Study Protocol

Official title:
Coronavirus disease 2019 (COVID-19) during pregnancy: prevalence of seroconversion, effect on maternal and perinatal outcomes and risk of vertical transmission

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Title of Project

Coronavirus disease 2019 (COVID-19) during pregnancy: prevalence of seroconversion, effect on maternal and perinatal outcomes and risk of vertical transmission

Keywords: 1) COVID-19; 2) pregnancy; 3) vertical transmission; 4) pregnancy loss; 5) ACE2 TMPRSS2

Project objectives

1) To determine the rate of SARS-CoV-2 seroconversion in unselected pregnant women in Hong Kong.

2) To determine the rate of SARS-CoV-2 infection in women presenting with miscarriage and stillbirth.

3) To follow the pregnancy course and perinatal outcome of confirmed COVID-19-infected pregnant cases.

4) To determine the risk and characteristics of vertical transmission.

5) To evaluate the placental barrier, immune response and fetal damage in vertical transmission of SARS-CoV-2.

- **Background of research and expected project commencement date**

Emerging infections have been shown to have an important impact on pregnant women and their fetuses[1] . Recent examples include the 2009 pandemic H1N1 influenza virus [2] and the Zika virus[3-5]. The emergence of a novel coronavirus not previously seen in humans, namely severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first reported in Wuhan, China, on December 31, 2019. The coronavirus disease 2019 (COVID-19), caused by the SARS-CoV-2, was declared by the World Health Organization (WHO) a pandemic on 11 March 2020. Since then, the number of reported cases has increased rapidly, with more than 10 million cases and 500000 deaths as of 1 July, 2020.

Coronaviruses are single-stranded RNA, non-segmented, enveloped viruses, which cause illness ranging in severity from a common cold to severe and fatal illness. The term coronavirus derives from the Latin word corona, which means crown or halo; the designation arises from the appearance of coronavirus virions viewed by electron microscopy (EM), in which the virus particles display a crown-like fringe typically referred to as spikes. In the past 2 decades, two notable coronaviruses, severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV), have caused severe respiratory illness in humans. Within a short period of time, the third coronavirus, SARS-CoV-2, has infected far more people than were reported for MERS-CoV and SARS-CoV combined.

It is recognized that pregnant women are at an increased risk of acquiring viral respiratory infection and developing severe pneumonia due to the physiologic changes in their immune and cardiopulmonary systems[6,7]. It is important to determine the effect of the SARS-CoV-2 infection

and its disease (COVID-19) on pregnant women and their fetuses as careful risk-benefit analysis and decisions regarding treatment and interventions during pregnancy are crucial. It is critical to acquire high-quality data on maternal and perinatal outcomes and risk of vertical transmission. The first study describing the clinical characteristics and investigating the possibility of vertical transmission of SARS-CoV-2 in 9 pregnant women with laboratory-confirmed COVID-19, demonstrated that the severity of COVID-19 in pregnant women was similar to that in non-pregnant adults and that there was no evidence of vertical transmission when the infection manifests during the third trimester of pregnancy as SARS-CoV-2 was not detected in amniotic fluid, cord blood and neonatal throat swab samples in six cases[8]. However, there remain many unanswered questions concerning mother-to-child-transmission of SARS-CoV-2.

Several studies have attempted to determine the potential risk of vertical transmission, however, relevant biological samples have not been collected in a systematic manner[9-18]. Paired maternal-perinatal testing i.e. with vaginal, cervical or rectal swabs, has been performed to detect genital tract viral shedding during vaginal delivery in 22.5% of the case-series. Breast milk has also been tested for SARS-CoV-2 using reverse transcription polymerase chain reaction (RT-PCR) in 22.5% of the studies. Five studies have reported immunoglobulin (Ig)G and IgM serology in the mothers and the neonates[10,12,19,20]with additional cytokine assays[19,21]. Several studies have evaluated the placenta for infectious pathology. Twenty-five percent of these reports have documented neonatal SARS-CoV-2 infection[10,16,19,20,22-25]. Reports from Italy, Peru and Iran attributed neonatal infection to horizontal transmission from RT-PCR positive mothers via breastfeeding without masks (respiratory droplets) and vaginal delivery (exposure to genital secretions).[16,20,24]

Two reports suggested possible transplacental infection due to the presence of neonatal IgM antibodies and abnormal cytokine levels after birth, since IgM is not transmitted transplacentally and may have been produced by the fetus in response to in utero exposure to SARS-CoV-2[10,19]. In both reports, RT-PCR of neonatal nasopharyngeal swab and maternal vaginal secretions were negative. Specifically, in the study of Dong et al., the observed decline of anti-SARS-CoV-2 IgG and IgM levels from 140.32 AU/mL (normal range is 0–10 AU/mL) and 45.83 AU/mL (normal range is 0–10 AU/mL), respectively, at 2 hours after birth to 69.94 AU/mL and 11.75 AU/mL, respectively, at 14 days of age, is not consistent with the typical profile of the body's antibody response to acute viral infection[10]. The rapid decline of anti-SARS-CoV-2 IgG antibody level in the infant within 14 days, in addition to the decline in IgM antibody level, strongly indicates that the neonatal anti-SARS-CoV-2 IgG antibodies were derived transplacentally from the mother and their production was not actively induced by the presumed neonatal infection.

Nevertheless, there are several limitations in addressing the issue of vertical transmission as most studies reported infections in the third trimester where the interval between infection and delivery is relatively short. The risks of transplacental infection in the first and second trimesters, with a potentially longer fetal exposure time, are unclear. While most reported cases have been delivered by Cesarean section, the evidence does not suggest a clear association between neonatal SARS-CoV-2 and vaginal delivery.

With regard to whether SARS-CoV-2 infection or its disease is associated with pregnancy loss,

existing evidence is unclear. Limited data on SARS-CoV (2003) reported a high first trimester miscarriage risk of 50%[26]. A recent high-quality systematic review evaluating the effect of COVID-19 on perinatal outcomes reported that there were only four reported cases of miscarriage among 295 pregnancies affected by COVID-19[15], suggesting that the miscarriage risk is not significantly increased by COVID-19. However, it is likely that cases of miscarriage associated with COVID-19 have been missed or underreported because pregnancy symptoms can mask COVID-19 symptoms, such as myalgia and fatigue. There was a case report of second trimester miscarriage associated with SARS-CoV-2 infection, where the virus was isolated in the placenta but not in the abortus[9]. This case raised the possibility of placental infection with SARS-CoV-2. Though there was no evidence of vertical transmission, absence of the virus in the abortus was not surprising given the short interval between maternal infection and miscarriage. Whether SARS-CoV-2 crosses the placental barrier warrants further investigation. In a non-consecutive case series of 9 cases of severe COVID-19 there were two pregnant women with intrauterine fetal death[27]. Further, latest evidence has demonstrated that placentas from pregnant women affected by COVID-19 show patterns of placental injury reflecting abnormalities in oxygenation within the intervillous space associated with adverse perinatal outcomes, such as stillbirth, fetal growth restriction (FGR)[28]. Based on data from the SARS-CoV (2003) case-series, 80% patients who presented after 24 weeks had preterm birth and 2 mothers recovered without delivery, but their ongoing pregnancies were complicated by FGR. It is important to determine if SARS-CoV-2 infection increases the risk of pregnancy loss, preterm birth, FGR or other pregnancy complications[26].

Emerging evidence suggests that a significant proportion of SARS-CoV-2 carriers are asymptomatic[29-31]. This poses significant challenges in regard to the management of pregnancy and childbirth during the COVID-19 pandemic. Pregnancy symptoms can mask COVID-19 symptoms, especially if they are mild. Using the approach of targeted testing based on a significant epidemiological history or symptoms, a cohort study of 116 pregnant women with COVID-19 in China demonstrated that 23.3% of cases were asymptomatic[32]. Using the approach of universal testing by RT-PCR in women admitted for delivery, the rates of positive SARS-CoV-2 result in asymptomatic women were 13.5% and 2.9% in and outside highly endemic regions in the United States, respectively, and 3.8% in Tokyo, Japan[31].

In this proposal, we plan to perform a systematic assessment of seroconversion, maternal and perinatal outcome and risk of vertical transmission of SARS-CoV-2 infection during pregnancy.

- **Research plan and methodology**

The research project will engage in collaborative research across obstetricians/maternal-fetal medicine (MFM) subspecialists (**Prof Poon, Prof Leung, Prof Yang, Dr Gil, Dr Chaemsaithong**), gynecologist (**Prof Chung**), infectious disease specialists (**Prof Chan, Prof Ng**), pediatrician (**Prof S Lam**), developmental and molecular biologists (**Prof Wang, Dr Leung**) and biostatistician/bioinformatician (**Prof Sahota**) between the **CUHK, China and Spain**.

<u>Objective 1:</u> Seroconversion during pregnancy

Methodology: a longitudinal study. Inclusion criteria: (1) pregnant women who attended for Down syndrome screening (DSS) at 11-13 weeks and had a serum sample (**A**) taken between 1 November 2019 and 1 June 2020; (2) consented for stored serum for future research; and (3) intended to deliver at the booking hospital. Ethics approval at Prince of Wales Hospital (PWH) has been acquired (CREC Ref 2020.214). A list of eligible patients has been identified from our DSS database. We have started recruiting eligible patients through telephone call. An alert is set within the Hospital Authority Clinical Management System and therefore when the participant attends for delivery, we will confirm informed consent and collect the second serum sample (**B**), which will be stored at -80^{o}C for subsequent testing. Ethics approval at Kwong Wah Hospital (KWH) has been submitted. The research team believes that it is critical to utilize stored serum samples from women who attended for routine DSS between November 2019 and June 2020 as this will capture the seroconversion data from the start of the outbreak until after the peak of the outbreak. Assuming a worst-case scenario of SARS-CoV-2 penetrance of 1% we estimate that **2400 women** will need to be recruited from amongst the 4800 women who attended for DSS during the study period. For all consented participants, **paired blood samples A and B** will be analyzed for anti-SARS-CoV-2 specific antibodies (Elecsys Anti-SARS-CoV-2, cobas e411 analyzer, Roche Diagnostics GmbH, Mannheim, Germany). Results on the rate of seroconversion will be summarized in counts and percentages. Target achievement: we aim to recruit 2400 unselected pregnant women to quantify the rate of seroconversion of SARS-CoV-2 during pregnancy from the start until after the peak of the COVID-19 outbreak in Hong Kong. This will allow us to determine the rate of women of childbearing age who have potentially been infected with SARS-CoV-2, but have remained asymptomatic or mildly symptomatic and undetected.

Objective 2: SARS-CoV-2 and pregnancy loss

Methodology: a cross-sectional study. We plan to recruit pregnant women presenting with first and second trimester miscarriage in Hong Kong and Spain, as well as those with stillbirth during periods of 6 and 12 months, respectively. The overall rates of clinical first trimester miscarriage, second trimester miscarriage and stillbirth are approximately 10%, 1% and 0.1-0.3%, respectively. We plan to recruit first trimester miscarriage cases undergoing surgical management in order to minimize the risk of contamination. Regarding second trimester miscarriage, a mixture of spontaneous, medical and surgical management cases will be recruited as the latter is not frequently performed due to the recognized increased risk of uterine perforation and cervical laceration. For cases of miscarriage, we anticipate a 50% uptake and accounting for women seeking private care (70%), we aim to recruit approximately **500 cases**. Regarding stillbirth, during a 1-year period we anticipate a 50% uptake and aim to recruit 5 cases. Biological samples to be collected and tested are listed in Table 1. For first trimester products of conception (POC), specimen will be washed in cold saline or PBS to remove blood, which will then be dissected into small pieces and stored in micro-tubes that will be frozen by snap freezing in liquid nitrogen or dry ice and subsequently stored at -80^{o}C. For second trimester POC and stillborn baby, placenta will be washed in cold saline or PBS to remove blood. Dissect the placenta into 5 full-thickness samples (1 x 1 cm) from 5 random sampling sites, away from cord insertion site and as far apart from each other as possible. One of these samples will be stored in 10% buffer-formalin and another 1 of them will be stored in EM fixative. For the remaining 3 samples, they will be dissected into 3 parts: chorionic plate, villous and maternal decidua; and store these samples into

separate micro-tubes that will be frozen by snap freezing in liquid nitrogen or dry ice and subsequently stored at -80^{o}C. Abortus/stillborn baby will be washed in cold saline or PBS to remove blood. Dissect the abortus/stillborn baby into different body parts, e.g. lungs, cartilage, skull, brain, muscle, intestines, and store them into separate micro-tubes that will be frozen by snap freezing in liquid nitrogen or dry ice and subsequently stored at -80^{o}C. Stillborn baby will also undergo routine autopsy and histopathological examination (HPE) of placenta. All placentas will be photographed against a scale bar. In addition, hematoxylin-eosin, myeloperoxidase immunohistochemistry, Gram, and periodic acid-Schiff colorations will be performed on POC and placenta that are tested positive for SARS-CoV-2 to exclude coexisting bacterial infection. Results on the rate of SARS-CoV-2 infection in women with pregnancy loss will be summarized in counts and percentages. Target achievement: we aim to recruit 500 and 5 pregnant women with miscarriage and stillbirth, respectively, to determine the rate of SARS-CoV-2 infection in women presenting with early and late pregnancy loss, and in cases with positive SARS-CoV-2, matched with negative cases, whether vertical transmission has contributed to these complications (see details in objective 5).

Objective 3: Pregnancy course and perinatal outcome

Methodology: a longitudinal study. For pregnant women affected by COVID-19, following recovery, 4-weekly ultrasound scan for assessing fetal size, amniotic fluid volume, fetal Dopplers (umbilical artery, middle cerebral artery, ductus venosus), uterine artery Doppler and placental morphology (echogenicity, thickness, appearance) and function will be done. For first and early second trimester infected cases, a detailed fetal morphological scan will be undertaken to determine the risk of structural malformation. The following information will be collected: (i) maternal demographics including: age, weight, height, racial origin, smoking, medical history of chronic hypertension, cardiovascular disease, diabetes mellitus and respiratory disease, drug history, method of conception, parity, past obstetric history; (ii) delivery outcome: gestational age at delivery, mode of delivery, complications including stillbirth, FGR, preterm birth, preeclampsia; (iii) neonatal data: birthweight, sex, Apgar scores, cord arterial and venous pH and base excess, neonatal outcome, admission to neonatal intensive care unit (NICU), complications such as sepsis, anemia, intraventricular hemorrhage, necrotizing enterocolitis, respiratory distress syndrome, need for respiratory support, laboratory findings; (iv) COVID-19 specific data: gestational age at clinical manifestation; symptoms and signs; epidemiological history; laboratory and radiological findings; treatment measures; COVID-19 severity and outcomes; and intervals from onset of disease to hospital admission, delivery and recovery will be recorded. Results on maternal and perinatal outcomes will be summarized in counts and percentages and median and ranges. Target achievement: To date, there are 5 reported pregnant cases affected by COVID-19 in Hong Kong. We anticipate that between Hong Kong, China and Spain, we will recruit 40-50 pregnancies affected by COVID-19 with complete data according to protocol. We will learn a complete picture of the pregnancy course and perinatal outcome of cases affected by COVID-19. Such data will form the basis of counselling regarding the impact of coronavirus on pregnancy and perinatal outcome.

Objective 4: Vertical transmission

Methodology: a cross-sectional and longitudinal study. Appropriate biological samples (Table 2) will be collected using aseptic technique and tested from consented pregnant women affected by COVID-19 from above objectives (Table 3). When the biological samples that have been collected immediately after birth are negative for SARS-CoV-2, but the IgM and IgG antibodies are positive in the newborn, longitudinal follow-up of the IgG antibody concentrations in the infant is required. If the IgG antibodies in the infant become negative within 6 months, the possibility of intrauterine infection can be ruled out, and if the IgG antibodies in the infant persist till the age of 18 months or beyond, the diagnosis of congenital infection can be confirmed after exclusion of infection during infancy. Results on the rate of positive results will be summarized in counts and percentages. Target achievement: Relevant testing (RT-PCR and/or anti-SARS-CoV-2 antibodies testing) of a comprehensive collection of biological samples from the mothers and the newborns will allow evaluation of whether vertical transmission has occurred.

Objective 5: Potential mechanisms for vertical transmission

Currently there is much controversy regarding the possibility of vertical transmission of SARS-CoV-2 infection. There is a need for a systematic approach to characterize the transmission potential of SARS-CoV-2. In order to successfully establish an infection and transmission of the virus in the target tissues, i.e. placenta and developing fetus, SARS-CoV-2 has to be able to dock and invade the placental barrier as well as to overcome the host immunologic response at the site of infection. In order to have a comprehensive investigation of these underlying mechanisms, laboratory tests will be divided into 3 parts to evaluate: 1) placental barrier, 2) immune response and 3) fetal damage of vertical transmission and mechanism in SARS-CoV-2 infection. Methodology: For the placenta collected from pregnant women affected by COVID-19 (objective 4), dissect it into 10 full-thickness samples (1 x 1 cm) from 10 random sampling sites, away from cord insertion site and far apart from each other. Three of these samples will be stored in 10% buffer-formalin and 2 of them will be stored in EM fixative. For the remaining 5 samples, they will be dissected into 3 parts: chorionic plate, villous and maternal decidua; and store these samples into separate micro-tubes that will be frozen by snap freezing in liquid nitrogen or dry ice and subsequently stored at -80^{o}C.

Placental barrier: we will look into localization, infection, replication and resistance of the viral infection in decidua, amnion, maternal-fetal interface, syncytium and placenta. Tissues and cells from COVID-19 affected pregnancies with live birth (objective 4) and pregnancy loss (objective 2) will be collected and isolated for the study; unaffected pregnancies with live birth and pregnancy loss will also be recruited as control for comparison. Standard scanning EM (SEM) and transmission EM (TEM) will be employed. SEM could provide detailed images of the surfaces of cells, so that the distribution and site of infection of the virion in the maternal, interface and fetal compartments could be located. While TEM provides details of interior structures within the cell, and thus any dysplasia or reconfiguration of cellular organelles due to viral infection could be studied.

Immune response: during viral infection, the virus has to employ host cell surface molecules as specific adhesive receptor. This interaction between the virus and the host cells induces conformational changes on both the viral protein as well as the host plasma membrane, and in turns, promotes viral entry. Viral entry could generally fall into two categories: 1) direct infection of the placenta or 2) indirect infection via transcytosis. Whether SARS-CoV-2 employs direct infection and/or indirect infection through virion transcytosis synctium will be determined. Results will also correlate with the viral +sense ssRNA load and histologic changes in the corresponding cells. Meanwhile, it has been confirmed that the SARS-CoV-2 invades human cells by utilizing two protein molecules: receptor of angiotensin-converting enzyme 2 (ACE2) which aids in host cell attachment, and a transmembrane serine protease (TRPRSS2) which further assists cell entry. The relative abundancy, distribution and co-localization of these cell surface proteins in different epithelial, stroma, endothelial, trophoblast, fibroblast and various immune cells will be measured and compared between COVID-19 cases and controls. Efficiency and resistance infection will be measured by viral replication in various human trophoblast primary cells and tissue explants using viral-specific neutralizing antibody titers. Tissue-specific spread, cell-to-cell spread, and bypass of the viral infection will be determined. Nonetheless, not all cells displaying these required receptors are permissive to productive infection. The presence or absence of specific host proteins may also play a critical role during viral infection. Little is known about how these intracellular host factors may influence successful SARS-CoV-2 infection. In order to address this question, we will look into the immunologic reaction of the cells in the decidua, amnion, maternal-fetal interface, syncytium, placental villi core and maternal and cord/fetal blood. Single cell RNA sequencing (scRNAseq) of maternal-fetal interface, maternal blood and cord blood will be conducted in the affected pregnancies with live birth and pregnancy loss. Gene expression of viral infection cascades (e.g. HLA-G, FcRn, TLR, NOD, defensin, etc) and surface molecules (e.g. ICAM, Cadherin, gap junction, etc) in epithelial, stroma, endothelial, trophoblast, fibroblast and various immune cells will be measured and compared with controls. Cell-specific mechanism of immune evasion and defense of SARS-CoV-2 infection for vertical transmission will be determined.

For fetal damage, we will look into the fetal barrier and immune response of the viral infection in the affected fetuses. POC and stillborn babies of affected pregnancies will be collected and isolated for SEM and TEM, ssRNA load qualification, and viral replication to confirm the localization, infection, replication and immunologic reaction as above. If the fetuses have any developmental structural abnormalities identified during antenatal ultrasound examinations, the affected fetal organs, tissues and cells will be specifically collected and studied as above. The results will be correlated with the pathology of the fetuses and compared with the POC and stillborn fetuses of unaffected pregnancies.

Target achievement: We aim to collect 5-10 affected cases with live birth and 5-10 affected cases with pregnancy loss, compared with gestational-age matched controls. We anticipate to characterize and reveal underlying mechanisms for vertical transmission associated with pregnancy loss and fetal complications.

Study coordination

Prof Poon has conceived and designed this project and will manage and coordinate all aspects of the study. For objective 1, together with **Prof Sahota**, who is the team bioinformatician, a list of eligible patients has been drawn from our DSS database. **Dr Chaemsaithong** is overseeing the recruitment of eligible patients for recruitment for objective 1. She will also collect and manage study data. For objective 3, there are currently 5 pregnant women in Hong Kong affected by COVID-19 and their pregnancy is ongoing. **Prof Leung** will provide clinical guidance for and oversee the follow up of the affected pregnant women. **Prof Yang** will contribute maternal and perinatal outcome data of the pregnant cases that had acquired SARS-CoV-2 infection during the first and second trimester of pregnancy from her existing cohort study in China. **Dr Gil** from Spain has started recruitment of patients affected by COVID-19 and collection of biological samples according to the proposed protocol (objective 4). **Prof Chung** is an academic gynecologist with an extensive research experience in pregnancy loss. She will organize for collection of POC and recruit stillbirth cases. **Prof Wang** is a clinician scientist and **Dr Leung** is a molecular biologist, both with a special interest in disease mechanism; together they will conduct all experiments for objective 5. **Prof Yang** will contribute and assist with the interpretation of data for objective 5. **Prof Chan** and **Prof Ng** are infectious disease specialists with extensive experience in COVID-19 research. Their laboratory will be in charge of analyzing all biological samples for objectives 2,4**.** **Prof Lam** is an academic pediatrician with expertise in neonatal intensive care research. He will coordinate follow up of the neonates born to mothers with COVID-19.

Work done by us

The multi-disciplinary research team includes experts in the fields of MFM, gynecology, infectious disease, pediatric, developmental and molecular biology and biostatistics/bioinformatics. Since March 2020, the PC has published 11 papers on COVID-19 during pregnancy, including a consecutive case series of 116 pregnant women with COVID-19 (with Prof Yang), and a high quality systematic review of 324 pregnant women in evaluating the effects of COVID-19 on maternal and neonatal outcomes (with Prof Yang and Dr Gil). She has also authored three international guidelines on the management of pregnant women with COVID-19, one consensus statement on organization of routine and specialist obstetric ultrasound services in the context of COVID-19, and two safety position statements regarding the appropriate use of personal protective equipment and safe performance of obstetric and gynecological scans and equipment cleaning.

Pregnancy outcome and vertical transmission

In the consecutive case series of 116 pregnant women with COVID-19 in China[32], the data were collected using a standardized methodology by a team of experienced clinicians, curated with

customized data collection form and verified independently by two investigators. The median gestational age on admission was 38+0 (interquartile range 36+0-39+1) weeks. The most common symptoms were fever (50.9%) and cough (28.4%). Abnormal radiologic findings were found in 96.3% cases. There were eight cases (6.9%) of severe pneumonia but no maternal deaths. One of eight patients (12.5%) that presented in the first and early second trimester had miscarriage. Ninety-eight pregnant women, including one with twin pregnancy, delivered their babies during hospitalization, 85 (86.7%) underwent Cesarean section. The Cesarean section was indicated for COVID-19 pneumonia in 38.8% of cases. The rates of preterm delivery before 34 weeks and 37 weeks were 2.0% and 21.4%, respectively. Among the 21 preterm deliveries, 6 (28.6%) had preterm premature ruptured of membranes. The rate of spontaneous preterm birth before 37 weeks was therefore 6.1% (6/98). There were no cases of fetal deaths but one case of neonatal death. 85/99 neonates were tested for SARS-CoV-2 on pharyngeal swab samples and the results were negative, of these nine neonates had paired amniotic fluid and cord blood samples that tested negative for COVID-19. At the time of publication, there were 17 ongoing pregnancies, of which 9 cases acquired SARS-CoV-2 during the first and second trimester.

In our high-quality systematic review[15], we have summarized data on clinical characteristics, including at least one maternal, perinatal or neonatal outcome, in 295 pregnant women affected by COVID-19 from 8 consecutive case series. Amongst these 295 cases, 210 cases originated from China. We have also identified a high number of possible duplicate reporting and excluded 327 cases from China. The most common symptoms at presentation were fever, cough, dyspnea/shortness of breath, fatigue and myalgia. The rate of severe pneumonia reported amongst the case series ranged from 0 to 14%. Almost all cases from the case series had positive computer tomography chest findings. Only 4 cases of spontaneous miscarriage or abortion were reported. In the consecutive case series, 219/295 women had delivered at the time of reporting, and of these, 78.1% had Cesarean section. The gestational age at delivery ranged from 28 to 41 weeks. Apgar scores at 1 and 5 min ranged from 7 to 10 and 7 to 10, respectively. Only eight (8/103; 7.8%) neonates had birth weight <2500 g and nearly one-third of cases (49/173; 28.3%) were transferred to the NICU. There was one case each of neonatal asphyxia and neonatal death. In 155 neonates that had nucleic-acid testing in throat swab, all, except three cases, were negative for SARS-CoV-2. Our systematic review has highlighted that despite the increasing number of published studies on COVID-19 in pregnancy, there are insufficient good-quality data to draw unbiased conclusions with regard to complications of COVID-19 in pregnant women as well as vertical transmission and perinatal complications. Our research to date will form the basis of our prospective studies described in this proposal. In addition, the PC is a project partner for a UK Research Institute (UKRI) and NIHR funded international registry of pregnancy and neonatal outcomes of COVID-19 (PAN-COVID; https://pan-covid.org). This registry is an international observational study with a web-based portal, collecting data on outcomes of pregnant women and their newborn babies who have suspected COVID-19 in pregnancy or confirmed SARS-CoV-2 infection. The registry will focus on reporting the incidence of the following outcomes: miscarriage, FGR and stillbirth, preterm delivery, vertical transmission and early-onset neonatal

SARS-CoV-2 infection. Data from our proposed project will have the opportunity to be compared with data collected through PAN-COVID.

SARS-CoV-2 target cells

Together with Prof Yang, we have conducted a pilot study and applied bioinformatic analysis on published scRNAseq datasets of early trophectoderm (TE) and first and second trimester placentas and identified the existence of ACE2 and TMPRSS2 expression in human TE and first and second trimester placentas (Figure 1). In human TE data, 54.4% of TE cells, 9.0% of cytotrophoblasts (CTBs), 3.2% of extravillous trophoblasts (EVTs) and 29.5% of syncytiotrophoblasts (STBs) were ACE2 positive. As for TMPRSS2, 90.7% of TE cells, 31.5% of CTBs, 22.1% of EVTs and 70.8% of STBs were TMPRSS2 positive. Amongst the placental cells, 20.4% of CTBs, 44.1% of STBs, 3.4% of EVT at 8 weeks and 63% of EVT at 24 weeks were ACE2 positive. And 1.6% of CTBs, 26.5% of STBs, 1.9% of EVT at 8 weeks and 20.1% of EVT at 24 weeks were TMPRSS2 positive. Pathway analysis revealed associations to morphogenesis of branching structure, extracellular matrix interaction, oxygen binding and antioxidant activity in ACE2+TMPRSS2+ EVT at 24 weeks cells. The ACE2+TMPRSS2+ TE cells were correlated with an increased capacity of viral invasion, epithelial cell proliferation and cell adhesion. Based on immunohistochemical results, a higher expression level of ACE2 and TMPRSS2 in first and second trimester placentas was observed (Figure 2). Localization of virion in the maternal, interface and fetal compartments; and associated dysplasia or reconfiguration of cellular organelles will be examined by TEM as we have previously described[33].

Table 1. Biological samples from cases of pregnancy loss

Biological samples	**Tests to be done**
Miscarriage POC*$^{\infty}$, maternal blood$^{\#}$	*SARS-CoV-2 RT-PCR; $^{\#}$IgG; $^{\infty}$HPE;
Stillbirth Maternal vaginal swab†¶, amniotic fluid†¶, amnion-chorion interface swab†¶, placental tissue¶, fetal blood¶‡, fetal throat swab†¶, maternal blood‡	†Culture+PCR; ¶SARS-CoV-2 RT-PCR; ‡IgG

Table 2. Biological samples from pregnant cases affected by COVID-19

Biological samples	**Tests to be done**
Before delivery: maternal blood*$^{\#}$ At or after delivery: amniotic fluid*, cord blood*$^{\#}$, placental tissue*†, amnion-chorion interface swab*, neonatal throat swab*	*RT-PCR; $^{\#}$IgG and IgM; †HPE
Breast milk from both breasts for the duration of hospitalization	RT-PCR, IgG and IgM
Newborn throat swab at 24 hours of life	RT-PCR
Newborn stool samples for the duration of hospitalization	RT-PCR
Infant throat swab and stool sample at 7 days of age	RT-PCR
Infant blood at 14 days, 6 months & 18 months of age	IgG and IgM

Table 3. COVID-19 pregnant cases with biological samples collected or to be collected (for objectives 4&5)

	Cases	Age (yrs)	Gestational age on admission (wks)	COVID-19 severity	Clinical outcomes	Pregnancy status	Current gestational age (wks)
HK	1	33	16	Mild	Recovered	Ongoing	30
	2	38	19	Mild	Recovered	Ongoing	31
	3	34	25	Mild	Recovered	Ongoing	39
	4	47	33	Asymptomatic	In hospital	Ongoing	34
Spain	5	30	40	Mild	Recovered	Delivered	
	6	22	34	Mild	Recovered	Delivered	
	7	35	38	Mild	Recovered	Delivered	
	8	41	38	Mild	Recovered	Delivered	
	9	18	38	Mild	Recovered	Delivered	
	10	28	19	Pneumonia	Recovered	Ongoing	34
	11	38	24	Asymptomatic	Recovered	Ongoing	38
	12	39	40	Mild	Recovered	Delivered	
	13	35	21	Mild	Recovered	Delivered	
	14	36	25	Mild	Recovered	Ongoing	34
	15	29	8	Pneumonia	Recovered	Ongoing	20
	16	40	11	Pneumonia	Recovered	Ongoing	25
	17	35	14	Mild	Recovered	Ongoing	36
	18	30	10	Mild	Recovered	Ongoing	23
	19	27	39	Asymptomatic	Recovered	Delivered	
	20	33	37	Asymptomatic	Recovered	Delivered	
	21	22	17	Pneumonia	Recovered	Ongoing	32
	22	31	32	Asymptomatic	Recovered	Ongoing	36
	23	24	27	Asymptomatic	Recovered	Ongoing	31
	24	33	1	Mild	Recovered	Ongoing	15
	25	34	32	Asymptomatic	Recovered	Ongoing	36
	26	34	33	Asymptomatic	Recovered	Ongoing	36
	27	29	34	Mild	Recovered	Ongoing	38
	28	36	11	Mild	Recovered	Ongoing	20
	29	37	36	Mild	Recovered	Ongoing	41
	30	37	35	Mild	Recovered	Ongoing	37
	31	37	5	Mild	Recovered	Ongoing	19
	32	31	37	Asymptomatic	Recovered	Delivered	
	33	32	20	Asymptomatic	Recovered	Ongoing	23
	34	43	29	Asymptomatic	Recovered	Ongoing	34
	35	32	32	Asymptomatic	Recovered	Delivered	

	36	31	33	Asymptomatic	Recovered	Ongoing	38
	37	39	29	Asymptomatic	Recovered	Ongoing	34
	38	39	16	Mild	Recovered	Ongoing	29
	39	28	29	Asymptomatic	Recovered	Ongoing	35
	40	36	14	Asymptomatic	Recovered	Ongoing	24
	41	40	1	Mild	Recovered	Ongoing	14
	42	40	22	Mild	Recovered	Ongoing	37
	43	30	25	Mild	Recovered	Ongoing	36

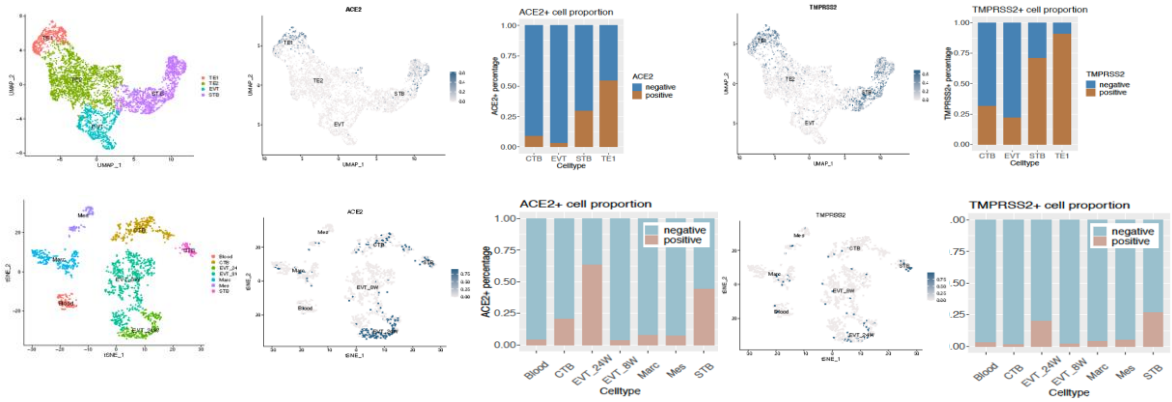

Figure 1. Single-cell RNA sequencing of SARS-CoV-2 related molecules in early trophectoderm and the first and second trimester placenta. UMAP distributions of ACE2 and TMPRSS2 in different placental cell types from embryonic TEs obtained 6-14 days after fertilization (upper panels) and T-SNE distributions of ACE2 and TMPRSS2 in 8 week and 24 weeks placentas (lower panels).

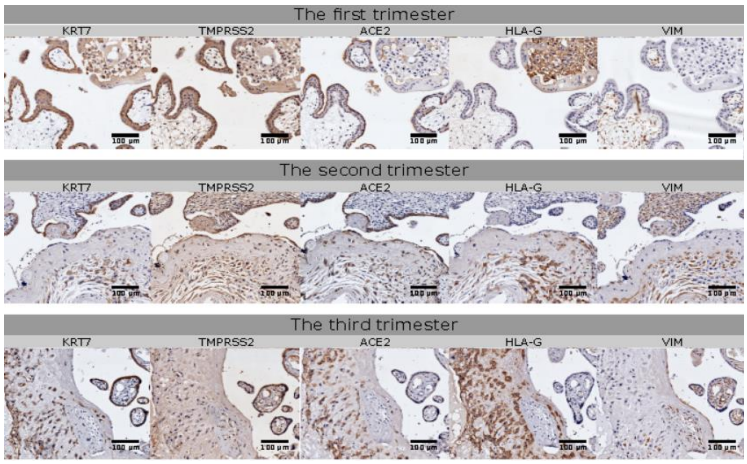

Figure 2. Immunohistochemistry staining of ACE2 and TMPRSS2 in the first (upper panels), second (middle panels) and third (lower panels) trimesters of human placenta. KRT7 (marker of trophoblasts), HLA-G (marker of EVTs) and VIM (marker of stromal cells) are included as controls.

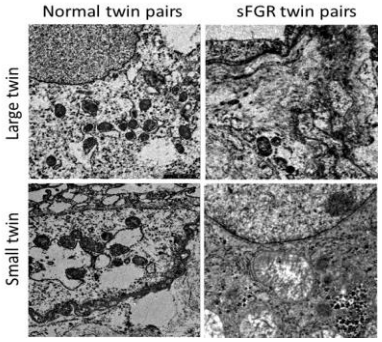

Figure 3. Transmission electron microscopy of trophoblast cells in placenta from normal and selective fetal growth restricted (sFGR) pregnancies. Damage of mitochondrial and endoplasmic reticulum are shown.

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