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NEONATAL OUTCOMES FOLLOWING MATERNAL SARS-CoV-2 INFECTION IN PREGNANCY

Longitudinal follow-up and assessment of transplacental antibody transfer

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ABSTRACT

BACKGROUND: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection in pregnancy has been associated with multiple adverse pregnancy outcomes, including the risk of *in utero* mother-to-child transmission. Short- and long-term outcomes of SARS-CoV-2 exposed neonates and the extent to which maternal SARS-CoV-2 antibodies are transferred to neonates are still unclear.

METHODS: Prospective observational study enrolling neonates born to mothers with SARS-CoV-2 infection in pregnancy, between April 2020-April 2021. Neonates were evaluated at birth and enrolled in a 12-month follow-up. SARS-CoV-2 IgG transplacental transfer ratio was assessed in mother-neonate dyads at birth. Maternal derived IgG were followed in infants until negativizing.

RESULTS: Of 2745 neonates, 106 (3.9%) were delivered by mothers with SARS-CoV-2 infection in pregnancy. Seventy-six of 106 (71.7%) mothers were symptomatic. Median gestational age and mean birth weight were 39 weeks (range 25^{+5}-41^{+4}) and 3305 grams (SD 468). Six of 106 (6%) neonates were born preterm, without significant differences between asymptomatic and symptomatic mothers (P=0.67). No confirmed cases of *in utero* infection were detected. All infants had normal cerebral ultrasound and clinical evaluation at birth and during follow-up, until a median age of 7 months (range 5-12). All mothers and 96/106 (90.5%) neonates had detectable SARS-CoV-2 IgG at birth. Transplacental transfer ratio was higher following second trimester maternal infections (mean 0.94±0.46 versus 1.07±0.64 versus 0.75±0.44, P=0.039), but was not significantly different between asymptomatic and symptomatic women (P=0.20). IgG level in infants progressively decreased after birth: at 3 months 53% (51/96) and at four months 68% (63/96) had lost maternal antibodies respectively. The durability of maternal antibodies was positively correlated to the IgG level at birth (r=0.66; P<0.00001).

CONCLUSIONS: Maternal SARS-CoV-2 infection was not associated with increased neonatal or long-term morbidity. No cases of confirmed *in utero* infection were detected. Efficient transplacental IgG transfer was found following second trimester maternal infections.

INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was first described in Wuhan, in Hubei province in China, in December 2019 and has rapidly spread around the world.[1] In March 2020 the disease caused by SARS-CoV-2, named coronavirus disease 2019 (COVID-19), was declared a pandemic. As of October 2021, the World Health Organization (WHO) reports more than 200,000,000 confirmed cases of SARS-CoV-2 infections worldwide, with more than 4,000,000 confirmed deaths.[2] In Italy, as of October 2021, more than 4,700,000 confirmed cases have been diagnosed, with more than 130,000 deaths (Figure 1).[3]

In 2002 and 2012 respectively two other highly pathogenetic coronaviruses, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), caused fatal respiratory illness, making emerging coronaviruses a public health concern.[4] SARS-CoV-2 has overwhelming surpassed SARS and MERS in both terms of the number of infected people and the number of epidemic areas. The COVID-19 pandemic is impacting nearly every aspect of the society worldwide, both because of its morbidity and mortality and because of the efforts needed to mitigate its transmission, which lead to significant social and economic disruption.[5]

Pregnant women and their fetuses represent a high-risk and vulnerable population, both because of the possible consequences of SARS-CoV-2 infection on maternal health and because of the potential for mother-to-child transmission. Although respiratory viruses are usually not easily transmitted *in utero* and no previous evidence for *in utero* transmission of SARS-CoV and MERS-CoV has been reported, SARS-CoV-2 infection in pregnancy seems to pose a challenge to these knowledges, as *in utero* transmission seems to be a possible even if rare event.[6-9] The mechanisms of *in utero* transmission are unclear, but the presence in some infected patients of a viremic phase and the presence of the receptors for viral entry in placental tissues confer a biological plausibility to this route of transmission.[7] SARS-CoV-2 infection during pregnancy has also been associated with placental damage, which might both facilitate the passage of the virus to the fetus ad affects itself the perfusion of the fetus.[10,11] The possible neonatal and long-term outcomes of neonates born to mothers with SARS-CoV-2 infection during pregnancy are under investigation.

Previous studies reported that maternal antibodies produced in response to SARS-CoV-2 infection during pregnancy cross the placenta, likely conferring some degree of passive protection to the neonates.[12-16] The efficiency of placental transfer of maternal antibodies in relation to gestational age at infection and the duration of this possible protection in the neonate are still unclear.

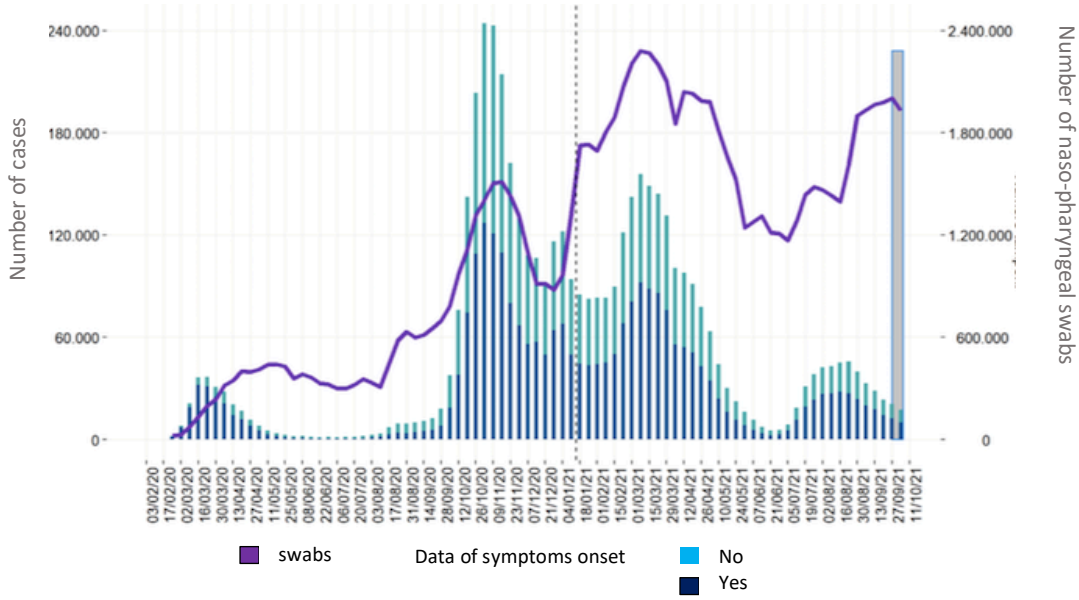

Figure 1. COVID-19 cases in Italy by week of diagnosis/swab, from March 2020 to October 2021 and number of naso-pharyngeal swab for SARS-CoV-2 RNA. From: Task force COVID-19 del Dipartimento Malattie Infettive e Servizio di Informatica. Istituto Superiore di Sanità. Epidemia COVID-19. Aggiornamento nazionale: 13 ottobre 2021.[3]

THE VIRUS

Coronaviruses are enveloped positive-stranded RNA viruses. Phylogenetic analyses for the whole genome showed that SARS-CoV-2 is clustered with SARS-CoV and SARS-related coronaviruses (SARSr-CoVs) found in bats, placing it in the subgenus Sarbecovirus of the genus Betacoronavirus. Within this clade, SARS-CoV-2 is grouped in a distinct lineage together with four horseshoe bat coronaviruses and novel coronaviruses recently identified in pangolins.[17] The closest RNA sequence similarity is indeed to two bat coronaviruses and to pangolin coronaviruses. It appears likely that bats, which carry coronaviruses healthily, were the primary source of infection, but whether SARS-CoV-2 may be transmitted directly from bats or through some other mechanism (e.g. intermediate host) remains to be fully clarified.[18] Unlike bats, infected pangolins show clinical signs and histopathological changes after infection, making unlikely these animals to be the reservoir of these viruses.

SARS-CoV-2 shares 79% genome sequence identity with SARS-CoV and 50% with MERS-CoV. Coronaviruses contain four structural proteins, including spike (S), envelope, membrane and nucleocapsid (N) proteins.

The host receptor for SARS-CoV-2 cell entry is the same as for SARS-CoV, the angiotensin-converting enzyme 2 (ACE2). The cell entry is mediated by the receptor binding domain (RBD) of the S protein of the virus.[19]

The binding of the S protein to the ACE2 receptor exposes a cleavage site on the S protein. Transmembrane protease serine 2 (TMPRSS2), which is a cellular protease, recognizes this cleavage site and cleaves the S protein to initiate fusion and endocytosis.[20] Other proteases, as cathepsin L and furin, may participate in the cleavage of the S protein. Cells in various human tissues, including kidney, heart, thyroid, adipose, small intestine and placenta show high ACE2 expression, whereas the lung cells show moderate expression.[21] TMPRSS2 is highly expressed in several tissues and body sites and is co-expressed with ACE2 in nasal epithelial cells, lungs and bronchial branches, which explains some of the tissue tropism of SARS-CoV-2.[22,23]

PATHOGENESIS AND TRANSMISSION

Direct person-to-person respiratory transmission is the primary route of SARS-CoV-2 transmission. It occurs mainly through close-range contact (within 2 meters), through inhalation of droplets and aerosol particles or contact of respiratory droplets and particles with exposed mucous membranes.[24] Reports of SARS-CoV-2 outbreaks have also highlighted the potential for the airborne route, especially in enclosed, poorly ventilated spaces.[24]

On binding to epithelial cells in the respiratory tract, SARS-CoV-2 starts replicating and migrating down the airways and enters alveolar epithelial cells in the lungs. After viral entry, the initial inflammatory response attracts virus-specific T cells to the site of infection, where infected cells may be rapidly eliminated, leading to recovery in most infected patients. However, the rapid viral replication may trigger a strong and aberrant immune response. A unique cytokine storm syndrome (CSS) may develop and cause acute respiratory distress syndrome (ARDS) and respiratory failure, which is considered the main cause of death in patients with COVID-19.[25,26] This CSS is characterized by a serum profile of inflammatory biomarkers different from more classic CSS, as hemophagocytic lymphohistiocytosis or macrophage-activation syndrome, with the elevation of multiple cytokine and chemokines, including interleukin (IL)-2, IL-7, IL-10, granulocyte colony-stimulating factor, interferon (IFN)-gamma-induced protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein 1 alpha and tumor necrosis factor-alpha (TNF-alfa).[27,28] This hyperinflammatory state is the rationale for the use of anti-inflammatory approaches to improve survival in severe COVID-19.[29]

Infection might also occur through contact with contaminated surfaces, even though this route does not seem to contribute substantially to new infections.[30] SARS-CoV-2 has been detected in non-respiratory specimens, such as stools, blood, ocular secretions and semen, but their role in transmission need to be further elucidated. The vertical transmission is considered a rare but possible event.

CLINICAL PRESENTATION

The spectrum of SARS-CoV-2 infection presentation varies from the absence of symptoms to severe respiratory involvement with multiorgan failure. The median incubation period for COVID-19 is 4-5 days, but it may extend to 14 days from exposure to symptoms onset.[31]

Based on available large population-based, cross-sectional surveys and longitudinal studies, asymptomatic infections may account for almost 33% of SARS-CoV-2 infections.[32] However, there is a wide range across studies in the proportion of reported asymptomatic infections and it is likely that its true impact is currently underestimated. Some studies also demonstrated that individuals who do not complain symptoms may have chest imaging abnormalities, with ground-glass opacities at chest computed tomography (CT), reported in as high as 50% of "asymptomatic" patients.[33]

Symptoms and signs of COVID-19 at illness onset vary, but the most common are: fever or chills, fatigue, myalgia, joint or bone aches, anosmia and/or ageusia, nasal congestion and rhinorrhea, sore throat, cough, shortness of breath or difficulty breathing, headache, gastrointestinal symptoms such as nausea, vomiting or diarrhea, dermatological manifestations such as a maculopapular rash, discolored lesions of the fingers and toes, hives.[34] After a median time from illness onset of 5-8 days, the clinical status of some patients may deteriorate and in the most severe cases may manifest as pneumonia, hypoxemic respiratory failure/ARDS, sepsis and septic shock, cardiomyopathy and arrhythmia, acute kidney injury, hypercoagulability.[35] A large cohort of more than 44,000 people with COVID-19 from China reported a mild to moderate disease in 81% of patients; a severe illness in 14% of patients; a critical disease characterized by respiratory failure, shock or multiorgan system dysfunction in 5% of patients. The case fatality rate was high among people with critical disease (49%) and overall was 2.3%.[36] Complications from prolonged hospitalizations, including bacterial and fungal superinfection, thromboembolism may occur.[34]

Clinical symptoms experienced by children are similar to adults, but illness severity is usually milder. Nonetheless there is increasing evidence that infants (<12 months of age) and children with underlying medical conditions may be at increased risk for severe illness.[37,38] Another

concern in pediatrics is the possibility of the multisystem inflammatory syndrome associated with COVID-19 (MIS-C), characterized by persistent fever, abdominal pain, gastrointestinal symptoms, skin rash, mucocutaneous lesions and in the most severe cases hypotension, cardiac and renal involvement and shock.[39] This condition may begin weeks after SARS-CoV-2 infection and may be the first manifestation of a previous asymptomatic SARS-CoV-2 infection. The complete clinical profile, laboratory characterization and long-term consequences of MIS-C are under investigation.

Several risk factors for severe illness have been identified: age, with higher case fatality rate among the oldest cohort, comorbidities including cardiovascular disease, diabetes, chronic respiratory disease.[34] Pregnant women seem to be at increased risk for severe illness, which in turn increases the risk of adverse pregnancy outcomes.

RESPIRATORY PATHOGENS IN PREGNANCY

Due to the physiological changes associated with pregnancy, like the altered cell-mediated immunity and changes in the cardiovascular and pulmonary function, including increased heart rate, stroke volume, oxygen consumption and decreased lung capacity, pregnant women are known to be more susceptible for complications of respiratory infections. Pneumonia arising from any infectious etiology is recognized as an important cause of morbidity and mortality in pregnant women, with the highest level of both morbidity and mortality described for viral pneumonia.[40]

According to this assumption previous epidemics, including influenza and other coronaviruses, showed a significantly more severe course in pregnant women as compared with non-pregnant women of reproductive age. However, the total number of infected individuals reached by both SARS and MERS epidemics were extremely lower than the number reached by the current SARS-CoV-2 pandemic (8422 probable SARS-CoV cases by July 2003 and 2574 confirmed MERS-CoV cases since 2012), which in turn lead to a lower number of SARS-CoV and MERS-CoV infected pregnant women.[41,42] Studies on clinical presentation and pregnancy outcomes of approximately 100 SARS-CoV infected pregnant women reported that the rate of admission to intensive care unit (ICU), mechanical ventilation, spontaneous abortion, intrauterine growth restriction, preterm delivery and deaths was higher than in infected non-pregnant women.[43-46] No cases of vertical transmission were identified. Data on MERS-CoV infected pregnant women are even more limited, as only 11 cases have been described: notably, adverse clinical and obstetrical outcomes were reported in 91% of these pregnancies.[47-52]

Given the paucity of data on the impact of coronaviruses infections in pregnancy, at the beginning of the SARS-CoV-2 pandemic multiple factors, regarding the pathogenesis of coronavirus infection in pregnancy, the relationship between timing of maternal infection and clinical presentation, gestational age of the fetus and pregnancy outcomes, the effects of maternal age and comorbidities on illness severity, were unclear.

SARS-CoV-2 INFECTION IN PREGNANCY

Available data are insufficient to determine if pregnancy increases susceptibility to SARS-CoV-2 infection. The reported prevalence of SARS-CoV-2 infection among pregnant women ranges from 2.7% to 20%.[53-55] Some studies report this prevalence to be higher than the prevalence observed in non-pregnant individuals: however, since these studies were not designed to evaluate the different susceptibility profile, which would require to compare individuals of the same sex, age, and with the same exposure to SARS-CoV-2, results are not definitive.[53-56]

The largest proportion of pregnant women infected with SARS-CoV-2 shows asymptomatic or mild courses, but there is increasing evidence that although the overall risk of severe illness is low, pregnant women might be at increased risk for severe disease as compared with non-pregnant women. The most interesting data on this issue are part of the Centers for Diseases Control and Prevention (CDC) COVID-19 surveillance system, which included 409,462 individuals of reproductive age with symptomatic COVID-19, of whom 23,434 pregnant women and 386,028 non-pregnant women, adjusted for age, race/ethnicity and underlying medical conditions.[57-59] Compared to non-pregnant women, pregnant women were more frequently admitted to ICU (10.5 versus 3.9 per 1,000 cases; aRR=3.0; 95% CI=2.6-3.4), received invasive ventilation (2.9 versus 1.1 per 1,000 cases; aRR=2.9; 95% CI=2.2-3.8) and received extracorporeal membrane oxygenation (0.7 versus 0.3 per 1,000 cases; aRR=2.4; 95% CI 1.5-4.0). A 70% increased risk for death associated with pregnancy was also reported (1.5 versus 1.2 per 1,000 cases; aRR=1.7; 95% CI=1.2-2.4).[58] Risk for severe disease was apparent in nearly all stratified analyses, but it was more evident in the age group 35-44 years. The most frequently reported signs and symptoms were cough, headache, muscle aches and fever.[58]

A similar conclusion was reached by a European multicenter case-control study using a propensity score: pregnant women were at higher risk of ICU admission than non-pregnant women (17.4% versus 11.08%; P<0.001), of needing oxygen supplementation (36.04% versus 17.24%; P=0.006) and endotracheal intubation (10.16% versus 1.67%; P=0.022).[60]

Data from the United Kingdom's Obstetric Surveillance System and from the Surveillance for Emergency Threats to Mothers and Babies Network (SET-NET) reported that risk factors for severe COVID-19 are similar among pregnant and non-pregnant women, and include age ≥ 35 years, black race, underlying medical conditions such as overweight/obesity, chronic lung disease, chronic hypertension and diabetes mellitus.[61,62]

Evidence is accumulating that COVID-19 in pregnancy, and particularly severe COVID-19, is associated with a number of adverse pregnancy outcomes, including preeclampsia, preterm birth, stillbirth, gestational diabetes and low birth weight.[63] Most of these complications may be placentally-mediated and/or placentally-reflected.[64] An observational study of 1,219 pregnant women also suggests that SARS-CoV-2 infected women with severe disease are at increased risk of cesarean section.[65] Interestingly, there are some reports that during the pandemic the overall rate of preterm birth has declined in some high-resource settings.[66-70]

SARS-CoV-2 RNA DETECTION IN BLOOD SAMPLES

The detection of SARS-CoV-2 RNA in blood samples is considered infrequent (pooled estimate 10%; 95% CI 5-18%, 200/1512 samples).[7,71]

Available studies with large sample size, mainly conducted in China, reported a wide range of SARS-CoV-2 RNA positive detections in blood samples (whole blood or serum) of infected patients, from 1% to 41%.[72-77] Most of these studies report a strong correlation of serum viral RNA with disease severity and progression with multiorgan involvement, including respiratory failure, cardiac damage, renal damage and coagulopathy. [72,74,78] One study reported the median duration of RNA detection in a positive blood sample as 16 days (range 11-21) without significant differences between patients with different disease severities.[74] A recent study, including both a review of 28 studies and original results, reported a high cycle threshold (Ct) in blood samples, reflecting low copy numbers and suggesting the detection of genomic fragments rather than replication-competent virus in blood. [71] However, the possibility of intact virions may not be definitively excluded.

Data on viral RNA detection in blood of pregnant women are limited. One study enrolling 64 subjects with SARS-CoV-2 infection, of whom 47 infected pregnant women, and 63 controls, reported no detectable viremia in any maternal blood but one case of detectable viremia among women of reproductive age in the non-pregnant cohort, detected 13 days after symptom onset.[16]

Notably, there is some methodological variability between the available studies, both for the sample used (whole blood versus serum) and because of the variable targets of the molecular assays and different thresholds for reporting positivity.

PLACENTAL INVOLVEMENT IN SARS-COV-2 INFECTION

It has been documented that in women with SARS-CoV-2 infection the virus can reach and affect the placenta. SARS-CoV-2 RNA and proteins have been detected in placental tissues in several studies, also in clinically recovered women.[79-81] However, the detection of the virus in the placenta does not necessarily mean that the virus is able *per se* to cross the placenta and to cause fetal infection, and does not clarify whether the placenta is susceptible to infection under normal physiological conditions or only under conditions of systemic inflammation.

Data regarding the expression of canonical SARS-CoV-2 receptor ACE2 and the co-expression of its classical co-factor TMPSSR2 in placental tissue are conflicting.

One study evaluating placentas from 28 patients between 14 and 40 weeks' gestation without SARS-CoV-2 infection and one placenta from a COVID-19 pregnant woman at 19 weeks' gestation using a monoclonal anti-ACE-2 antibody, demonstrated in situ expression of ACE2 at the maternal-fetal interface both in cytotrophoblast and syncytiotrophoblast cells of placental villi, as well as membranous expression in extravillous trophoblast.[82] ACE2 expression was present constantly throughout pregnancy.[82] Since trophoblastic cells are in direct contact with maternal blood in the villous space, these data may suggest that SARS-CoV-2 is able to infect the placenta via a receptor-mediated mechanism. Another study conducted on 27 placentas from SARS-CoV-2 infected women and 10 placentas from control patients, reported the detection of ACE2 by immunohistochemistry in syncytiotrophoblast cells during early pregnancy but rarely in healthily placentas at full term, but term placentas from COVID-19 affected women displayed increased ACE2 expression as compared with non-infected women.[11] The factors that may drive placental ACE2 expression during COVID-19 remain unknown. It is likely that placental cytotrophoblasts and syncytiotrophoblasts express ACE2 from 7 weeks onward.[83]

Also TMPRSS2 has been identified in placental cells, but there are not definitive data on its co-expression with ACE2. One study investigating the expression of both ACE2 and TMPRSS2 throughout the three trimesters of pregnancy at the transcriptional level found that very few cells co-express ACE2 and TMPRSS2 - four cells in any of the three trimesters - resulting in an estimated <1/10,000 cells.[84] The co-expression of ACE2 and TMPRSS2 was minimally detected also in the chorioamniotic membranes. Even though the possibility that SARS-CoV-2 may use alternate entry routes in placental cells, while interacting with other proteins, may not be excluded, this study suggests that transplacental transmission of SARS-CoV-2 in unlikely to occur unless facilitated by other concomitant pathological conditions resulting in a breach of the maternal-fetal crosstalk.

Several reports suggest the possibility of increased risk of placental lesions and impaired placental function due to hypoperfusion and inflammation in women with SARS-CoV-2. As mentioned above, this damage may both facilitate the virus spread to the fetus without requiring placental cells infection and may itself lead to an indirect damage to the fetus, depriving it from its oxygen and nutritional requirements, and leading to increased mortality and morbidity.

A recent review of 57 studies which included 1,009 pregnancies complicated by SARS-CoV-2 infection reported maternal vascular malperfusion in 31.4% and fetal vascular malperfusion in 26.9% of examined placentas.[10] Almost 25% of the placentas had features of acute or chronic inflammation. Only 17.4% of placentas did not present any abnormal histological findings. It is to be further defined if these pathological findings are related to a direct viral effect, to a compromised hemodynamic status of some infected women or to the effect of pro-inflammatory cytokines.

There is indeed a growing body of evidence that severe COVID-19 can trigger maternal inflammatory responses at the maternal-fetal interface, governed by T cells and macrophages, as well as a cytokine response in the fetal circulation.[11,85] Even if these responses are critical for protecting the developing fetus from pathogen invasion, placental inflammation itself may lead to pathological changes detrimental to pregnancy and fetal development.[11,85] A placental immune activation, and thus potential long-term consequences for the developing fetus, has been reported also in women with asymptomatic and/or mild infections occurring during late pregnancy.[81,85]

MOTHER-TO-CHILD TRANSMISSION OF SARS-COV-2

Transmission of SARS-CoV-2 to neonates is thought to occur primarily through respiratory droplets during the postnatal period when neonates are exposed to mothers or other caregivers with SARS-CoV-2 infection.

The extent and clinical significance of vertical transmission – in utero, intrapartum or early postnatal through breastfeeding - is unclear.[86]

Generally, *in utero* transmission of pathogen can occur through the hematogenous route or more rarely the ascending route. Most pathogens transmitted *in utero* are those in which systemic (bloodstream) infection occurs in a pregnant woman to permit the pathogen to obtain access to the placenta. Once the pathogen reaches the placenta, it must cross the maternal-placental interface to obtain access to fetal vessels, reach the fetus and cause infection. Various factors may play a role into this passage. These include direct damage to the villous tree with a break in the syncytiotrophoblast layer, which can be caused by virus-induced apoptosis and

vascular damage in the placenta, spread through the virus-infected maternal endothelium to the extravillous trophoblast, trafficking of infected maternal immune cells throughout the syncytiotrophoblast, paracellular or transcellular transport into fetal capillaries, transmission via swallowed or aspirated amniotic fluid.[87-89] In early life pathogens may also be transmitted during labor and delivery, when the pathogen in maternal blood or vaginal secretions or in maternal rectum gain access to an appropriate host cell in the neonate. Some pathogens may also reach the neonate through breast milk, and lead to infection if susceptible cells are encountered through the gastrointestinal system.

Based on previous knowledges on respiratory viruses during pregnancy, including SARS-CoV and MERS-CoV, from the beginning of the pandemic pregnant women and their fetuses were considered a vulnerable population mostly because of the possible higher maternal morbidity and mortality, which in turn may lead to obstetrical complications. Other respiratory viruses are indeed generally not easily transmitted, and *in utero* transmission of other coronaviruses has never been demonstrated. As the number reached by the COVID-19 pandemic increased however, single case reports or small case series suggesting possible *in utero* transmission of SARS-CoV-2 have been increasingly published.[90-93] Even if the reported number of cases remains low and in most of these cases diagnosis is not unquestionable, there is a biological plausibility for *in utero* transmission of SARS-CoV-2, as the virus may reach the placenta, placental tissues may express the virus receptors, and also fetal tissues and organs may express SARS-CoV-2 receptors during gestation. ACE2 is expressed in fetal kidney, ileum, and rectal cells from as early as 15 weeks, is barely detectable at 15 weeks in the lungs with undetectable expression thereafter, and undetectable in the cerebral ependymal, parenchymal and cardiac cells.[83] A proportion of cells located in the fetal adrenal glands and the kidneys seems to co-express ACE2 and TMPRSS2.[83]

Some cases of *intrapartum* transmission have also been suggested and are plausible both because SARS-CoV-2 has been detected in vaginal secretions of some mothers and because SARS-CoV-2 shedding in stools of infected individuals is well documented.[94-96] However, viral contamination of the environment, droplets and aerosols generated by the infected woman during labor may account for early neonatal infection.[97]

Since defining the true impact of vertical transmission of SARS-CoV-2 based on available literature has been difficult, due in part to the variety of methods and the different timing used for diagnosis, on February, 2021 the WHO proposed a classification system to determine timing of vertical transmission of SARS-CoV-2, which is based on three elements:[7]

- documented maternal infection, using the WHO COVID-19 case definition anytime during pregnancy for *in utero* infection; near the time of birth for intrapartum and early postnatal infection;
- tests to evaluate the likelihood of early *in utero* or intrapartum exposure;
- tests to evaluate the later exposure/persistence of the virus or virus-specific immune response in the fetus/neonate.

The timing of vertical transmission (*in utero*, intrapartum and early postnatal) is classified in:

1) confirmed;
2) possible (evidence is suggestive but not confirmatory for infection);
3) unlikely (little support for diagnosis but infection cannot be completely ruled out);
4) indeterminate (when tests required to define classification have not been performed).[7]

As pointed out by the WHO document, the diagnosis of *in utero* SARS-CoV-2 infection may be challenging. Since *in utero* infection would be the result of a bloodstream infection with transplacental passage of the virus, the mechanism by which it should lead to a positive nasopharyngeal swab in the neonate in unclear. Serological assays have several limitations: immunoglobulin (Ig) G levels found in the neonate are primarily reflective of maternal antibodies and even if IgM and IgA are generally thought to represent a fetal response, maternal IgM and IgA may be found in neonatal blood in some cases of placental disruption. The sensitivity and specificity of IgM assays vary by disease, but usually are less reliable than molecular assays and prone to both false negative and false positive results.[98] Thus for the purpose of diagnosing *in utero* infection, a first positive serological test always requires confirmatory testing, preferably using molecular diagnostic tests or otherwise a later serological test showing persistent immunological response in the neonate (Figure 2).[7]

NEONATAL OUTCOMES FOLLOWING MATERNAL SARS-COV-2 INFECTION

Currently there are insufficient data to draw solid conclusions about possible neonatal complications after maternal SARS-CoV-2 infection in pregnancy. One large cohort study conducted in Sweden reported the neonatal outcomes of 2,323 neonates born to 2,286 mothers with SARS-CoV-2 infection, with SARS-CoV-2 infection defined as a test positivity from conception to one week after birth.[99] The median time from a maternal positive test result to delivery was 36 days (interquartile range, 5-85), with a minority of women infected from 2 weeks before to one week after delivery (710/2,286 - 31%). Overall 0.9% of 2,323 infants tested positive for SARS-CoV-2, with most of the infected infants born to mothers with a positive SARS-CoV-2 test at delivery (17/21 - 81%).

	(#1) Maternal infection: Suspect, probable or confirmed case of SARS-CoV-2 infection anytime during pregnancy, as defined by WHO COVID-19 case definitions *(25)*.	
Category	**(#2) Evidence *in utero* fetal exposure**	**(#3) Viral persistence/immune response**
Confirmed	One or more of following samples at *age <24 hours* **positive** for SARS-CoV-2: • RT-PCR from sterile sample[1] • Placental tissue (RT-PCR or ISH) • RT-PCR from non-sterile sample[2] • Serology (IgM or IgA)	One or more of following samples *at age 24-48 hours* **positive** for SARS-CoV-2: • RT-PCR from sterile sample[1]
Possible	One or more of following samples at *age <24 hours* **positive** for SARS-CoV-2: • RT-PCR from sterile sample[1] • Placental tissue (RT-PCR, ISH, IHC or microscopy), placental swab RT-PCR • RT-PCR from non-sterile sample[2] • Serology (IgM or IgA)	One or more of following samples *at age 24-48 hours* **positive** for SARS-CoV-2: • RT-PCR from non-sterile sample[2] **OR** • **Positive** serology (IgM or IgA) at *age 24 hours to <7 days*
Unlikely	One or more of following samples at *age <24 hours* **positive** for SARS-CoV-2: • RT-PCR sterile sample[1] • Placental tissue (RT-PCR, ISH, IHC or microscopy), placental swab RT-PCR • RT-PCR from non-sterile sample[2] • Serology (IgM or IgA)	All tests that were performed on samples *at age 24-48 hours* are **negative** for SARS-CoV-2: • RT-PCR from sterile sample[1] • RT-PCR from non-sterile sample[2] **OR** • **Negative** serology (IgM or IgA) at *age 24 hours to <7 days*
	All *in utero* fetal exposure tests (above) that were performed are **negative** for SARS-CoV-2	One or more of following samples at *age 24-48 hours* **positive** for SARS-CoV-2: • RT-PCR from sterile sample[1] • RT-PCR from non-sterile sample[2] **OR** • **Positive** serology (IgM or IgA) at *age 24 hours to <7 days*
Indeterminate	One or more of following samples at *age <24 hours* **positive** for SARS-CoV-2 • RT-PCR from sterile sample[1] • Placental tissue (RT-PCR, ISH, IHC or microscopy), placental swab RT-PCR • RT-PCR from non-sterile sample[2] • Serology (IgM or IgA)	No viral persistence/immune response tests (above) performed
	No *in utero* fetal exposure tests (above) performed	One or more of following samples at *age 24-48 hours* **positive** for SARS-CoV-2: • RT-PCR from sterile sample[1] • RT-PCR from non-sterile sample[2] **OR** **Positive** serology (IgM or IgA) at *age 24 hours to <7 days*

IHC: immunohistochemistry; ISH: in-situ hybridization; RT-PCR= reverse transcription polymerase chain reaction.
[1] Sterile sample: amniotic fluid (sterile collection caesarean section prior to rupture of membranes or amniocentesis), neonatal blood (cord blood needs confirmation with peripheral blood or other sample), lower respiratory tract samples obtained by bronchoscopic or non-bronchoscopic bronchoalveolar lavage, bronchial or tracheal aspirate, or cerebrospinal fluid; [2] Non-sterile sample: upper respiratory tract samples (e.g. naso- or oropharyngeal swab or aspirate) or other non-sterile samples (e.g. stool).

Figure 2. Categorization of in utero SARS-CoV-2 transmission in live births proposed by WHO. *In utero* SARS-CoV-2 infection requires: #1) evidence of maternal SARS-CoV-2 in pregnancy AND #2) *in utero* SARS-CoV-2 exposure AND #3) SARS-CoV-2 persistence or immune response in the neonate. From: WHO. Definition and categorization of the timing of mother-to-child transmission of SARS-CoV-2. Scientific brief.[7]

Comparing the outcomes of neonates born to SARS-CoV-2 infected and non-infected women, this study suggests that maternal SARS-CoV-2 infection is associated with increased risk of some neonatal morbidities, such as lower gestational age (gestational age <37 weeks 8.8%

versus 5.5%) and birth weight, even if the number of neonates small for gestational age was comparable between the two groups (2.4% versus 2.2%). Neonates born to mothers with SARS-CoV-2 infection were also more likely to have some respiratory disorders, but this finding was mediated by preterm birth, which could also explain the more frequent use of antibiotics, hyperbilirubinemia and need for assisted ventilation in this cohort.[99] Unfortunately, in this study no information on maternal clinical status and severity were provided. Even if preterm birth, low birth weight and admission for neonatal care are the most commonly reported neonatal outcomes, there are conflicting results in literature.[100-103] A study from the prospective German registry on obstetric and neonatal outcome following maternal COVID-19 in pregnancy, reporting data from neonates born to women with early and late COVID-19 (early if women tested positive for SARS-CoV-2 more than 2 weeks prior to delivery, late if tested positive for SARS-CoV-2 less than 2 weeks prior to delivery), found no significant differences in birth weight, congenital malformations (2.5% versus 1.9%), cesarean section, rate of prematurity (10.4% versus 14.9%), neonatal intensive care unit (NICU) admission (12.5% versus 15.4%) or need for ventilatory support between the two groups.[103] Interestingly, in addition to the low neonatal morbidity, Authors report that 9/323 (2.8%) neonates tested positive for SARS-CoV-2 RNA, all born to women with late SARS-CoV-2 infection, and that all these neonates were asymptomatic.

A recent systematic review and metanalysis of large cohort and case series including 31,016 pregnant women from 44 countries of the six continents, mostly infected during the third trimester of gestation reported the highest risk of preterm birth (<37 weeks), NICU admission and low birth weight in severe COVID-19.[104]

To date no clinically detectable congenital abnormalities have been described in infants born to mothers with SARS-CoV-2 infection in pregnancy. Most studies evaluating hematological parameters and coagulation in neonates reported values in the normal range for age, even if studies are limited by small sample sizes.[100, 105,106] Some Authors raised questions about the possible long-term effects on neonatal health of both the maternal inflammatory state related to COVID-19 and the possible neurotropic effects of the virus, including the possible effect on the inner ear structures,[107] but conclusive data are still lacking.

TRANSPLACENTAL ANTIBODIES TRANSFER

One of the most important aspect of immunity against pathogens in early life relies on effective maternal antibody production in response to the pathogen, efficient transfer across the placenta to the fetus, and persistence of this passive immunity in the infant for the first months

of life. Transplacental antibody transfer begins during the first trimester but increases exponentially during pregnancy, with the majority of transfer occurring during the third trimester.

The majority of maternal antibodies transferred to the fetus are of the IgG isotype, and across the IgG subclasses there is a certain variability in the transfer efficiency ($IgG_1 > IgG_3 > IgG_2 > IgG_4$); also the glycosylation profile of the IgG, which varies with the maturity of the IgG, drives the efficiency of the transplacental transfer. Upon transfer, IgG antibodies may be detected in neonatal blood in a finite amount that declines over time. These maternally derived antibodies have an important effective function in protecting neonates and infants against infectious diseases, as demonstrated in infants with agammaglobulinemia, but might also interfere with vaccines, as it may suppress vaccine-induced immune responses.[108] For most pathogens, umbilical cord IgG titers are higher than IgG titers in maternal blood, leading to a cord-to-maternal IgG ratio equal or above 1, with the highest ratio of approximately 1.5 typically observed for vaccinable pathogens, including influenza, pertussis and measles.[109-112] This higher titer observed in neonates as compared with their mothers, is partly due to endosomal transport of IgG across the syncytiotrophoblast layer from maternal to fetal circulation. IgG are transferred by the neonatal Fc receptor, which is present in high concentrations on the placental syncytiotrophoblast. It has been suggested that some maternal acute infection, as malaria and HIV, may compromise the efficiency of transplacental transfer of non-disease-specific antibodies, because of alterations in antibody glycosylation and because hypergammaglobulinemia can result in competition for binding the neonatal Fc receptor. [112]

Most individuals infected with SARS-CoV-2 develop antibodies to the S and N proteins, which are used as antigens in the serology assays. The S protein is an important target for neutralizing antibodies, and these antibodies can prevent viral entry into the host cells. SARS-CoV-2 IgM, IgA and IgG antibodies typically become detectable at similar median times of about 2 weeks post-infection onset. IgG titers peaks around weeks 3 to 7 post-infection, then plateau.[113] Current information on the role of antibodies in viral clearance and modulation of the disease severity and the longevity of these responses are limited. Both a rapid waning of SARS-CoV-2 IgG by approximately 3 months after infection and stable titers over several weeks or months have been reported.[113-115] One study on a limited number of pregnant women (17) reported that neutralizing antibody titers remained stable since 12-weeks' gestation throughout pregnancy.[116]

Recent publications have provided further evidence on maternal SARS-CoV-2 antibody production after infection and antibody transplacental transfer. Studies enrolling women with

third trimester SARS-CoV-2 infections and their neonates suggested compromised SARS-CoV-2-specific antibody transfer.[12-15,112] Some Authors suggested that this compromised transfer may be likely related to inflammation-induced alteration in IgG glycosylation, which however did not affect the transfer of non-SARS-CoV-2-specific antibodies to the neonate.[112] Authors suggested that this perturbed glycosylation might be aimed at leveraging immune functions to protect the mothers, and maybe the fetus, from the virus. However, the reduced transplacental passage might also be due to the short time period between maternal infection and delivery, because of the SARS-CoV-2 IgG kinetics. Interestingly, higher transplacental transfers were reported in women with SARS-CoV-2 infection in early stages of pregnancy.[12] Whether this change is related to a longer time for the antibody response to be produced in the woman and to be transmitted to the fetus or if there is a resolution of the inflammation-induced perturbation of IgG profile over time in unclear.

Few studies have followed the dynamics of maternally derived IgG in infants in the first months of life: one study reported results from four infants, suggesting that IgG levels decreased sharply in the first two months of life, accounting for only 10.7% of the titer at birth.[13] The persistence of maternal-derived IgG seems to be positively correlated to the initial cord blood levels.

RESEARCH PROJECT

AIMS OF THE STUDY

- To evaluate the possible *in utero* transmission of SARS-CoV-2 in a large series of mother-neonate dyads
- To evaluate the neonatal and long-term outcomes of infants born to mothers with SARS-CoV-2 infection during pregnancy
- To assess the efficiency and dynamics of transplacental transfer of maternal SARS-CoV-2 antibodies to the neonate and to evaluate the longevity of these maternal derived antibodies in infants

MATERIALS AND METHODS

STUDY POPULATION

Neonates born to mothers with SARS-CoV-2 infection during pregnancy, delivered at IRCCS Azienda Ospedaliero Universitaria di Bologna in Bologna, Italy, between April 2020 and April 2021 were included in the study.

During the study period all women were routinely screened for SARS-CoV-2 infection on admission for delivery. Between April 2020 and October 2020, given to the recent spread of the virus, SARS-CoV-2 infection during pregnancy was defined by the presence of SARS-CoV-2 antibodies in maternal serum at the time of delivery with or without clinical data consistent with COVID-19 during pregnancy and/or previous history of SARS-CoV-2 exposure. Between November 2020 and April 2021, clinical data consistent with COVID-19 during pregnancy and/or history of SARS-CoV-2 exposure confirmed by a positive nasopharyngeal swab for SARS-CoV-2 RNA were required to confirm SARS-CoV-2 infection during pregnancy.

Neonates born to mothers with peripartum SARS-CoV-2 infection, defined as a positive result of the SARS-CoV-2 RNA on nasopharyngeal swab by real-time polymerase chain reaction (PCR) between 2 weeks prior delivery and 2 days post-delivery, and without SARS-CoV-2 antibodies, were excluded from the study.

The study was approved by the Institutional Review Board of the IRCCS Azienda Ospedaliero Universitaria di Bologna.

STUDY DEFINITIONS AND DATA COLLECTION

For mothers, data regarding demographics, trimester of infection, illness severity, prenatal ultrasound and comorbidities were collected at the time of delivery.

The timing of maternal infection was based on the first positive SARS-CoV-2 test during pregnancy.

Maternal illness severity was defined per definition provided by the US CDC: (1) asymptomatic: no history of COVID-19 symptoms on review of prenatal history; (2) mild disease: symptoms that do not include shortness of breath, dyspnea or radiographic evidence of pneumonia; (3) moderate disease: evidence of lower respiratory disease by clinical assessment or imaging and a saturation of oxygen (SpO2) >= 94% on room air; (4) severe disease: evidence of lower respiratory diseases with SpO2 < 94% on room air, or lung infiltrated >50%; (5) critical disease: respiratory failure, septic shock and/or multiple organ dysfunction.[117]

For neonates, data regarding gestational age, birth weight, length, head circumference and clinical signs at birth were collected. All neonates underwent nasopharyngeal swab for SARS-CoV-2 RNA by real-time PCR during the first 24 hours of life and blood tests for total and differential blood cell count, hemoglobin and platelet evaluation, liver function tests and SARS-CoV-2 antibodies during the first 48 hours of life. All infants were enrolled in a follow-up program with clinical evaluation at 1, 3 (± 30 days), 6, 9 (± 30 days) and 12 months of age. Cerebral ultrasound (US), fundoscopy evaluation and abdominal US were performed in all infants during the first 3 months of life. Results of the audiological screening tests were also collected.

SARS-CoV-2 antibodies were assessed in both maternal and neonatal serum during the first 48 post-delivery. SARS-CoV-2 antibodies were repeatedly assessed in infant serum at 1 month, 3 months (± 30 days), 6 months, 9 months (± 30 days), 12 months or until two consecutive negative results performed at least 1 month apart. At the same time-points also the mothers were evaluated for SARS-CoV-2 antibodies.

Preterm birth was defined as birth before 37 weeks' gestation.

In utero infection was defined per definition provided by the WHO: *in utero* SARS-CoV-2 transmission requires evidence of maternal SARS-CoV-2 infection anytime during pregnancy AND *in utero* fetal SARS-CoV-2 exposure AND SARS-CoV-2 persistence or immune response in the neonate. The timing of vertical transmission is classified in confirmed, possible, unlikely, indeterminate.[7]

LABORATORY METHODS

Blood samples were collected in ethylenediamine tetraacetic acid-anticoagulated tubes. The tests' procedures and the interpretation of results adopted were those reported in the manufacturer instructions for the assays.

For the qualitative detection of SARS-CoV-2 IgG and IgM, the SARS-CoV-2 IgM and IgG CLIA kits (Shenzen YHLO Biotech Co., Ltd., China) were used. The assays were performed on the iFlash3000 CLIA analyzer. The amount of SARS-CoV-2 IgM or IgG in the serum/plasma sample is in proportion to the relative light unit (RLU) measured by the CLIA analyzer that automatically calculates the antibody concentration (in arbitrary units (AU)/ml) on the basis of the RLU and the calibration curve. The cut-off value for positivity is equal to 10.0 AU/mL for both IgG and IgM. The assay detects antibodies to N and S proteins of SARS-CoV-2.[118]

For the quantitative detection of SARS-CoV-2 antibodies the LIAISON® SARS-CoV-2 S1/S2 IgG CLIA assay (DiaSorin S.p.A., Saluggia, Italy) performed on the LIAISON® XL Analyzer (DiaSorin) was used. Antibody concentration in serum/plasma sample, expressed as AU/mL, was automatically calculated by the analyzer on the basis of the RLU and the calibration curve. The cut-off value for a positive result is equal to 15 AU/mL; the cut-off value for a negative result is < 12 AU/mL. The magnetic beads of the assay are coated with recombinant antigens representing the S1 and S2 subunits of the S protein of SARS-CoV-2. Given this target, potential neutralizing antibodies could be detected.[118]

Detection of SARS-CoV-2 RNA in oro-and/or naso-pharyngeal swab specimens and in other biological samples was performed by real-time PCR targeting regions in the *N* gene, following the U.S. CDC protocol.[119]

STATISTICAL ANALYSES

Demographics, clinical and serological data were summarized using descriptive analyses. Categorical data were reported using numbers and percentages. Continuous variables were reported using mean and standard deviation or median and range and/or interquartile range (IQR), as appropriate for the normality of the data. Transplacental transfer ratio was calculated as infant IgG concentration divided by maternal IgG concentration at birth. Correlations between maternal and neonatal IgG concentrations and between transplacental transfer ratio and persistence of IgG in the first months of life were reported using the Pearson correlation coefficient (*r*). Standard descriptive analyses, including Fisher's exact test, unpaired t test, Mann Whitney U test and Wilcoxon signed rank test were used as appropriate to compare

demographics, clinical characteristics, transplacental transfer ratio and maternally derived IgG persistence between groups based on disease severity and timing of infection. Statistical significance was set at P<0.05. STATA version 15 (StataCorp, College Station, TX, USA) was used for analysis.

RESULTS

During the study period, 2745 neonates were delivered. One hundred and thirty out of 2745 (4.7%) were born to 130 mothers with SARS-CoV-2 infection during pregnancy. Twenty-four of these 130 mother-neonate dyads were excluded because of peripartum SARS-CoV-2 infection. Thus, 106/2745 (3.9%) neonates and their 106 mothers with SARS-CoV-2 infection during pregnancy, diagnosed more than two weeks before delivery, were included in this study.

Sixteen of 106 (15%) infants were born between April 2020 and October 2020; 90/106 (85%) were born between November 2020 and April 2021. The month of maternal infection is reported in Figure 3.

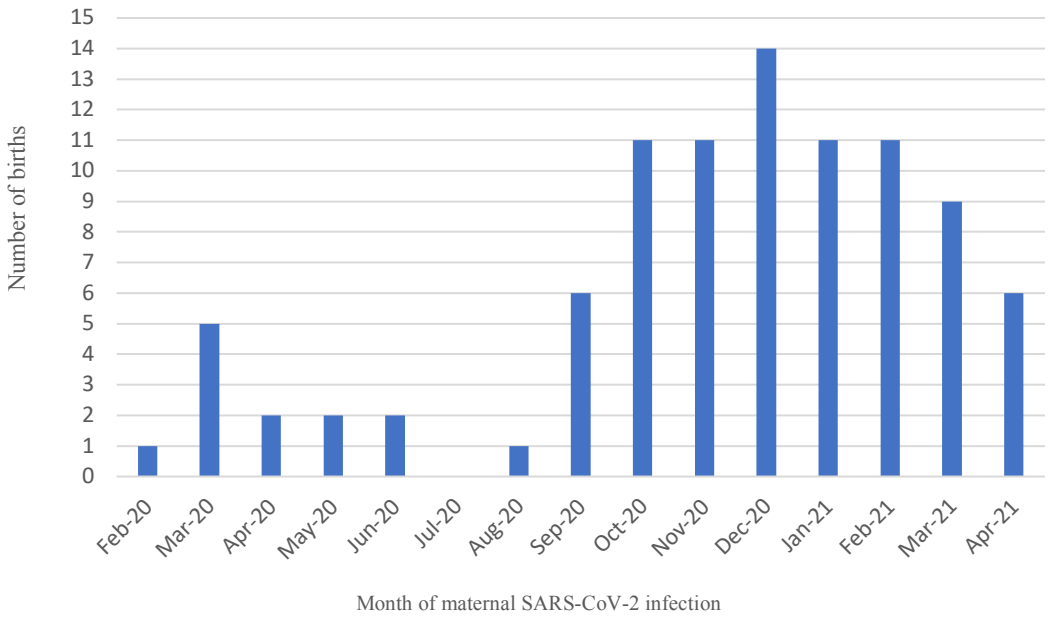

Figure 3. Number of births from the women with SARS-CoV-2 infection by month of infection. The month of SARS-CoV-2 infection was known in 92/106 (86.7%) women.

Most neonates were born via vaginal delivery (82/106, 77.3%), while 24/106 (22.6%) were born by cesarean section. All cesarean sections were performed because of pregnancy-related indications. The rate of cesarean section was similar between neonate born to mother with and without SARS-CoV-2 infection (22.6% versus 31.8%, Figure 4). Even when including in the

SARS-CoV-2 infected cohort the women with peripartum SARS-CoV-2, the rate of cesarean sections was not higher between neonate born to mother with SARS-CoV-2 infection as compared with women without SARS-CoV-2 infection (22.3% versus 32.2%).

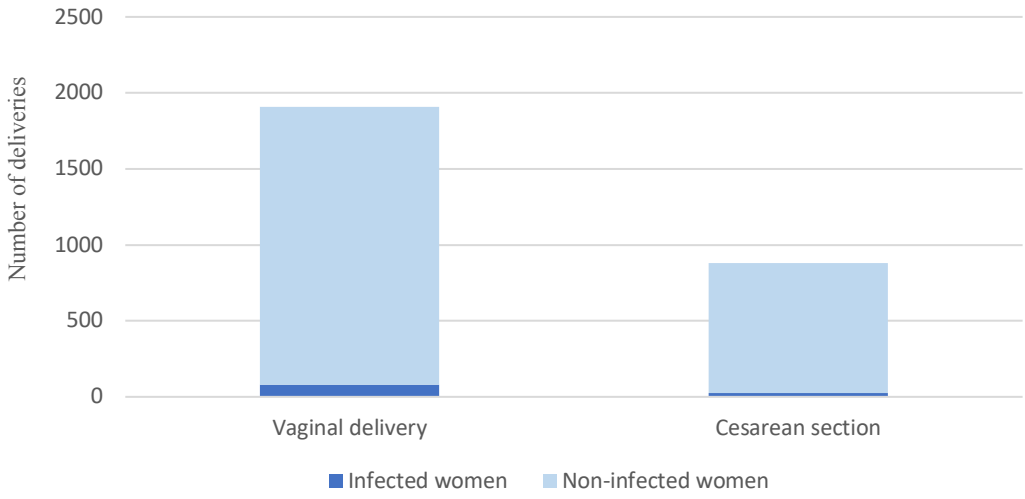

Figure 4. Type of delivery (vaginal and cesarean section) in women with SARS-CoV-2 infection and in women without SARS-CoV-2 infection in the study period.

MATERNAL SYMPTOMS AND DIAGNOSIS

Demographic characteristics of the mothers are summarized in Table 1.

Most women (76/106, 71.7%) were symptomatic during pregnancy: 67/106 (63.2%) had a mild disease; 4/106 (3.7%) had a moderate disease; 5/106 (4.7%) had a severe disease with radiologic evidence of pneumonia requiring hospitalization for oxygen supplementation. The signs and symptoms of COVID-19 in these women are summarized in Table 2.

The remaining 30/106 (28.3%) women were asymptomatic. Of these asymptomatic women, 14 were identified because of serological screening during the first phase of the study and were unaware of their serological status for SARS-CoV-2 at delivery. The other asymptomatic women were tested during pregnancy because of close contacts with SARS-CoV-2 infected individuals.

None of the enrolled women was vaccinated against SARS-CoV-2.

The trimester of infection was known in 92/106 (86.7%) women: 12/92 (13%) were first trimester infections, 33/92 (36%) were second trimester infections, 47/92 (51%) were third trimester infections.

NEONATES

All 106 neonates were tested for SARS-CoV-2 RNA by real-time PCR on nasopharyngeal swab within 24 hours after birth and all were negative.

All infants but one extremely preterm baby had an Apgar score at 10 minutes equal or above 9.

Characteristics	Asymptomatic	Symptomatic			Total
	N=30	Mild	Moderate	Severe	N=106
		N=67	N=4	N=5	
Age at delivery, y					
- Mean (SD)	30.9 (6.3)	33.6 (5.4)	31.7 (4.9)	32.8 (2.3)	32.7 (5.7)
- No. (%)					
18-24	7 (23)	4 (6)	0	0	11 (10)
25-29	5 (17)	8 (12)	1 (25)	0	14 (13)
30-34	8 (27)	23 (34)	2 (50)	3 (60)	36 (34)
35-39	9 (30)	26 (39)	1 (25)	2 (40)	38 (36)
≥40	1 (3)	6 (9)	0	0	7 (7)
Birth country, No (%)					
- Europe	16 (53)	57 (85)	3 (75)	5 (100)	81 (76)
- Asia	10 (33)	6 (9)	0	0	16 (15)
- Africa	1 (3)	3 (4)	1 (25)	0	5 (5)
- South America	3 (10)	1 (2)	0	0	4 (4)
- Other	0	0	0	0	0
Gravidity, median (IQR)	1 (1-2)	2 (1-2)	2	1 (1-2)	2 (1-2)
Pre-pregnancy comorbidities, No (%)	9 (30)*	11 (16)*	0	1 (20)*	21 (20)*
- Thrombocytopenia	1 (3)	1 (1)	0	0	2 (2)
- Hypothyroidism	5 (17)	5 (7)	0	1 (20)	11 (10)
- Factor V Leiden	0	1 (1)	0	0	1 (1)
- Factor II mutation	1 (3)	1 (1)	0	0	2 (2)
- Polycythemia	1 (3)	0	0	0	1 (1)
- Lupus	1 (3)	0	0	0	1 (1)
- Venous thrombosis	1 (3)	2 (3)	0	0	3 (3)
- Obesity	0	1 (1)	0	0	1 (1)
- Cutaneous mastocytosis	0	1 (1)	0	0	1 (1)
- Myasthenia gravis	0	1 (1)	0	0	1 (1)
- Asthma	0	1 (1)	0	0	1 (1)
Trimester of infection, No (%)					
- I trimester	4 (13)	6 (9)	1 (25)	1 (20)	12 (11)
- II trimester	3 (10)	28 (42)	2 (50)	0	33 (31)
- III trimester	9 (30)	33 (49)	1 (25)	4 (80)	47 (44)
- Not known	14 (47)	0	0	0	14 (13)
Gestational diabetes, No (%)	9 (30)	11 (16)	0	2 (40)	22 (21)
IgG+ IgM- , No (%)	22 (73)	63 (94)	3 (75)	4 (80)	92 (87)
IgG+ IgM+, No (%)	8 (27)	4 (6)	1 (25)	1 (20)	14 (13)

*some women may have more than one pre-pregnancy comorbidity
None of the women had pre-eclampsia.

Table 1. Maternal characteristics by clinical presentation of COVID-19 during pregnancy.

Median gestational age at birth was 39 weeks (range 25^{+5}-41^{+4} weeks). Mean birth weight was 3305 grams (SD ± 468 grams), mean length at birth was 49.8 cm (SD ± 2.5 cm), mean occipital-frontal circumference was 33.9 cm (SD ± 1.6 cm). Six of 106 infants (5.6%) had a birth weight below the 10° centile, without significant differences between neonates born to asymptomatic and to symptomatic mothers (P=0.67, Table 3). Six of 106 (5.6%) neonates were born preterm, without significant differences in the rate of preterm birth between asymptomatic and symptomatic mothers (P=0.67, Table 3). The reason for the preterm birth was a preterm rupture of the membranes in three cases and the spontaneous onset of labor in the other three cases. The rate of preterm birth among neonates born to mothers with and without SARS-CoV-2 infected women in the study period was comparable (6/106, 5.6% versus 216/2639, 8.2%, P=0.57).

Signs and symptoms	**Number of women** (N=76)*
Fever, No (%)	31 (41)
Fatigue, No (%)	18 (24)
Muscle, joint, bone aches, No (%)	9 (12)
Anosmia/ageusia, No (%)	40 (53)
Nasal congestion/rhinorrea, No (%)	18 (24)
Sore throat, No (%)	2 (3)
Cough, No (%)	22 (29)
Shortness of breath/difficulty breathing, No (%)	5 (7)
Headache, No (%)	9 (12)
Pneumonia, No (%)	6 (8)
Gastrointestinal symptoms, No (%)	4 (5)
Dermatological signs, No (%)	0
Other non-specific signs, No (%)	2 (3)

*most women experienced a combination of multiple signs and symptoms

Table 2. Signs and symptoms of COVID-19 in the enrolled women. Most women experienced a combination of multiple signs and symptoms.

All infants were clinically evaluated at birth and no congenital abnormalities potentially related to SARS-CoV-2 were detected. One infant had congenital heart disease (pulmonary atresia) diagnosed during pregnancy before maternal COVID-19.

Laboratory parameters at birth are reported in Table 3 and were in the normal range for age in all infants but one, found to have a low hemoglobin value (Hb 9.8 g/dL) at birth. This neonate

was born to a mother with a third trimester infection and required a blood cell transfusion at 34 days of life; the anemia gradually resolved over the first few months of life. All the most common causes of neonatal anemia were excluded.

Characteristics	Asymptomatic mothers (N=30)	Symptomatic mothers (N=76)	P value
Gestational age, median (range), wk:d	39 (32:0-41:0)	39 (25:5-41:4)	0.39
Preterm delivery (GA<37 wk), No (%)	1 (3)	5 (6.5)	0.67
Birth weight, grams, mean (SD)	3,309 (447)	3,304 (477.2)	0.96
Small for gestational age, No (%)	1 (3)	5 (6.5)	0.67
Mode of delivery, No (%)			
- Vaginal	21 (70)	61 (80)	0.30
- Cesarean section	9 (30)	15 (20)	
Apgar score at 5 min ≥9, No (%)	30 (100)	75 (99)	1
Hemoglobin value, g/dL, mean (SD)	17.0 (2.7)	18.4 (1.9)	0.06
White blood cell count, mean (SD), /mmc	14,505 (4,991)	18,405 (5,510)	0.07
- Neutrophil, mean (SD), /mmc	9,209 (4,253)	11,446 (5,156)	0.06
- Lymphocyte, mean (SD), /mmc	4,240 (1,031)	4,630 (1,509)	0.24
Platelet count $x10^9$, mean (SD), /mmc	318 (71)	325 (96)	0.75
Aspartate aminotransferase, mean (SD), IU/ml	18.5 (8)	20 (0.7)	0.17
IgG + IgM -, No (%)	28 (93)	67 (88)	0.72
IgG+ IgM +, No (%)	0	1 (1)	1
IgG- IgM-, No (%)	2 (7)	8 (11)	0.72
Transfer ratio, mean (SD)	0.74 (0.37)	0.95 (0.58)	0.2

Table 3. Neonatal characteristics by maternal clinical presentation of SARS-CoV-2 infection during pregnancy.

All infants underwent cerebral US, and no abnormalities potentially related to SARS-CoV-2 infection were detected. One preterm infant (gestational age 25^{+5}, birth weight 802 grams) had pathological cerebral findings related to prematurity (intraventricular hemorrhage).

A fundoscopy evaluation was performed in 83/106 (78%) neonates and no pathological findings were detected. Seventy-one of 106 (67%) neonates underwent an abdominal US, showing normal hepatic, renal and splenic parenchyma.

All infants had a pass result of the neonatal hearing screening (otoacoustic emissions).

The median age at the last follow-up evaluation was 7 months (range 5-12 months). Sixteen of 106 infants (15%) are 12 or more months old and have already completed the scheduled follow-up; 28/106 (16%) are between 9 and 11 months old; 46/106 (43%) are between 6 and 8

months old; the remaining 16/106 (15%) infants were younger than 6 months of age at the last evaluation.

SEROLOGY

Matched maternal-neonatal blood sera collected during the first 48 hours post-delivery were available for all 106 dyads.

All the 106 mothers had detectable SARS-CoV-2 IgG; 14/106 mothers (13.2%) were both IgG and IgM positive at delivery. For 9 of these 14 women with both SARS-CoV-2 IgG and IgM positivity, the trimester of infection was known (first trimester 1/9, 11%; second trimester 1/9, 11%; third trimester 7/9, 78%).

Maternal SARS-CoV-2 S1/S2 IgG level at the time of delivery was significantly higher in women with SARS-CoV-2 infection during the third trimester of pregnancy as compared to women with SARS-CoV-2 infection during the first two trimesters of pregnancy (median IgG level 46.5 AU/mL, IQR 23-76.5 AU/mL, versus 24.5 AU/mL, IQR 15-49 AU/mL, P=0.046, Figure 5). The SARS-CoV-2 S1/S2 IgG level at delivery was not significantly different between asymptomatic and symptomatic mothers (median IgG level 35.5 AU/mL, IQR 18-54 AU/mL, versus 36.5 AU/mL, IQR 20-75.5 AU/mL, P=0.22, Figure 6).

Ninety-six neonates had detectable SARS-CoV-2 IgG at birth (Table 3). One neonate had both detectable SARS-CoV-2 IgG and IgM during the first 24 hours of life, further confirmed at a second sample taken within the first week of life. This neonate was born to a mother with a third trimester infection and both SARS-CoV-2 IgG and IgM positive at delivery; neonatal nasopharyngeal swab, meconium and stools, blood and urine were negative for SARS-CoV-2 RNA by real-time PCR. Also placental tissue and maternal serum were negative for SARS-CoV-2 RNA by real-time PCR at the time of delivery. This infant was the only one who fulfilled the WHO criteria for possible *in utero* infection.

There was a significant positive correlation between maternal and neonatal SARS-CoV-2 S1/S2 levels at first sampling (r = 0.81, P<0.00001).

Neonatal SARS-CoV-2 S1/S2 IgG level at birth was not significantly different in relation to the trimester of maternal infection (median SARS-CoV-2 S1/S2 IgG level 25.5 AU/mL, IQR 17.5-68 AU/mL, versus 19.5 AU/mL, IQR 15-59.7 AU/mL, versus 31 AU/mL, IQR 15-70 AU/mL, P=0.09, Figure 5) nor between neonates born to asymptomatic mothers and those born to symptomatic mothers (median IgG level 18.5 AU/mL, IQR 12-49.2 AU/mL, versus 25 AU/mL, IQR 12-73 AU/mL, P=0.13, Figure 6).

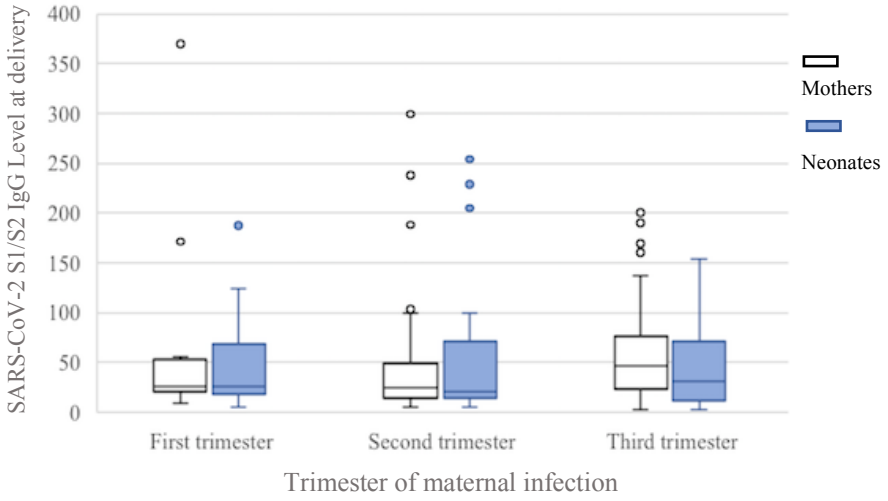

Figure 5. Box plots of the distribution of maternal and neonatal SARS-CoV-2 IgG level at the time of delivery based on the trimester of infection.

Note: the box represents the middle 50% of the data; the line within the box represents the median; whiskers indicate variability outside the upper and lower quartile, excluding outliers.

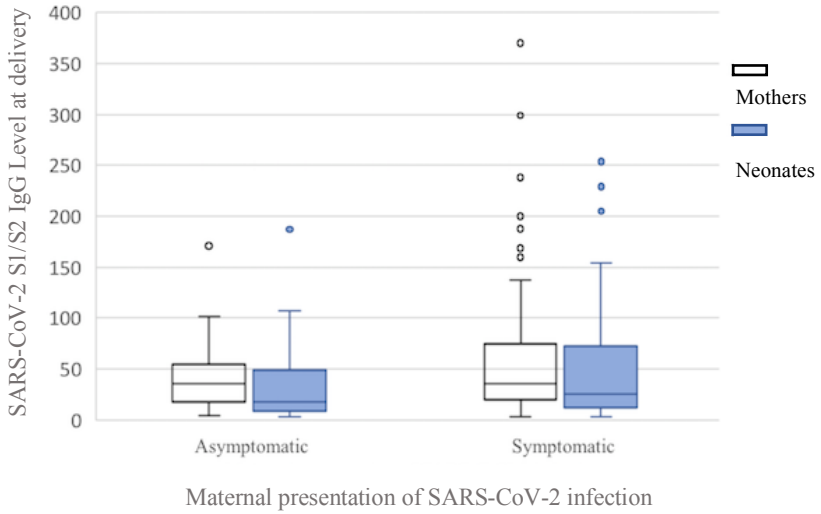

Figure 6. Box plots of the distribution of maternal and neonatal SARS-CoV-2 IgG level at the time of delivery based on maternal presentation of SARS-CoV-2 infection in pregnancy.

Note: the box represents the middle 50% of the data; the line within the box represents the median; whiskers indicate variability outside the upper and lower quartile, excluding outliers.

The transplacental transfer ratio was significantly higher in neonatal-mother dyads when maternal infection had occurred in the second trimester compared with infections occurred during the first and third trimester (mean transfer ratio 0.94±0.46 versus 1.07±0.64 versus

0.75±0.44, P=0.039), but was not significantly different between asymptomatic and symptomatic women (P=0.20). The transfer ratio was also not significantly different between term and preterm infants (0.89±0.53 vs 0.84±0.59, P=0.83).

Characteristics	Transplacental transfer ratio			
	<0.50 (N=20)	**0.50-1.00 (N=47)**	**1.01-2.00 (N=26)**	**>2.00 (N=3)**
Maternal presentation, No (%):				
- asymptomatic	7 (35)	15 (32)	8 (31)	0
- symptomatic	13 (65)	32 (68)	18 (69)	3 (100)
Trimester of infection, No (%):				
- I trimester	2 (10)	4 (8)	5 (19)	0
- II trimester	3 (15)	15 (32)	7 (27)	2 (67)
- III trimester	12 (60)	21 (45)	10 (38)	1 (33)
- not known	3 (15)	7 (15)	4 (15)	0
Maternal S1/S2 IgG level, median (IQR)	44 (28-74)	35 (22-75)	41 (19-59)	24 (N/A)
Gestational age, No(%):				
- preterm	1 (5)	1 (2)	2 (8)	0
- term	19 (95)	46 (98)	24 (92)	3 (100)

Table 4. Transplacental transfer ratio in relation to maternal and neonatal characteristics in the 96 neonates with SARS-CoV-2 positive antibodies at birth. N/A: not assessed.

The SARS-CoV-2 S1/S2 IgG level in neonatal blood progressively decreased during the first months of life in all enrolled infants (Figure 7). At 3 months of life 53% (51/96) of the infants had lost maternal antibodies and this percentage increased to 68% (63/96) at four months of life. Considering the previously seropositive infants who have already been tested at six and 8 months of age, 73/80 (91%) and 44/44 (100%) had lost maternal antibodies respectively, meaning that none of the infants who are currently more than 8 months old still have SARS-CoV-2 S1/S2 IgG. The neonate with possible *in utero* infection was SARS-CoV-2 S1/S2 IgG negative at 6 months of age.

The persistence of maternal antibodies was positively correlated to the SARS-CoV-2 S1/S2 IgG level at first sampling ($r = 0.66$; $P<0.00001$).

The SARS-CoV-2 S1/S2 IgG level was followed post-delivery in a subset of women. Between 1 and 3 months post-delivery 81/106 (76.4%) samples were available, and the trimester of infection was known in 69 of these women (first trimester 7/69, second trimester 24/69, third trimester 38/69 respectively). The median IgG level in mothers between 1 and 3 months post-delivery was similar to their IgG level at delivery (median IgG level at delivery 36

AU/mL, IQR 20-65, versus 44 AU/mL, IQR 22-99, P=0.18). At 6 months post-delivery only 17/106 samples were available and the trimester of infection was known in 13 of these women (first trimester 1/13, second trimester 4/13, third trimester 8/13). Notably, the median IgG level at this time point (124 AU/mL, IQR 43.5-306.5 AU/mL) was significantly higher than the IgG level measured both at delivery (P=0.001) and between 1 and 3 months post-delivery (P=0.01), even if the median days between SARS-CoV-2 infection and sampling was 255.5 (range 126-371).

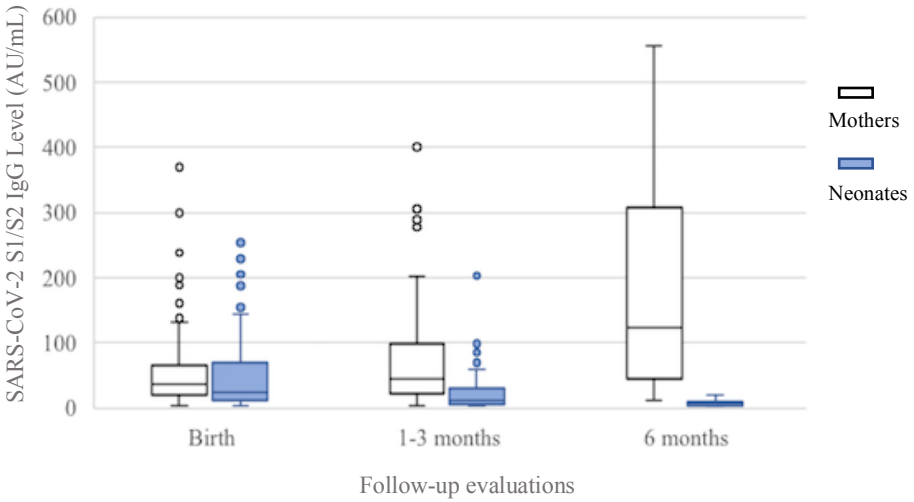

Figure 7. Box plots of the distribution of maternal and neonatal SARS-CoV-2 IgG during follow-up.
Note: the box represents the middle 50% of the data; the line within the box represents the median; whiskers indicate variability outside the upper and lower quartile, excluding outliers.

DISCUSSION

Current evidence suggests that *in utero* transmission of SARS-CoV-2 infection is a possible but rare event. In line with this assumption, we did not confirm *in utero* infection in any of the neonate enrolled in the present study. Only one neonate, born to a mother with a third trimester infection, was defined as possible *in utero* infection based on the WHO classification,[7] although the low sensitivity and specificity of the presence of IgM to diagnose congenital infection suggest caution in the interpretation of this finding, as it could reflect an aberrant transplacental transfer of IgM related to placental compromise. Even though the diagnosis of *in utero* SARS-CoV-2 infection remains possible, the neonate had no abnormalities detected at birth or during follow-up, virology investigations performed at birth on placental tissues, maternal and neonatal blood, meconium and stools, upper respiratory tract specimens and urine tested negative for SARS-CoV-2 RNA by real-time PCR, and SARS-CoV-2 S1/S2 IgG levels slightly decreased during the first few months of life, until negativizing at 6 months of age. Similarly

to this case, other Authors reported on the possible presence of IgM in cord blood of neonates born to mothers with SARS-CoV-2 infections during pregnancy and negative birth specimens for SARS-CoV-2 RNA, underlining the complexity of providing a clear categorization of possible *in utero* infections.[16]

All the enrolled exposed neonates had no clinical abnormalities detected at birth or during follow-up, including those born to mothers with severe COVID-19 and with first trimester infections. Most women were infected during the second wave of the pandemic in Italy, in line with the rate of infections in the general population (Figure 1 and Figure 3).[3] During the study period, the rate of preterm birth was comparable between neonates born to mothers with and without SARS-CoV-2 infection, as well as it was the rate of cesarean sections. Previous studies lead to conflicting results: while some Authors suggest a significant increase of adverse pregnancy outcomes, including preterm birth, low birth weight, cesarean sections among SARS-CoV-2 infected women,[63-65] others did not show such an association,[103] in line with our findings. Even when including women with peripartum SARS-CoV-2 infection, the results of the lack of higher risk of preterm birth and cesarean section did not change. This study however did not include potential cases of SARS-CoV-2 related miscarriages or stillbirths.

Neonatal outcomes following *in utero* SARS-CoV-2 exposure have been mainly described in neonates born to mothers infected during late pregnancy. One of the strength of this study is that the trimester of maternal infection was known in most infected women, and neonates born to mothers with infections in all three trimesters of gestation were included and extensively studied, providing reassuring evidence of the lack of signs and symptoms directly or indirectly related to maternal infection during pregnancy. Indeed, even though intrauterine transmission is recognized as a possible but rare event, previous studies revealed the presence of both SARS-CoV-2 RNA and protein in the placenta, suggesting that the placenta might be vulnerable to SARS-CoV-2 infection.[81] Recent studies also suggest the possibility of perinatal morbidity without infection related to placental damage.[11,85] Severe COVID-19 has been shown to trigger maternal inflammatory responses at the maternal-fetal interface; recently also placental tissues from women with asymptomatic and/or mild infections occurring during late pregnancy have been analyzed, documenting a placental immune activation with potential long-term consequences for the developing fetus also in these circumstances.[81,85] In light of the possible unfavorable outcome related to both direct infection of the fetus and/or to placental involvement, all infants underwent a cerebral US and an abdominal US, and no abnormalities were detected. Unfortunately, one limitation of in this study is that placental tissues and maternal serum were not systematically analyzed.

As already suggested by previous studies, hematological parameters were in the normal range for age in all but one neonate.[100,105,106] This neonate was born with a low Hb value (9.8 g/dL), which required a blood cell transfusion. Whether the anemia may be related to maternal SARS-CoV-2 infection is unclear. Autoimmune hemolytic anemia has been rarely described in adults with COVID-19, mostly in moderate to severe disease, and is considered even rarer in children.[120,121] The underlying mechanism has yet to be elucidated, but it is likely related to the immunological and inflammatory activation secondary to the viral infection, which would be unlikely to explain anemia in an exposed uninfected neonate. Some Authors however suggested that a molecular mimicry might be a determinant factor, with the erythrocyte membrane protein ankyrin 1 (ANK-1) and the viral S protein being the central players.[122] Indeed, ANK-1 and the S protein have structural similarities that might be responsible of potential immunological cross-reactivity: since this neonate had maternally derived anti SARS-CoV-2 S1/S2 IgG, the hypothesis of a link between maternal infection and neonatal anemia may not be completely rejected.

Available literature on SARS-CoV-2 infection during pregnancy has been mainly focused on the possible pregnancy complications and on short-term neonatal outcomes, without providing information on the long-term outcomes of both infected and exposed infants. In line with previous cohort studies, the majority of enrolled mothers had asymptomatic or mild infections, while the hospitalization rate for COVID-19 was lower than reported - 4.7% in this study versus 21.7% recently reported by CDC Data Tacker, which however includes a much higher number of pregnant women.[123] The long-term outcomes of all the enrolled infants, up to a median age of 7 months (range 5-12) was favorable, but it will be interesting to confirm this observation on larger sample size.

Transplacental acquired antibodies are considered a useful arm of the neonatal immune defense. The extent to which maternal SARS-CoV-2 antibodies cross the placenta might be important for understanding a potential neonatal mechanism of protection from COVID-19 and ideally for developing appropriate maternal vaccination strategies aimed at protecting both the mothers and the offspring. Indeed, even if to date SARS-CoV-2 vaccination during pregnancy has been mainly aimed at protecting the woman, in the future it could be advisable that maternal vaccination would aim at both maternal and fetal/neonatal protection, as there is increasing evidence that COVID-19 may have a severe course in the first few months of life.[37,38] In this study, even if maternal IgG level at delivery was higher after SARS-CoV-2 infection occurring during the third trimester, higher transplacental IgG transfer ratios were observed when maternal SARS-CoV-2 infection occurred in the second trimester. This result is in line with

previous reports, showing an impaired transplacental transfer ratio when maternal infection occurred during the last 60-70 days of gestation:[12,15,112] this may be due to both the kinetics of IgG development after infection and the limited time available for the transplacental IgG transfer, even if some Authors suggest a negative effect of acute inflammation on transplacental IgG transfer.[112] Matching the higher IgG transplacental transfer ratio and the peak response after infection might result in the higher and most durable neonatal passive immunity. Whether the transplacental passage of vaccine-induced anti-SARS-CoV-2 antibodies overlaps with that seen after natural infection remains to be established, as well as the dynamic changes of maternal-derived vaccine-induced IgG in the infant in the first few months of life.

Previous studies on antibody production following infection showed that IgG production starts 12-14 days after infection, peaks 3-7 weeks after infection, and then plateaus and persists for at least eight weeks after infection, even if peak timing has been reported as longer in pregnancy, around 60-120 days.[14] In the present study, even if post-delivery IgG levels were followed only in a minority of women, a delayed IgG peak was documented in some women, with a progressive increase of the IgG level until 56 weeks post-infection. The reason of this delayed antibodies rise is unknown, but theoretically it may be related to multiple factors, such as the peripheral immune adaptation during pregnancy, needed to balance fetal tolerance and growth with host defense. Also this finding would need to be verified on larger sample size.

The level of neonatal maternally derived IgG at birth correlated with maternal IgG level, and this level correlated with the duration of passive immunity in infants. The persistence of these antibodies in infants showed a wide variability, but most infants lost maternal antibodies at 3 months of age and all infants tested till now lost maternal antibodies within 8 months of age. No previous studies on large sample size are available on the dynamic of maternally derived IgG in maternal SARS-CoV-2 infection, but it is possible that there may be some variability based on the sensitivity and specificity of the serological assay.[118] Even though it remains to be fully elucidated whether the presence of maternally derived SARS-CoV-2 S1/S2 IgG correlates with some degree of protection in the neonate, it is known that vertically transferred immunity can influence the response to vaccination.[108] High titer maternal antibodies have often been associated with diminished primary antibody response of infants to vaccines, although the T cell response is usually unaffected. Interference is variable between studies and individual vaccines. Maternal antibodies against SARS-CoV-2 in this study were rapidly eliminated naturally after birth. These results may contribute to helping understand the vulnerability of infants to SARS-CoV-2 and could assist in the planification of appropriate vaccination

strategies in infants, as soon as studies on the safety and efficacy of anti-SARS-CoV-2 vaccines in this population become available.

CONCLUSIONS

This study may contribute to a better delineation of the possible neonatal and long-term outcomes of maternal SARS-CoV-2 infection. In this cohort no cases of confirmed *in utero* SARS-CoV-2 infection have been detected and all exposed infants have had a favorable neonatal course and a favorable long-term outcome, even if the median age at the last follow-up evaluation is 7 months (range 5-12 months). SARS-CoV-2 infection in pregnant women was not associated with an increased risk of preterm delivery or cesarean section. An efficient transplacental antibody transfer has been demonstrated, mainly in women who experienced SARS-CoV-2 infection in the second trimester. The rapid waning of maternal derived antibodies in infants may contribute to the vulnerability of infants to SARS-CoV-2 in the first months of life. Understanding how antibody transfer varies by trimester of pregnancy and the longevity of maternally derived antibodies in the first months of life might be particularly relevant to point a critical window during pregnancy that may be most desirable for the induction of antibodies, ideally optimizing protection for the neonate.

REFERENCES

1. Zhu N, Zhang D, Wang W, et al. China Novel Coronavirus Investigating and Research Team. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med*. 2020;382(8):727-733.
2. WHO Coronavirus (COVID-19) Dashboard. Available at https://covid19.who.int [accessed on October 15, 2021].
3. Task force COVID-19 del Dipartimento Malattie Infettive e Servizio di Informatica. Istituto Superiore di Sanità. Epidemia COVID-19. Aggiornamento nazionale: 13 ottobre 2021. Available at https://www.epicentro.iss.it/coronavirus/bollettino/Bollettino-sorveglianza-integrata-COVID-19_13-ottobre-2021.pdf [accessed on October 20, 2021]
4. Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. *Nat Rev Microbiol*. 2019:17(3):181-192.
5. Nicola M, Alsafi Z, Sohrabi C, et al. The socio-economic implications of the coronavirus pandemic (COVID-19): a review. *Int J Surg*. 2020;78:185-193.
6. 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, parainfluenza, metapneumovirus respiratory syncytial virus, and influenza. *Arch Pathol Lab Med*. 2020;144(8):920-928.
7. WHO. Definition and categorization of the timing of mother-to-child transmission of SARS-CoV-2. Scientific brief. Available at: WHO/2019-nCoV/mother-to-child_transmission/2021.1 [accessed on September 30, 2021]
8. Di Mascio D, Khalil A, Saccone G, et al. Outcome of coronavirus spectrum infections (SARS, MERS, COVID-19) during pregnancy: a systematic review and meta-analysis. *Am J Obstet Gynecol MFM*. 2020;2(2):100107.
9. Musa SS, Bello UM, Zhao S, Abdullahi ZU, Lawan MA, He D. Vertical transmission of SARS-CoV-2: a systematic review of systematic reviews. *Viruses*. 2021;13:1877.
10. Di Girolamo R, Khalil A, Alameddine S, et al. Placental histopathology after SARS-CoV-2 infection in pregnancy: a systematic review and meta-analysis. *Am J Obstet Gynecol MFM*. 2021; doi:https://doi.org/10.1016/j.ajogmf.2021.100468.
11. Lu-Culligan A, Chavan AR, Vijayakumar P, et al. Maternal respiratory SARS-CoV-2 infection in pregnancy is associated with a robust inflammatory response at the maternal-fetal interface. *Med (N Y)*. 2021;2(5):591-610.e10.

12. Flannery DD, Gouma S, Dhudasia MB, et al. Assessment of maternal and neonatal cord blood SARS-CoV-2 antibodies and placental transfer ratios. *JAMA Pediatrics*. 2021;175(6):594-600.
13. Wang X, Yang P, Zheng J, et al. Dynamic changes of acquired maternal SARS-CoV-2 IgG in infants. *Sci Rep*. 2021;11(1):8021.
14. Song D, Prahl M, Gaw SL, et al. Passive and active immunity in infants born to mothers with SARS-CoV-2 infection during pregnancy: prospective cohort study. *BMJ Open*. 2021;11(7):e053036.
15. Rathberger K, Hausler S, Wellmann S, et al. SARS-CoV-2 in pregnancy and possible transfer of immunity: assessment of peripartal maternal and neonatal antibody levels and longitudinal follow-up. *J Perinat Med*. 2021;49(6):702-708.
16. Edlow AG, Li JZ, Collier AY, et al. Assessment of maternal and neonatal SARS-CoV-2 viral load, transplacental antibody transfer, and placental pathology in pregnancies during the COVID-19 pandemic. *JAMA Netw Open*. 2020;3(12):e2030455.
17. Hu B, Guo H, Zhou P, Shi ZL. Characteristics of SARS-CoV-2 and COVID-19. *Nat Rev Microbiol*. 2021;19(3):141-154.
18. Perlman S. Another Decade, Another Coronavirus. *N Engl J Med*. 2020; 382(8):760-762.
19. Gorbalenya AE, Baker SC, Baric RS, et al: Coronaviridae Study Group on the International Committee on Taxonomy of Viruses. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat Microbiol*. 2020;5(4):536-544.
20. Benton DJ, Wrobel AG, Xu P, et al. Receptor binding and priming of the spike protein of SARS-CoV-2 for membrane fusion. *Nature*. 2020;588(7837):327-330.
21. Li MY, Li L, Zhang Y, Wang XS. Expression of the SARS-CoV-2 cell receptor gene ACE2 in a wide variety of human tissues. *Infectious diseases of poverty*.2020;9:45.
22. Lukassen S, Chua RL, Trefzer T, et al. SARS-CoV-2 receptor ACE2 and TMPRSS2 are primarily expressed in bronchial transient secretory cells. *EMBO J*. 2020;39(10):e105114.
23. Sungnak W, Huang N, Bécavin C, et al. SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. *Nat Med*. 2020;26(5):681-687.
24. Centers for Diseases Control and Prevention (CDC). Scientific Brief: SARS-CoV-2 transmission. Available at https://www.cdc.gov/coronavirus/2019-ncov/science/science-briefs/sars-cov-2-transmission.html#print. [accessed on October 14, 2021]
25. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395(10223), 497–506.

26. Mehta P, McAuley DF, Brown M, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet*. 2020;395;1033–1034.
27. Kessel C, Vollenberg R, Masjosthusmann K, et al. Discrimination of COVID-19 from inflammation-induced cytokine storm syndromes using disease-related blood biomarkers. *Arthritis Rheumatol*. 2021:73(10):1791-1799.
28. Cron RQ, Caricchio R, Chatham WW. Calming the cytokine storm in COVID-19. *Nat Med*. 2021;27(10):1674-1675.
29. RECOVERY Collaborative Group, Horby P, Lim WS, Emberson JR, et al. Dexamethasone in Hospitalized Patients with Covid-19. *N Engl J Med*. 2021;384(8): 693–704.
30. Kampf G, Bruggermann T, Kaba H, et al. Potential sources, modes of transmission and effectiveness of prevention measures against SARS-CoV-2. *J Hosp Infect*. 2020;106(4):678-697.
31. Lauer SA, Grantz KH. Bi Q, et al. The incubation period of coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: estimation and application. *Ann Intern Med*. 2020;172(9):577-582.
32. Oran DP, Topol EJ. The Proportion of SARS-CoV-2 Infections That Are Asymptomatic: A Systematic Review. *Ann Intern Med*. 2021;174(5):655-662.
33. Hu Z, Song C, Xu C, et al. Clinical characteristics of 24 asymptomatic infections with COVID-19 screened among close contacts in Nanjing, China. *Sci China Life Sci*. 2020; 63(5):706-711.
34. Centers for Diseases Control and Prevention (CDC). Interim Clinical Guidance for Management of Patients with Confirmed Coronavirus Disease (COVID-19). Available at: https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-guidance-management-patients.html [accessed on September 26, 2021]
35. Merad M, Martin JC. Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. *Nat Rev Immunol*. 2020;20:355–362.
36. Wu Z, McGoogan JM. Characteristics of and Important Lessons from the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72314 Cases from the Chinese Center for Disease Control and Prevention. *JAMA*. 2020;323(13):1239-1242.
37. Kim L, Whitaker M, O'Halloran A, et al. Hospitalization Rates and Characteristics of Children Aged <18 Years Hospitalized with Laboratory-Confirmed COVID-19 — COVID-NET, 14 States, March 1–July 25, 2020. *MMWR Morb Mortal Wkly Rep*. 2020;69(32):1081–1088.

38. Bellino S, Punzo O, Rota MC, et al. COVID-19 Disease Severity Risk Factors for Pediatric Patients in Italy. *Pediatrics*. 2020 Oct;146(4):e2020009399.
39. Feldstein LR, Rose EB, Horwitz SM, et al. Multisystem Inflammatory Syndrome in U.S. children and adolescents. *N Engl J Med*. 2020;383(4):334-346.
40. Rigby FB, Patorek JG. Pneumonia during pregnancy. *Clin Obstet Gynecol*. 1996;39:107-119.
41. WHO. Consensus Document on the Epidemiology of Severe Acute Respiratory Syndrome (SARS). Available online at: https://www.who.int/csr/sars/en/WHOconsensus.pdf [accessed on September 2,2021].
42. WHO. Middle East Respiratory Syndrome (MERS) Background. Available online at: https://www.who.int/health-topics/middle-east-respiratory-syndrome-coronavirus-mers [accessed on September 2, 2021].
43. Wong SF, Chow KM, de Swiet M. Severe acute respiratory syndrome and pregnancy. *BJOG*. 2003;110(7):641–642.
44. Wong SF, Chow KM, Leung TN, et al. Pregnancy and perinatal outcomes of women with severe acute respiratory syndrome. *Am J Obstet Gynecol*. 2004;191:292–297.
45. Ng PC, So KW, Leung TF, et al. Infection control for SARS in a tertiary neonatal centre. *Arch Dis Child Fetal Neonatal Ed*. 2003;88(5):F405–F409.
46. Ng PC, Leung CW, Chiu WK, Wong SF, Hon EK. SARS in newborns and children. *Biol Neonate*. 2004;85(4):293–298.
47. Assiri A, Abedi GR, Almasry M, Bin Saeed A, Gerber SI, Watson JT. Middle East respiratory syndrome coronavirus infection during pregnancy: A report of 5 cases from Saudi Arabia. *Clin Infect Dis*. 2016:63(7):951-953.
48. Jeong SY, Sung SI, Sung JH, et al. MERS-CoV infection in pregnant woman in Korea. *J Korean Med Sci*. 2017;32(10):1717–1720.
49. Alserehi H, Wali G, Alshukairi A, Alraddadi B. Impact of Middle East respiratory syndrome coronavirus (MERS-CoV) on pregnancy and perinatal outcome. *BMC Infect Dis*. 2016;16:105.
50. Malik A, El Masry KM, Ravi M, Sayed F. Middle East respiratory syndrome coronavirus during pregnancy, Abu Dhabi, United Arab Emirates 2013. *Emerg Infect Dis*. 2016;22: 515–517.
51. Alfaraj SH, Al-Tawfiq JA, Memish ZA. Middle East respiratory syndrome coronavirus (MERS-CoV) infection during pregnancy: Report of two cases & review of literature. *J Microbiol Immunol Infect*. 2019;52(3): 501–503.

52. Payne DC, Iblan I, Alqasrawi S, et al. Stillbirth during infection with Middle East respiratory syndrome coronavirus. *J Infect Dis*. 2014;209(12):1870–1872.
53. LaCourse SM, Kachikis A, Blain M, et al. Low Prevalence of Severe Acute Respiratory Syndrome Coronavirus 2 Among Pregnant and Postpartum Patients With Universal Screening in Seattle, Washington. *Clin Infect Dis*. 2021;72(5):869-872.
54. Vintzileos WS, Muscat J, Hoffmann E, et al. Screening all pregnant women admitted to labor and delivery for the virus responsible for coronavirus disease 2019. *Am J Obstet Gynecol*. 2020;223(2):284-286.
55. Kelly JC, Raghuraman N, Carter EB, Palanisamy A, Stout MJ. Preprocedural asymptomatic coronavirus disease 2019 cases in obstetrical and surgical units. *Am J Obstet Gynecol*. 2021;224(1):114-116.
56. Lokken EM, Taylor GG, Huebner EM, et al. Washington COVID-19 in Pregnancy Collaborative. Higher severe acute respiratory syndrome coronavirus 2 infection rate in pregnant patients. *Am J Obstet Gynecol*. 2021;225(1):75.e71-75.e16.
57. Ellington S, Strid P, Tong VT, et al. Characteristics of Women of Reproductive Age with Laboratory-Confirmed SARS-CoV-2 Infection by Pregnancy Status - United States, January 22-June 7, 2020. *MMWR Morb Mortal Wkly Rep*. 2020;69(25):769-775.
58. Zambrano, LD, Ellington S, Strid P, et al. Update: Characteristics of Symptomatic Women of Reproductive Age with Laboratory-Confirmed SARS-CoV-2 Infection by Pregnancy Status - United States, January 22-October 3, 2020. *MMWR Morb Mortal Wkly Rep*. 2020;69(25):1641-1647.
59. Takemoto MLS, Menezes MO, Andreucci CB, et al. The tragedy of COVID-19 in Brazil: 124 maternal deaths and counting. *Int J Gynaecol Obstet*. 2020;151(1):154-156.
60. Badr DA, Mattern J, Carlin A, et al. Are clinical outcomes worse for pregnant women at >/=20 weeks' gestation infected with coronavirus disease 2019? A multicenter case- control study with propensity score matching. *Am J Obstet Gynecol*. 2020;223(5):764- 768.
61. Knight M, Bunch K, Vousden N, et al. Characteristics and outcomes of pregnant women admitted to hospital with confirmed SARS-CoV-2 infection in UK: national population based cohort study. *BMJ*. 2020;369:m2107.
62. Galang RR, Newton SM, Woodworth KR, et al. Risk factors for illness severity among pregnant women with confirmed SARS-CoV-2 infection - Surveillance for Emerging Threats to Mothers and Babies Network, 22 state, local, and territorial health departments, March 29, 2020 -March 5, 2021. *Clin Infect Dis*. 2021;73(Suppl 1):S17-s23.

63. Wei SQ, Bilodeau-Bertrand M, Liu S, Auger N. The impact of COVID-19 on pregnancy outcomes: a systematic review and meta-analysis. *CMAJ*. 2021;193(16):E540-E548.
64. Roberts DJ, Edlow AG, Romero RJ, et al. A standardized definition of placental infection by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a consensus statement from the National Institutes of Health/Eunice Kennedy Shriver National Institute of Child Health and Human Dvelopment (NIH/NICHD) SARS-CoV-2 placental workshop. *Am J Obstet Gynecol*. 2021;S0002-9378(21)00832-2.
65. Metz TD, Clifton RG, Hughes BL, et al. Eunice Hennedy Shriver National Institute of Child Health and Human Development (NICHD) Maternal-Fetal Medicine Units (MFMU) Network. Disease Severity and Perinatal Outcomes of Pregnant Patients With Coronavirus Disease 2019 (COVID-19). *Obstet Gynecol*. 2021;137(4):571-580.
66. Been JV, Burgos Ochoa L, Bertens LCM, Schoenmakers S, Steegers EAP, Reiss IKM. Impact of COVID-19 mitigation measures on the incidence of preterm birth: a national quasi-experimental study. Lancet Public Health. 2020;5(11):e604-e611.
67. Harvey EM, McNeer E, McDonald MF, et al. Association of Preterm Birth Rate With COVID-19 Statewide Stay-at-Home Orders in Tennessee. JAMA Pediatr. 2021;175(6):635-637.
68. Hedermann G, Hedley PL, Baekvad-Hansen M, et al. Danish premature birth rates during the COVID-19 lockdown. Arch Dis Child Fetal Neonatal Ed. 2021;106(1):93-95.
69. Maeda Y, Nakamura M, Ninomiya H, Ogawa K, Sago H, Miyawaki A. Trends in intensive neonatal care during the COVID-19 outbreak in Japan. Arch Dis Child Fetal Neonatal Ed. 2021;106(3):327-329.
70. Philip RK, Purtill H, Reidy E, et al. Unprecedented reduction in births of very low birthweight (VLBW) and extremely low birthweight (ELBW) infants during the COVID-19 lockdown in Ireland: a 'natural experiment' allowing analysis of data from the prior two decades. BMJ Glob Health. 2020;5(9):e003075.
71. Andersson MI, Arancibia-Carcamo CV, Auckland K, Baillie JK, Barnes E, Beneke T, et al. SARS-CoV-2 RNA detected in blood products from patients with COVID-19 is not associated with infectious virus. *Wellcome Open Res*. 2020;5:181.
72. Chen W, Lan Y, Yuan X, et al. Detectable 2019-nCoV viral RNA in blood is a strong indicator for the further clinical severity. *Emerg Microbes Infect*. 2020;9:469-473.
73. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in Different Types of Clinical Specimens. *JAMA*. 2020;323(18):1843-1844.

74. Zheng S, Fan J, Yu F, et al. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study. *BMJ*. 2020; 369:m1443.
75. Fang Z, Zhang Y, Hang C, Ai J, Li S, Zhang W. Comparisons of viral shedding time of SARS-CoV-2 of different samples in ICU and non-ICU patients. *J Infect*. 2020;81(1):147–178.
76. Zhang W, Du RH, Li B, et al. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. *Emerg Microbes Infect.* 2020;9(1):386–389.
77. Wu J, Liu J, Li S, et al. Detection and analysis of nucleic acid in various biological samples of COVID-19 patients. *Travel Med Infect Dis.* 2020;37:101673.
78. Xu D, Zhou F, Sun W, et al. Relationship Between Serum Severe Acute Respiratory Syndrome Coronavirus 2 Nucleic Acid and Organ Damage in Coronavirus 2019 Patients: A Cohort Study. *Clin Infect Dis*. 2021;73(1):68-75.
79. Patanè L, Morotti D, Giunta MR, et al. Vertical transmission of coronavirus disease 2019: severe acute respiratory syndrome coronavirus 2 RNA on the fetal side of the placenta in pregnancies with coronavirus disease 2019-positive mothers and neonates at birth. *Am J Obstet Gynecol MFM*. 2020;2(3):100145.
80. Algarroba GN, Rekawek P, Vahanian SA, et al. Visualization of severe acute respiratory syndrome coronavirus 2 invading the human placenta using electron microscopy. *Am J Obstet Gynecol*. 2020;223(2):275–278.
81. Wu H, Liao S, Wang Y, et al. Molecular evidence suggesting the persistence of residual SARS-CoV-2 and immune responses in the placentas of pregnant patients recovered from COVID-19. *Cell Prolif*. 2021;54(9):e13091.
82. Gengler C, Dubruc E, Favre G, Greub G, de Leval L, Baud D. SARS-CoV-2 ACE-receptor detection in the placenta throughout pregnancy. *Clin Microbiol Infect*. 2021:27(3):489-490.
83. Faure-Bardon V, Isnard P, Roux N, et al. Protein expression of angiotensin-converting enzyme 2, a SARS-CoV-2 specific receptor, in fetal and placental tissues throughout gestation: new insight for perinatal counselling. *Ultrasound Obstetrics Gynecol*. 2021; 57(2):242-247.
84. Pique-Regi R, Romero R, Tarca AL, Luca F, XuY, Alazizi A, et al. Does the human placenta express the canonical cell entry mediators for SARS-CoV-2? *Elife*. 2020;9:e58716.

85. Garcia-Flores V, Romero R, Xu Y, et al. Maternal-Fetal Immune Responses in Pregnant Women Infected with SARS-CoV-2. *Res Sq*. 2021:rs.3.rs-362886. Doi: 10.21203/rs.3.rs-362886/v1. Preprint.
86. Centers for Diseases Control and Prevention. Evaluation and Management Considerations for Neonates at Risk for COVID-19. Available at https://www.cdc.gov/coronavirus/2019-ncov/hcp/caring-for-newborns.html [accessed on October 5, 2021]
87. Blumberg DA, Underwood MA, Hedriana HL, Lakshminrusimha S. Vertical transmission of SARS-CoV-2 infection in pregnancy: what is the optimal definition? *Am J Perinatol*. 2020;37(08):769-772.
88. Zamaniyan M, Ebadi A, Aghajanpoor S, Rahmani Z, Haghshenas M, Azizi S. Preterm delivery, maternal death, and vertical transmission in a pregnant woman with COVID-19 infection. *Prenat Diagn*. 2020;40(13):1759-1761.
89. Rad HS, Rohl J, Stylianou N, et al. The effects of COVID-19 on the placenta during pregnancy. *Front Immunol*. 2021;12:743022.
90. Vivanti A, Vauloup-Fellous C, Prevot S, et al. Transplacental transmission of SARS-CoV-2 infection. *Nat Commun*. 2020;11(1):3572.
91. Dong L, Tian J, He S, et al. Possible vertical transmission of SARS-CoV-2 from an infected mother to her newborn. *JAMA*. 2020;323(18):1846-1848.
92. Zeng H, Xu C, Fan J, et al. Antibodies in infants born to mothers with COVID-19 pneumonia. *JAMA*. 2020;323(18):1848-1849.
93. 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. *JAMA Pediatr*. 2020;174(7):722-725.
94. Yap M, Debenham L, Kew T, et al. Clinical manifestations, prevalence, risk factors, outcomes, transmission, diagnosis and treatment of COVID-19 in pregnancy and postpartum: a living systematic review protocol. *BMJ Open*. 2020;10(12):e041868-e.
95. Carosso A, Cosma S, Borella F, Marozio L, Coscia A, Ghisetti V, et al. Pre-labor anorectal swab for SARS-CoV-2 in COVID-19 pregnant patients: is it time to think about it? *Eur J Obstet Gynecol Reprod Biol*. 2020;249:98-9.
96. vanDoorn AS, Meijer B, Frampton CMA, Barclay ML, DeBoer NKH. Systematic review with meta-analysis: SARS- CoV-2 stool testing and the potential for faecal-oral transmission. *Aliment Pharmacol Ther*. 2020;52(8):1276-88.

97. Hermesch AC, Horve PF, Edelman A, et al. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Environmental Contamination and Childbirth. *Obstet Gynecol*. 2020;136(4):827-9
98. Kimberlin D, Stagno S. Can SARS-CoV-2 infection be acquired in utero? More definitive evidence is needed. *JAMA*. 2020;323(18):1788-1789.
99. Norman M, Navér L, Soderling J, et al. Association of maternal SARS-CoV-2 infection in pregnancy with neonatal outcomes. *JAMA*. 2021;325(20):2076-2086.
100. Murphy CA, O'Reilly DP, Edebiri O, et al. Haematological parameters and coagulation in umbilical cord blood following COVID-19 infection in pregnancy. *Eur J Obst Gynecol Reprod Biol*. 2021;266:99-105.
101. Biasucci G, Cannalire G, Raymond A, et al. Safe perinatal management of neonates born to SARS-CoV-2 positive mothers at the epicenter of the Italian epidemics. *Front Pediatr*. 2020;8:565522.
102. Bahrami R, Schwarts DA, Karimi-Zarchi M, et al. Meta-analysis of the frequency of intrauterine growth restriction and preterm premature rupture of the membranes in pregnant women with COVID-19. *Turk J Obstet Gynecol*. 2021;18:136-244.
103. Mand N, Iannaccone A, Longardt AC, et al. CRONOS Network. Neonatal outcome following maternal infection with SARS-CoV-2 in Germany: COVID-19- related obstetric and neonatal outcome study (CRONOS). *Arch Dis Child Fetal Neonatal Ed*. 2021;fetalneonatal-2021-322100.
104. Lassi ZS, Ana A, Das JK, et al. A systematic review and meta-analysis of data on pregnant women with confirmed COVID-19: clinical presentation, and pregnancy and perinatal outcomes based on COVID-19 severity. *J Glob Health*. 2021;11:05018.
105. Chen Y, Peng H, Wang L, et al. Infants born to mothers with a new coronavirus (COVID-19). *Front Pediatr*. 2020;8:104.
106. 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.
107. Mostafa BE, Mostafa A, El Fiky LM, Omara A, Teaima A. Maternal COVID-19 and neonatal hearing loss: a multicentric survey. *Eur Arch Otorhinolaryngol*. 2021;1-4. Doi:10.1007/s00405-021-07098.
108. Niewiesk S. Maternal antibodies: clinical significance, mechanism of interference with immune responses, and possible vaccination strategies. *Front Immunol*. 2014;5:446.
109. Goncalves G, Cutts FT, Hills M, Rebelo-Andrade H, Trigo FA, Barros H. Transplacental transfer of measles and total IgG. *Epidemiol Infect*. 1999;122(2):273-279.

110. Heininger U, Riffelmann M, Leineweber B, Wirsing von Koenig CH. Maternally derived antibodies against Bordetella pertussis antigens pertussis toxin and filamentous hemagglutinin in preterm and full term newborns. *Pediatr Infect Dis J*. 2009;28(5):443-445.

111. Munoz FM, Patel SM, Jackson LA, et al. Safety and immunogenicity of three seasonal inactivated influenza vaccines among pregnant women and antibody persistence in their infants. *Vaccine*. 2020;38(33):5355-5363.

112. Atyeo C, Pullen KM, Bordt EA, et al. Compromised SARS-CoV-2- specific placental antibody transfer. *Cell*. 2021;184:628-642.

113. Roltgen K, Powell AE, Wirz OF, et al. Defining the features and duration of antibody responses to SARS-CoV-2 infection associated with disease severity and outcome. *Sci Immunol*. 2020;5(54):eabe0240.

114. Ibarrondo FJ, Fulcher JA, Goodman-Meza J, et al. Rapid decay of anti-SARS-CoV-2 antibodies in person with mild Covid-19. *N Engl J Med*. 2020;383:1085-1087.

115. Gudbjartsson DF, Norddahl GL, Melsted P, et al. Humoral immune response to SARS-CoV-2 in Iceland. *N Engl J Med*. 2020; 383:1724-1734.

116. Cosma S, Carosso AR, Corcione S, et al. Longitudinal analysis of antibody response following SARS-CoV-2 infection in pregnancy: from the first trimester to delivery. *J Reprod Immunol.* 2021;144:103285.

117. Centers for Disease Control and Prevention. Interim Infection Prevention and Control Recommendations for Healthcare Personnel During the Coronavirus Disease 2019 (COVID-19) Pandemic. Available at https://www.cdc.gov/coronavirus/2019-ncov/hcp/disposition-hospitalized-patients.html#definitions. [accessed on October 5, 2021].

118. Chiereghin A, Zagari RM, Galli S, et al. Recent Advances in the evaluation of serological assays for the diagnosis of SARS-CoV-2 infection and COVID-19. *Front Public Health*. 2021;8:620222.

119. Centers for Diseases Control and Prevention. CDC's Diagnostic Test for COVID-19 Only and Supplies. Available at https://www.cdc.gov/coronavirus/2019-ncov/lab/virus-requests.html [accessed on October 5, 2021].

120. Lazarian G, Quinquenel A, Bellal M, et al. Autoimmune haemolytic anaemia associated with COVID-19 infection. *Br J Haematol*. 2020;190(1):29-31.

121. Rosenzweig JD, McThenia SS, Kaicker S. SARS-CoV-2 infection in two pediatric patients with immune cytopenias: a single institution experience during the pandemic. *Pediatr Blood Cancer*. 2020;67(9):e28503.

122. Angileri F, Légaré S, Gammazza AM, Conway de Macario E, Macario AJL, Cappello F. Is molecular mimicry the culprit in the autoimmune haemolytic anaemia affecting patients with COVID-19? *Br J Haematol*. 2020;190(2):e92-93.

123. Centers for Diseases Control and Prevention. COVID Data Tracker. Data on COVID-19 during pregnancy: severity of maternal illness. Available at https://covid.cdc.gov/covid-data-tracker/#pregnant-population. [accessed on October 15, 2021].