and amplified in a GeneAmp System 9700 thermal cycler (Applied Biosystems). The thermal cycling conditions were as follows: 45 for 10 min (reverse transcription); 95 for 5 min; and 40 cycles of 95 for 15 sec, and 58 for 30 sec. The cycled plate was then transferred to the QX200 Droplet Reader (Bio-Rad) and analyzed using the QuantaSoft droplet reader software (Bio-Rad). Reactions containing more than 10,000 droplets were treated as effective and involved in data analysis.

DdPCR data analysis will be performed using Quanta Soft analysis software v 1.7.4.0917 (Bio-Rad) which was included with the droplet reader and will be used to calculate the concentration of the target DNA sequences,

along with their Poisson-based 95 % confidence intervals. The positive populations for each primer/probe are identified using positive and negative controls with single (i.e., not multiplexed) primer–probe sets. The concentration reported by QuantaSoft equals copies of template per microliter of the final 1× ddPCR reaction, which will also used in all the results. In addition, plots of linear regression will be conducted with GraphPad Prism 7.00, and probit analysis for lower limit of detection (LLoD) will be conducted with StatsDirect software v3.2.9. Lower limit of quantitation (LLoQ) and LLoD are defined as the lowest concentration at which 95 % and 50 % of positive samples are detected, respectively.

We will summarize the nucleic acid test results of all clinical samples collected (vaginal mucosa, amniotic fluid, cord blood, placenta, maternal nasopharyngeal/oropharyngeal and neonatal nasopharyngeal swab, breastmilk) to ascertain the possibility of intrauterine vertical transmission and post-partum feeding transmission. A positive test result reflects the presence of coronavirus in these clinical samples and will be indicative of possible mother-to-infant transmission. Laboratory test results however will be considered within the context of clinical observations and epidemiological data when making the final diagnosis decisions. In addition, binary logistic regression will be conducted to further investigate the association between coronavirus in placenta and the presence of fetal/neonatal complications.

4.2.2 *Antibody Testing*

IgG, IgM and IgA antibodies to coronavirus will be evaluated in maternal and cord blood samples to assess whether patients (mothers and infants) have been recently exposed to the virus. IgG, IgM and IgA levels will be measured using a commercially available colorimetric enzyme-linked immunosorbent assay (ELISA), with all reagents prepared as per the manufacturer's protocol.

[redacted]

6. Addition of maternal and cord blood sample data from the INVIVO-P Biobank to the COPE Network dataset

6.1 Rationale

During the COVID-19 pandemic, many research initiatives sought to address important questions pertaining to the impact of SARS-CoV-2 infections in pregnancy. A separate initiative conducted in Quebec, Canada included the collection of biological samples into the Integrated View of Immune Variation on Outcomes of Pregnancy (INVIVO-P) Biobank. INVIVO-P samples were collected from pregnant patients of the Centre Hospitalier Universitaire Sainte-Justine who had a confirmed SARS-CoV-2 infections in pregnancy. Maternal and cord blood samples from INVIVO-P were previously analyzed by Dr. Langlois at the University of Ottawa for assessment of SARS-CoV-2-specific antibodies (IgG, IgM, and IgA) against the spike (S), receptor binding domain (RBD) and nucleocapsid (N) proteins, as was done for COPE Network Samples.

The study team is requesting the use existing data from the INVIVO-P dataset and COPE Network Study dataset to create a combined dataset from which to evaluate the perinatal transmission potential of SARS-CoV-2 and patterns of antibody transfer following prenatal infection.

6.2 Data Sources

- INVIVO-P Dataset: The INVIVO-P biobank included samples from 29 individuals with confirmed SARS-CoV-2 infections who delivered at the Centre Hospitalier Universitaire Sainte-Justine. The INVIVO-P dataset includes maternal, fetal and neonatal baseline characteristics and outcomes and COVID-19

disease characteristics. Also included are the findings from serological analysis of maternal and cord blood plasma samples.

The INVIVO-P dataset will be combined with the COPE Network Study dataset to create a single dataset of maternal, fetal and newborn characteristics, outcomes and COVID-19 profiles including serology results.

6.3 Contracts and ethics

INVIVO-P participants provided informed consent to the sharing of the data and samples for research.

A material and data transfer agreement is already in place to between OHRI and Centre Hospitalier Universitaire Sainte-Justine to cover the transfer of de-identified data (MTA E8817).

7. ETHICAL CONSIDERATIONS

The study will be submitted for Research Ethics Board (REB) approval. The study will be initiated by the OMNI Research Group upon written Research Ethics Board approval. The Site Investigator will obtain their site ethics approval and as applicable, ongoing renewals of the study protocol, protocol amendments, and all other relevant documents from their local REB. Copies of REB approvals must be forwarded to the Study Coordinating Center prior to the study initiation or continuation at the site.

Revisions and/or amendments to the protocol must be documented and approved by the REB prior to implementation. In addition, the study will be conducted in accordance with the protocol, ICH/GCP as applicable, and applicable local regulatory requirements and laws.

7.1 Risks

Participation in this study poses minimal risk to the participants or to their infants. There is no risk to the mother or infant to have placenta samples collected, as standard of care will be followed, placenta tissue will be sent for pathology for evaluation as per the existing procedure for maternal cases with infection. If the study participant has decided to breastfeed, they will be asked to provide a breastmilk sample which can be collected by the mother in isolation, should they agree to.

7.2 Benefits

The results of this may be beneficial to the participants and their infants, as COVID-19 case ascertainment information may be made available by sample analysis. While the results of sample analysis are research results, any results that may impact the care of a participants will be communicated to the staff and/or participant as they become available.

The study may not be directly beneficial to the participants or their infants in the cases of confirmed maternal diagnosis or negative sample ascertainment in infants, however the results of this study will help to guide clinicians in determining best mode of delivery and feeding practices in future COVID-19 pregnancies. Clarification of vertical transmission and mother-to-infant feeding transmission will provide essential information to health care providers globally for care management in pregnancy. The results from this study will be shared with health care professionals including general practitioners, obstetricians and gynecologists, policy makers and health care planners.

7.3 Data Management Plan

A master file which will contain a comprehensive list of all participants, with their associated study ID, will be held by the SCC at the OHRI. This file will be updated as samples arrive from participating centres and will be used to assign study IDs and link collected samples to participants. This spreadsheet will be stored on internal

hospital shared drive with limited user access to the study team. Authorized personnel at participating sites will have access to their master file that is stored on the SCC hospital shared drive.
All study data will be securely stored for a period of 10 years after study termination. After this period of time, all study documents will be securely destroyed and/or deleted from all hospital servers.
A data sharing agreement will be in place prior to any study conduct. All data released from the OHRI or participating sites, including CHEO and the University of Ottawa, will be provided via electronic file transfer and will include only de-identified data, with the exception of data sent to BORN Ontario which is required to be identified for linkage purposes External data transfer will be conducted to comply with N2 SOPs on Secure File Transfers. Data received by the study investigators will be stored securely on encrypted, institutional servers.

7.4 Confidentiality

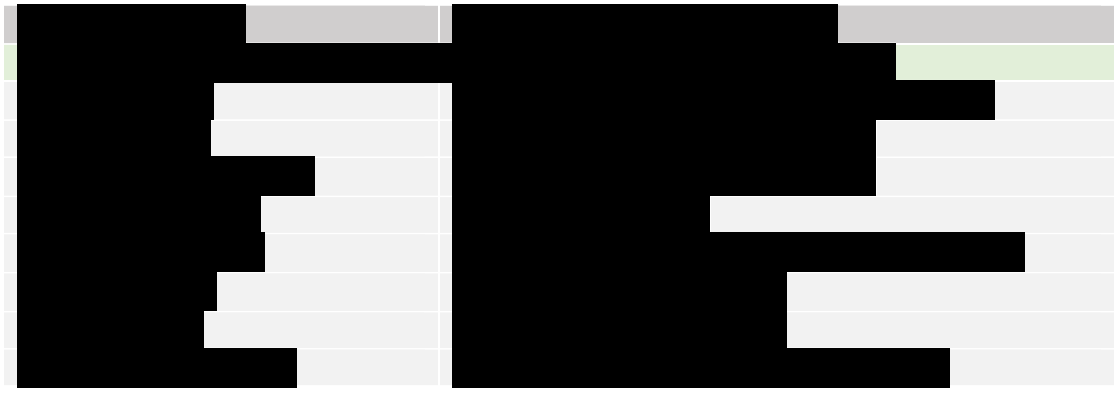
Privacy is a fundamental value, perceived by many as essential for the protection and promotion of human dignity (TCPS). Every effort should be made to protect the right of the participant for privacy according to jurisdictional regulations. All personal health information will be treated in a confidential manner and will be subject to all legal and regulatory legislation within the jurisdiction of the research participant regarding the collection, use and disclosure.

Multiple measures will be put in place to protect participant confidentiality and privacy during the study. Prior to analysis, a unique patient study ID number will be generated that contains no identifiable information relating to the patient. A master list will be kept in a password-protected document stored on the OHRI secure network with restricted access available only to necessary study personnel. Applicable government regulations, CHEO, The Ottawa Hospital and the University of Ottawa research policies and procedures will be followed.
As with any multi-centre study conduct, sample and data transfer between participating institutions poses small confidentiality risks. Mitigiation strategies are in place including de-identification of data where possible, execution of a Sample/Data Sharing Agreement with each institution prior to study conduct, and ensuring that all study staff are compliant to local institutional and regulatory body privacy policies and data management processes.

8. STUDY TEAM & TIMELINE

8.1 Study Team

The team at SCC will consist of researchers with clinical and epidemiological expertise in perinatal and infectious disease research. **Dr. Darine El-Chaâr** (Maternal-fetal Medicine Specialist at TOH with expertise in clinical and epidemiologic obstetric research) will lead both the COPE Network and this project. [redacted]



9. KNOWLEDGE TRANSLATION (KT) and SIGNIFICANCE

9.1 Knowledge Translation

Results of interest will be disseminated to appropriate clinical care team. To reach health care professionals, who expect clear, action-oriented messaging delivered through their professional organizations, we intend to leverage our various knowledge user-based partnerships (e.g. BORN, SOGC) to permit local, provincial, and national disseminations of findings to be delivered through various modalities. In keeping with more traditional forms of knowledge translation, we plan to distribute the project results to HCP and researchers in the maternal and fetal health fields through local, national, and international presentations, CME activities, and publications. Where possible, we will have involved trainees present internally at their local institution supported by their local supervisor.

9.2 Strengths and Significance

As of April 15th, 2020, we have limited information on the clinical course and consequences of COVID-19 infection in pregnant women. Early case series reports from China show no evidence of severe adverse outcomes among infected pregnant women.[13,14] [13,14] There is currently insufficient evidence concerning possible vertical transmission from mother-to-infant. To date, there is no information from other countries in Europe where the burden of illness has been high. Additional data are rapidly needed to robustly evaluate the health of pregnant women and infants following COVID-19 infection during pregnancy. We expect the new evidence generated by our research to be of high interest and relevance for public health officials and health care professionals providing maternal and newborn care during this extraordinary global outbreak of a novel viral respiratory illness.

Our decision to collect several varieties of clinical samples from the COVID-19 infected women will allow for a complete investigation of the maternal-infant transmission potential of the virus. In Ontario, many hospitals are fortunate to have an electronic hospital information system to identify cases in real-time without the requirement for any additional clinical or hospital resources. The ability to compile this clinical data on a provincial level is a strength, given that many case series reports during epidemic/pandemic time periods lack a large enough sample for clinical significance. Further, collaboration of sites will aid to facilitate rapid generation of evidence during the pandemic. We expect the new evidence generated by our research to be of high interest and relevance for public health officials and health care professionals providing maternal and newborn care during this extraordinary global outbreak of a novel viral respiratory illness.

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APPENDIX 1 BORN Ontario COVID-19 Case Report Form

Please refer to the Case Report Form on the BORN Ontario website, as amended
https://www.bornontario.ca/en/news/born-data-collection-on-covid-19.aspx

APPENDIX 2 Placenta Pathology Report

Synoptic framework for placental pathology

Gross examination	
State □ Fresh □ Fixed	
Trimmed placental weight : _____ grams **Placenta weight percentile :** _____ *derived from local population standards **Complete basal plate**: Yes or no	
Placental Disk	
Maximal linear length	_____ cm
Maximal width (perpendicular to liner length)	_____ cm
Minimal thickness	_____ cm
Maximal thickness	_____ cm
Accessary lobes?	Yes or no Size: ____ cm
Umbilical Cord	
Maximal diameter	_____ cm
Cord length	_____ cm
Velamentous cord insertion?	Yes or no If yes, _____ cm from disc margin
Color	_____
Marginal cord insertion? *<1cm from nearest margin*	Yes or no
Peripheral cord insertion? *<3cm from nearest margin*	Yes or no
Handedness of coiling	□ Right or □ left or □ Could not be determined
Hypercoiling of cord? *>3 coils per 10cm*	Yes or no
Segmented hypercoiling?	Yes or no
Presence of deep grooves between coils?	Yes or no
Hypocoiling of the cord? *<1 coil per 10cm*	Yes or no
True knots?	Yes or no If yes, tight or loose
Membranes	
Colour	_____
Completeness	_____
Extrachorialis?	Yes or no Circumvallate or circummarginate
Localised lesions *separate entry for each distinct lesion observed	
Location: central or peripheral **Number of lesions:** _____ **Maximal dimension:** _____ x _____ x _____ **Percentage of placental disc volume involved:** _____ % Specified or non-specified Textbox - description	
Retroplacental hematoma (compression of overlying parenchyma) **Location:** central or peripheral **Maximal dimension:** _____ x _____ x _____	
Diffuse parenchymal consolidation? Yes or no Greatest thickness : _____ mm Estimated volume as a percent of total disc volume : _____ %	

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