A Serological Analysis of SARS-CoV-2 Infection in the Obstetric Population

Sophia Rose

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A Serological Analysis of SARS-CoV-2 Infection in the Obstetric Population

A Thesis Presented

by:

Sophia Rose

To the Keck Science Department

Of Claremont McKenna, Pitzer, and Scripps Colleges

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The degree of Bachelor of Arts

Senior Thesis in Biology

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Abstract

In December 2019, the surfacing and spread of a novel coronavirus, SARS-CoV-2, resulted in the global COVID-19 pandemic. As a viral antigen, SARS-CoV-2 poses a particular threat to the obstetric population due to physiological and immunological changes that women face during pregnancy. While recent studies have found that SARS-CoV-2 may have better clinical outcomes as compared to other betacoronaviruses, adverse pregnancy events such as ICU admission, preeclampsia, and/or preterm birth have been associated with COVID-19. Progress has been made in better understanding the pathophysiology of SARS-CoV-2 in pregnancy, but there is still much to be known about the interaction between the two conditions. As SARS-CoV-2 spreads largely through asymptomatic and pre-symptomatic carriers, serological surveys of the virus help us better understand the actual transmission rates and can also provide important information about the mechanisms of the humoral immune response. These antibody specific studies of SARS-CoV-2 are of particular interest to the obstetric population as inclusion of umbilical cord serum allows researchers to gain insight on vertical transmission, or the maternal-fetal transfer of antibodies across the placenta. As a neonate’s immune defense system is dependent on the immune cells it receives from its mother, the potential for vertical transmission of SARS-CoV-2 antibodies is critical as the pandemic continues. Using data from serological experiments carried out at the University of Massachusetts Chan Medical School and its partnering Memorial Medical Center, this study further investigates antibody levels in the umbilical cord blood across various clinical factors in the mother. Cord blood RBD IgG antibody levels did not significantly vary from antibody levels of nonpregnant individuals, nor were they
impacted by diagnosis of preeclampsia. A strong correlation was found between trimester of maternal SARS-CoV-2 infection and neonatal antibody levels with second trimester infections resulting in higher levels. The results of this study have significant implications for SARS-CoV-2 infection in the obstetric population with the potential to impact best vaccine administration practices.

**Introduction**

As this paper investigates SARS-CoV-2 antibody levels in the obstetric population, the introduction will provide an overview on three main sections: SARS-CoV-2 and the COVID-19 pandemic, COVID-19 and pregnancy, and seroprevalence of COVID-19 in the obstetric population. The first section will discuss the origins of SARS-CoV-2, compare the novel coronavirus to other viruses in the *Coronaviridae* family, and explain viral transmission. The second section will outline the potential threat of COVID-19 to pregnant women and the results of preliminary studies in the field. The final section will explain serological surveys, the benefits they provide during viral outbreaks, and how they can inform studies of maternal-fetal transfer across the placenta. Together, these topics are essential in understanding the serological analyses of umbilical cord blood that were conducted in this thesis project.

*SARS-CoV-2 and the COVID-19 Pandemic*

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) emerged as a novel coronavirus in 2019 and has since spread rapidly across the globe resulting in the
coronavirus disease-19 (COVID-19) pandemic. As of January 12\textsuperscript{th}, 2022, the COVID-19 pandemic has resulted in 312.2 million infections and 5.5 million associated deaths (WHO, 2021d). SARS-CoV-2 stands out from other coronaviruses due to its robust ability to transmit through pre-symptomatic and asymptomatic carriers which allows it to transfer undetected and spread in a seemingly uncontrollable manner. The virus was originally discovered in Wuhan, China in December of 2019 due to an outbreak linked to a seafood and wildlife market. By January 5\textsuperscript{th}, the complete genome of the virus was sequenced, and it was classified as a \textit{betacoronavirus} within the \textit{Coronaviridae} family (Lofti et al., 2020). SARS-CoV-2, unnamed at the time, was officially identified as the causative agent of the outbreak in Wuhan on January 7\textsuperscript{th}. Less than a week later, cases began to appear in countries across Asia and, by January 20\textsuperscript{th}, 2020, the first confirmed case of COVID-19 was reported in the United States (CDC, 2021c).

There are seven other known viruses in the \textit{Coronaviridae} family that are capable of human infection including SARS-CoV-2 (Table 1; Wang et al., 2021). The \textit{Coronaviridae} family is characterized by large, positive-sense, single-stranded RNA viruses that have long spike proteins that typically range from 16–21 nm. In fact, the family consists of the largest known RNA viruses (Payne, 2017). Including SARS-CoV-2, all known coronaviruses that can result in human infection have been identified as zoonotic viruses meaning that they originated in a different animal species and eventually infected humans (Zhang & Holmes, 2020). In the case of known coronaviruses, all studies have pointed to bats as the specific species of origin (Hamady et al., 2021). Among the seven human coronaviruses, four have low pathogenicity (229E, NL63, OC43, and HKU1), referred to as HCoVs, and three are highly pathogenic (SARS-CoV,
MERS-CoV, and SARS-CoV-2; Azkur et al., 2020). Infection of one of the low pathogenic coronaviruses results in mild, cold-like symptoms. These viruses are seasonally-circulated like the common flu and have been classified as endemic in humans (Wang et al., 2021). SARS-CoV, MERS-CoV, and SARS-CoV-2 all result in more severe symptoms and outcomes such as fever, cough, shortness of breath in addition to cold-like symptoms. All three of the more pathogenic coronaviruses belong to the same genus, betacoronavirus, and are known to cause respiratory infections and can lead to death (Wang et al., 2021). Genome-wide studies have also found that SARS-CoV and MERS have ~79.5% and 51% sequence similarity to the protein-coding regions of SARS-CoV-2 respectively (Lofti et al., 2020).

<table>
<thead>
<tr>
<th>Genus</th>
<th>Betacoronavirus</th>
<th>Betacoronavirus</th>
<th>Alphacoronavirus</th>
<th>Betacoronavirus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spike similarity to SARS-CoV-2</td>
<td>77–97.7%</td>
<td>32.79%</td>
<td>229E, NL63, HKU1, OC43</td>
<td>229E, 30% NL63, 29% OC43</td>
</tr>
<tr>
<td>Host cellular receptor</td>
<td>ACE2</td>
<td>DPP-4</td>
<td>ACE2</td>
<td>ACE2</td>
</tr>
<tr>
<td>Reservoir: intermediate host</td>
<td>Respiratory droplet, close contact with infected individual, aerosol, possibly faecal/oral</td>
<td>Respiratory droplet, close contact with infected individual, aerosol, consumption of unpasteurized camel milk</td>
<td>Respiratory droplet, close contact with infected individual, aerosol</td>
<td>Respiratory droplet, close contact with infected individual, aerosol, possibly faecal/oral</td>
</tr>
<tr>
<td>Mode of transmission</td>
<td>Respiratory droplet, close contact with infected individual, aerosol, possibly faecal/oral</td>
<td>Respiratory droplet, close contact with infected individual, aerosol</td>
<td>Respiratory droplet, close contact with infected individual, aerosol</td>
<td>Respiratory droplet, close contact with infected individual, aerosol, possibly faecal/oral</td>
</tr>
<tr>
<td>Emergence</td>
<td>February 2003</td>
<td>June 2012</td>
<td>229E, 2016</td>
<td>December 2019</td>
</tr>
<tr>
<td>Current status*</td>
<td>Contained as of May 2004</td>
<td>Sporadic</td>
<td>Redomic</td>
<td>Pandemic</td>
</tr>
<tr>
<td>Infected cases*</td>
<td>&gt; 8000</td>
<td>&gt; 2500</td>
<td>N/A</td>
<td>&gt; 178 million</td>
</tr>
<tr>
<td>Number of deaths*</td>
<td>&gt; 770</td>
<td>&gt; 80</td>
<td>N/A</td>
<td>&gt; 3.8 million</td>
</tr>
<tr>
<td>Case fatality rate*</td>
<td>~10%</td>
<td>~34%</td>
<td>N/A</td>
<td>~ 2%</td>
</tr>
<tr>
<td>Risk factors for severe disease</td>
<td>Male sex, age ≥ 65 years, comorbidities (heart disease and diabetes mellitus), elevated lactate dehydrogenase and neutrophil count at admission</td>
<td>Male sex, age ≥ 65 years, comorbidities, comorbid infection, low serum albumin (&lt;35 g/L)</td>
<td>Immunosuppression, age &lt; 5 years and ≥ 65 years, respiratory co-infection</td>
<td>Male sex, age ≥ 60 years, non-white ethnicity, comorbidities, dyspnea, hematocrit abnormalities, respiratory rate &gt; 24 breaths/min, Sp02 &lt; 90% at admission</td>
</tr>
<tr>
<td>Clinical manifestations</td>
<td>Fever, headache, muscle aches, malaise, non-productive cough, dyspnea, respiratory failure in 10–20%</td>
<td>Fever, cough, dyspnea, pneumonia, vomiting or diarrhea, fatigue, myalgia, respiratory failure. 21.1% of cases are mild/asymptomatic</td>
<td>Most develop mild-moderate severity illness. Fever, non-productive cough, fatigue, anemia, dyspnea, chest pain, pneumonia, respiratory failure, cough/lopalphy</td>
<td>Most develop mild-moderate severity illness. Fever, non-productive cough, fatigue, anemia, dyspnea, chest pain, pneumonia, respiratory failure, cough/lopalphy</td>
</tr>
</tbody>
</table>

* MERS-CoV cases and deaths correct as of April 2021, and SARS-CoV-2 cases and deaths correct as of 20th June 2021

**Table 1.** A comparison of clinical and non-clinical characteristics of coronaviruses SARS-CoV, MERS, HCoVs, and SARS-CoV-2 (Hamady et al., 2021).
Due to similar symptomatology, their shared genus, and high percentages of genome similarity, many early studies use previous knowledge of SARS-CoV and MERS to guide research on SARS-CoV-2.

SARS-CoV, or Severe Acute Respiratory Syndrome Coronavirus, first emerged as an abnormal case of pneumonia in November 2002 in the Guangdong Province of China. In February 2003, after similar cases also appeared in Vietnam and Hong Kong, SARS-CoV was officially identified as a betacoronavirus (CDC, 2021c). Originating from bat species, SARS-CoV was found to have an intermediary host of Himalayan palm civets which were commonly farmed in Southern China (Payne, 2017). Although spreading to 29 countries across five continents and resulting in 774 deaths globally, SARS-CoV was confined within the year and maintained its status as an epidemic (WHO, 2021b). In January of 2004, the CDC issued the “Notice of Embargo of Civets” which banned the importation of civets of any kind into the United States which is still in effect today (CDC, 2021c).

MERS, or the Middle Eastern Respiratory Syndrome coronavirus, was discovered in 2012 in several Middle Eastern countries. The virus was identified as having an intermediary host of dromedary camels (Hamady et al., 2021). MERS can be transmitted from camel to camel, camel to human, or human to human when in close contact. Since 2012, there have been 2578 confirmed cases of MERS resulting in 888 deaths globally (WHO, 2021a). MERS was not contained as quickly as SARS, but it is slightly less widespread geographically with only 27 countries reporting cases outside of the Eastern Mediterranean Region – which reported cases in 12 countries. Although case counts have generally decreased since peak infection rates in 2014, the virus remains prevalent
globally and particularly in Saudi Arabia where cases remained well over 100 until 2020 (WHO, 2021e). A breakthrough peak in 2019 resulted in 204 cases in Saudi Arabia alone, but since then case numbers have universally declined with only 11 cases in Saudi Arabia and 14 cases globally in 2021 (WHO, 2021e).

Relative to SARS-CoV and MERS, two clear distinctions in SARS-CoV-2 have quickly emerged: lower mortality and higher morbidity rates. SARS-CoV and MERS both have higher mortality rates of approximately 10% and 35% respectively (Henss et al., 2021 and Wang et al., 2021). The mortality rate of SARS-CoV-2 remains at approximately 2% globally although it appears to be lowering with the Omicron variant which recently emerged (Petrosillo et al., 2020). Relatively, the reproductive number ($R_0$) of SARS-CoV-2 is higher than both SARS and MERS, but more so for the latter. $R_0$ values serve as a measure of disease transmissibility or contagiousness as a function of the biological character of a pathogen and human behavior. In short, the reproductive number represents the estimated number of patients likely to become infected from an individual COVID+ patient and can serve as a measure of morbidity. $R_0$ values are particularly important when diseases result in epidemics or pandemics, such as the COVID-19 pandemic, as they are crucial in understanding the necessary precautions needed to stop the spread. For example, government mandated quarantines and the scope of contact tracing are often based on $R_0$ values (Achaiah et al., 2020). Previous studies have found that MERS and SARS have average $R_0$ values of < 1 and 1.7-1.9 respectively (Petrosillo et al., 2020). Comparably, although an exact SARS-CoV-2 $R_0$ value is still controversial and under consideration, a meta-analysis conducted in February 2020 by Liu et al. found a $R_0$ range of 1.5-6.68 across 12 studies with median and mean $R_0$ values
coming out to be 2.79 and 3.28 respectively (Liu et al., 2020a). In a similar study conducted by Park et al. in February 2020, the authors found that 13 out of 20 studies observed yielded a SARS-CoV-2 $R_0$ within a range of 2 and 3 (Park et al., 2020). Therefore, most individuals who have contracted SARS-CoV-2 are expected to transmit the virus to 2 to 3 additional people. Although the variant is relatively new and the $R_0$ of the omicron variant is still under review, the rapid increase of infection rates across the globe suggest that omicron is even more contagious than previous variants.

The increased morbidity and decreased mortality in SARS-CoV-2 relative to SARS-CoV and MERS, in addition to the significant prevalence of pre-symptomatic and asymptomatic carriers, has resulted to the rapid transmission and exponential rise in COVID cases globally. Pre-symptomatic carriers are capable of transmitting the virus prior to experiencing symptoms. The exact definition of pre-symptomatic patients varies slightly across studies which has resulted in slightly varied results. In a study conducted by He et al. in July 2021, the authors defined pre-symptomatic carriers as patients who are asymptomatic at the time of their COVID diagnosis, but then went on to develop symptoms later on. With this definition, He et al. found that the pooled percentage of pre-symptomatic patients from 10 articles published between March and May 2020 was 48.9% with a 95% confidence interval range of 31.6%-66.2% (He et al., 2021).

Meyerowitz et al. conducted a study in June 2021 that used the same definition of pre-symptomatic carriers but found a slightly lower percentage range for pre-symptomatic patients of 25-40% (Meyerowitz et al., 2021). While both He et al. and Meyerowitz et al.’s meta-analyses represented studies from across the globe, He et al.’s study included primarily studies from China and other Asian countries whereas Meyerowitz et al.’s
study primarily represented American studies. The definition of asymptomatic carriers is more universal as it encompasses all patients who are diagnosed with COVID but never experience symptoms throughout the course of their infection. He et al. and Meyerowitz et al. both investigated asymptomatic carriers as well and found pooled asymptomatic proportions of 15.6% and 17%-20% respectively. While asymptomatic spread is possible for SARS-CoV and MERS as well, previous studies have found that it is less prevalent as more than 90% of infected patients experienced symptoms for both SARS-CoV and MERS (Wilder-Smith et al., 2005 and Al-Tawfiq, 2020).

SARS-CoV-2 is primarily transmitted from human-to-human through the respiratory droplets (>5-10 μm in diameter) of an infected individual. Early studies found that the respiratory droplets typically dissipate quickly and traverse up to ~6 feet (Lofti et al., 2020). The virus can also remain intact in aerosolized droplets (<5 μm in diameter) and is capable of remaining in the air for approximately three hours. Airborne inhalation is the most common mode of transmission and is possible from respiratory droplets of all sizes. However, transmission through contact with contaminated surfaces is also possible and takes place when the larger respiratory droplets, which are capable of settling before evaporation, land on surfaces within 6 feet of an infected individual (Prather et al., 2020). Droplets are most efficiently spread through coughing or sneezing, but can also be spread simply from speaking or breathing. Initial infection typically occurs when the contaminated droplets make contact with the mucous membranes of a healthy individual’s eyes, nose, or mouth (Lofti et al., 2020). For this reason, social distancing precautions and mask mandates are implemented as some of the main modes of defense against SARS-CoV-2 transmission.
COVID-19 and Pregnancy

Novel viral antigens such as SARS-CoV-2 are of particular concern in the obstetric population due to the physiological changes that women face throughout their pregnancy that can contribute to a shift in their immune systems away from a pro-inflammatory response. This increased risk of morbidity along with the potential of negative fetal impact in pregnant women places the obstetric population on the list of vulnerable populations as the pandemic surges on. Previous studies specifically on respiratory viral infections (SARS-CoV, MERS, and the influenza viruses) have found that infected pregnant women are more likely to develop critical disease and have perinatal complications (Chen, G et al., 2021). Increased mortality rates are often observed in pregnant patients infected with SARS-CoV and MERS as well (Fathi et al., 2020). The study of SARS-CoV-2 infection in the obstetric population has been prioritized to better understand the impact of the virus during pregnancy and to determine whether the virus causes similar outcomes in pregnant women to those observed in SARS and MERS. Just over two years after the virus was identified, a variety of studies on the effect of COVID-19 on pregnancy have been published. However, there is still uncertainty in the pathophysiology of SARS-CoV-2 in pregnancy with a variety of findings, many varying, and challenges associated with an evolving virus.

Through an early systematic review of the Center for Disease and Control’s COVID-19 database from January 2020 – June 2020, Ellington et al. (2020) investigated the discrepancies between pregnant and non-pregnant women of reproductive age (15 – 44 years of age). The authors found that of the cases where symptom status was
reported, 97.1% of pregnant women and 96.9% of nonpregnant women were symptomatic. These statistics remained consistent across most symptoms: cough and shortness of breath whereas headache, muscle aches, fever, chills, and diarrhea were reported with minimal difference across pregnant and nonpregnant women (Ellington et al., 2020). One of the most substantial findings of the study was the large difference in hospitalization rates between the two populations. Of all reported COVID-19 cases in the obstetric population, 31.5% resulted in a hospitalization compared to only 5.8% reported within nonpregnant patients. Ellington et al. also found that, in addition to pregnant patients being 5.4 times more likely to be hospitalized, they were also 1.5 times more likely to be admitted to the ICU, and 1.7 times more likely to require mechanical ventilation. However, the authors suggested that a possible explanation for the higher rates of hospitalization in the obstetric population beyond pregnancy status could be the implementation of universal screening that has become commonplace in labor and delivery units. Finally, the authors found that there was no significant difference in mortality rates among the two populations (Ellington et al., 2020).

Of particular interest in the study of SARS-CoV-2 in pregnancy is the possibility of “vertical transmission” – the spread of a pathogen, in this case a SARS-CoV-2 infection, from the mother to the fetus or neonate. Within this definition vertical transmission can take place during the antepartum, intrapartum, or even the postpartum period if the neonate comes in contact with bodily fluid through the placenta in utero or through breast milk while breastfeeding (Petrosillo et al., 2020). Vertical transmission is frequent among several common pathogens that have been denoted as “TORCH” pathogens, those that are currently monitored in the hospital course of all pregnancies.
TORCH pathogens include *Toxoplasma gondii*, *Other* (*Listeria monocytogenes*, *Treponema pallidium*, parvovirus, HIV, varicella zoster virus), *Rubella*, *Cytomegalovirus*, and *Herpesviruses* type-1 and type-2. More recently, syphilis and the *Zika* virus are also commonly included in this category of infections (Arora et al., 2017). As antenatal fetal infections of these TORCH viruses are major contributors to morbidity and mortality measures globally, monitoring for the viruses in both the mother and fetus is crucial (Arora et al., 2017). While there is currently no guaranteed safeguard against fetal infection, further precautions can be taken during the pregnancy course and delivery of infected mothers to avoid vertical transmission.

Prior to the publication of studies on vertical transmission of SARS-CoV-2 specifically, analyses of maternal-fetal transmission were revisited and newly conducted for both SARS-CoV and MERS. Literature suggests that there was minimal to no evidence of vertical transmission present in cases of mothers infected with SARS-CoV or MERS (Di Mascio et al., 2020). These findings are promising as they suggest there may be a lack of substantial vertical transmission with the infection of *betacoronaviruses*. Luckily, the implications of previous literature were supported by the majority of initial studies on maternal-fetal transmission of SARS-CoV-2 (Karimi-Zarchi et al., 2020, Schwartz, 2020, and Cribiù et al., 2021). These findings suggest that the placenta likely serves as a functional barrier between maternal and fetal infection. However, while most studies show no evidence of vertical transmission whatsoever, some studies have observed maternal-fetal transmission at low rates. In an early 2020 study conducted by Petrosillo et al., the authors found that 13 out of 1287 neonates observed across 60 studies tested
positive for COVID following delivery (~1%; Petrosillo et al., 2020). Therefore, while vertical transmission of SARS-CoV-2 has been found to be highly unlikely, it is possible, and it is important to continue to monitor the infection status of mothers and neonates for the duration of the pandemic.

Preliminary findings of COVID-19 symptomatology in women have also led to further research on SARS-CoV-2 infections in pregnant women relative to hypertensive disorders such as preeclampsia. Preeclampsia is a hyperinflammatory condition that takes place during pregnancy and is characterized by high blood pressure due to an overproduction of proinflammatory cytokines (Illi et al., 2021). Preeclampsia is clinically defined as “the new onset of hypertension in pregnancy after 20 weeks’ gestation with proteinuria in a previously normotensive woman” and it affects 0.6-1.2% of pregnancies in the US (Society for Maternal-Fetal Medicine, 2013). Preeclampsia refers to blood pressure that rises above 140/90 and proteinuria is diagnosed when 0.3 grams or more of protein is detected in urine in a 24-hour collection time. Preeclampsia can also be defined by elevated blood pressure with evidence of end-organ damage in the absence of proteinuria and can become severe when the hypertensive metrics rise or other significant clinical factors are met (Figure 1; Wagner, 2004).

Figure 1. Diagnostic criteria for preeclampsia (Wagner, 2004).
2004). Early research on both preeclampsia and COVID-19 suggests that many of the same pro-inflammatory cytokines that contribute to preeclampsia have been found to be responsible for severe COVID-19-induced cytokine storms. Cytokine storms take place when the body releases an abundance of cytokines in response to a pathogen and they typically result in damage to organs or tissue death (Illi et al., 2021). Through further investigation, Illi et al. identified that the renin angiotensin system (RAS), a hormone-based system that aids in blood pressure control, seemed to be a key link between SARS-CoV-2 infection and preeclampsia as the presence of either results in uncommon levels of expression of many central RAS components and hyperinflammation (Figure 2). The authors also identified significant endothelial damage as an additional commonality (Illi et al., 2021). These similarities observed at the cellular level prompted researchers to further explore the relationship between SARS-CoV-2 and preeclampsia and their clinical outcomes.

As complications such as premature birth, intrauterine growth restrictions, or immune system defects are characteristic of preeclampsia, it is important to investigate whether similar complications are common with SARS-CoV-2 infection as well (Dang et al., 2020). In a June 2020 case study, Hosier et al. investigated the symptomatology of a COVID-19 positive patient whose pregnancy was terminated due to severe preeclampsia that was resulting in critical health conditions. The findings of the study suggested that the patient’s SARS-CoV-2 infection progressed inflammation in her placenta. Hosier et al. claimed that it is very possible that the COVID-related hyperinflammation is what prompted the early onset of preeclampsia. These results were supported by a study conducted by Beys-da-Silva et al. in March 2021 that concluded that the manifestation of
SARS-CoV-2 in pregnant patients induces a “preeclampsia-like syndrome” (Beys-da-Silva et al., 2021). There are several key implications of these findings: rates of preeclampsia diagnosis may be higher in pregnant women with SARS-CoV-2 infections and devastating preeclampsia-like complications are a possible result of SARS-CoV-2 and pregnancy.

While these implications may be disheartening, Ghi et al. conducted a study in 2020 that suggests a more hopeful outcome. Through additional investigation of the specific physiological and immunological changes that take place during pregnancy, the authors suggest that changes in expression of specific T-cells might actually allow for enhanced protection against the cytokine storms commonly associated with SARS-CoV-2 infections (Ghi et al., 2020). The authors describe that the obstetric population is typically more susceptible to viral antigens because pregnant women experience a shift from T helper type 1 (Th1) cells to (T helper type 2) Th2 cells dominated immunity as their Th1 levels are suppressed during pregnancy. Th1 cells are typically the first line of defense against intracellular pathogens, so the suppression of these cells

![Figure 2. Comparison of activated RAS pathway leading to endothelial dysfunction (ED) during SARS-CoV-2 infection (left) and Preeclampsia (right; Illi et al., 2021).]
largely drives the vulnerability associated with pregnancy. The implications of such an immunological shift are grim, so Ghi. et al were surprised when they found no indication of higher morbidity or mortality rates among pregnant versus nonpregnant individuals (Ghi et al., 2020). In fact, the authors found that COVID-induced pneumonia resulting in tissue damage was less common in pregnant than nonpregnant patients. With this in mind, Ghi et al. suggest that pregnant women are more likely to avoid severe cytokine storms due to their immunological shift from Th1 cells, which stimulate proinflammatory cytokines, to Th2 cells, which largely produce cytokines with an anti-inflammatory response (Figure 3; Ghi et al., 2020).

Overall, the implications of the previously discussed studies suggest the need for a more comprehensive understanding of the virus's manifestation in this vulnerable population. Studies that seek to further investigate the effects of SARS-CoV-2 infection on pregnancy are crucial in identifying the best ways to optimize treatment and care for the obstetric population while limiting the adverse effects of the COVID-19 pandemic.

Figure 3. The protective effect of pregnancy on the natural history of Covid-19 infection (Ghi et al., 2020).
Seroprevalence of COVID-19 in the Obstetric Population

This study specifically investigates SARS-CoV-2 infections within the obstetric population through seroprevalence surveys. Seroprevalence surveys, or serosurveys, are used to study the humoral immune response to viruses from an antibody perspective and are particularly useful for understanding viruses with asymptomatic spread such as SARS-CoV-2. Serosurveys allow researchers to analyze the true transmission state of a virus and track its prevalence over time (Murhekar & Clapham, 2021). In a recent interview with Dr. Soumya Swaminathan, WHO’s Chief Scientist, Dr. Swaminathan revealed that seroprevalence studies revealed that 5-10x more people have been exposed to SARS-CoV-2 than has been indicated by PCR tests. This number increases as high as 40x in some regions (Swaminathan, 2021). However, although these numbers may heighten the hopes of those suggesting the possibility of herd immunity, Dr. Swaminathan also explained that serosurvey data also suggests that only about 5-10% of the population in most regions have antibodies present within their systems – even in locations that have had the largest outbreaks. Therefore, the vast majority of the globe is still susceptible to the virus if they are exposed, and herd immunity remains far from an effective solution at the current state of the pandemic (Swaminathan, 2021).
Serological surveys are conducted by extracting antibodies from blood specimens. Functional and neutralizing antibodies that are extracted from serum are called immunoglobulins and several different classes are typically observed in serosurveys: IgA, IgG, and IgM (Figure 4). Making up 75-85% of all antibodies in the body, IgGs are the most common antibodies and are crucial in fighting viral and bacterial infections. IgA and IgM antibodies make up 10-15% and 5-10% of antibodies in the body respectively. IgM antibodies present themselves and peak prior to IgA and IgG as they are the first response to infection (Healthwise Staff, 2020). Additionally, various antibody targets can...
be monitored within the immunoglobulin types. In the analyses of SARS-CoV-2, spike protein (S), receptor binding domain (RBD), and nucleocapsid protein (N) targets are the most commonly analyzed (Ortega et al., 2021).

Although IgA, IgG, and IgM have all been identified in COVID serosurveys, only IgGs were analyzed in this study as the neonatal Fc receptor (FcRn) in the placenta specifically selects only IgGs for maternal-fetal transfer and therefore they are the most abundant antibodies present in the collected umbilical cord blood (Jennewein et al., 2019). Additionally, to narrow the scope of the study, only RBD antibodies were analyzed as they are less likely to have cross-reactivity with the commonly circulated HCoVs. Using RBD as an antigen is particularly useful and important for SARS-CoV-2 because it isolates neutralizing antibodies that have been identified as essential to COVID therapeutics (Min & Sun, 2021). In fact, there is currently an RBD-based COVID vaccine.

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Figure 5. Graphical representation of the longevity and magnitude of the nAb antibody response to coronaviruses according to disease severity: (a) antibody response to SARS-CoV, MERS, and HCoVs (b) SARS-CoV-2 antibody response (Hamady et al., 2021).
in phase three clinical trials in China that engages those RBD neutralizing antibodies to protect against the virus (Yang et al., 2020).

Looking back to SARS-CoV and MERS, serosurveys suggest that antibody titres to SARS-CoV-2 will likely not maintain high levels as both betacoronaviruses had undetectably low titres within two years following infection (Figure 5; Hamady et al., 2021). In a March 2021 study, Hamady et al. expanded on the previous findings of the antibody course in patients infected with SARS-CoV and MERS and compared it to recent SARS-CoV-2 antibody data. The authors reported that IgG antibodies reached their peak titres at ~2-4 months post symptom onset (PSO) for SARS-CoV and ~3 weeks PSO for MERS. Comparably, the authors found that IgGs for SARS-CoV-2 peaked at ~2-3 PSO, plateau, and began to decline within 2-3 months PSO (Hamady et al., 2021). Through mathematical modeling, Hamady et al. estimated that IgG levels one-year PSO reduce to 31% of their levels at 2 weeks PSO. In a similar study published in August 2021, Ortega et al. found that SARS-CoV-2 IgG antibody levels antibody levels peaked ~50 days PSO and remained steady for up to ~230 days PSO whereas IgA and IgM antibodies peaked within the first month post symptom onset and steadily declined (Ortega et al., 2021). Although they were unable to find a concrete explanation, the authors also observed an uptick in IgG antibody levels ~150 days PSO with a constant, slight increase in levels up to 230 days PSO (Ortega et al., 2021). Both Ortega et al. and Hamady et al. additionally observed a correlation between the longevity of IgG antibody responses with increased COVID severity. Hamady et al. found that the majority of study participants who had severe SARS-CoV-2 infections experienced detectable IgG levels.
one-year PSO whereas 67% of participants with mild symptoms had IgG levels that were undetectable (Hamady et al., 2021).

Serological surveys can also be used to evaluate how viruses manifest within particular populations relative to others. In the case of this study, antibodies were extracted from the umbilical cord blood of a group of pregnant study participants directly following the delivery of their babies. Isolating cord blood antibodies allows for an analysis of the IgGs that have been transferred from the mother to the baby through vertical transmission as umbilical cord blood is from the neonate rather than the mother. From this perspective, the various factors that influence a mother’s pregnancy and COVID course can be used to inform analyses of maternal-fetal transfer and the antibody abundance of the neonate. To date, there is still a lack of robust knowledge of SARS-CoV-2 antibody transfer across the placenta due to the relative newness of the virus. However, significant progress has been made in the last decade in understanding the mechanisms that drive the trans-placental transfer and selection of antibodies across the placenta from the mother to the fetus through the study of other viral antigens. The findings of these studies have significant implications for how to best navigate SARS-CoV-2 infections and vaccinations in the obstetric population.

Dr. Galit Alter, Professor of Immunology and Virology at Harvard Medical School and group leader at the Ragon Institute of Mass General Hospital, MIT and Harvard, and her team have reshaped the scope of this field through technology they have developed in systems serology. Dr. Alter’s first major contribution to the understanding of the mechanisms behind maternal-fetal antibody transfer involved the selective transfer of IgGs and the general humoral immune response in women with HIV infections. Along
with the fascinating implications of the results from this study, Dr. Alter and her team were able to create comprehensive antibody profiling tools using machine learning technology and systems serology that allowed them to adapt their programs very quickly to SARS-CoV-2 in 2020.

In a February 2021 presentation as part of the Howard Hughes Medical Institute’s speaker series, Dr. Alter explained more about what she and her team have recently discovered about placental antibody transfer generally using their modern technology as well as early findings from their analysis of the humoral immune response to SARS-CoV-2 and the subsequent vertical transmission. She described that the taking off point for her team was largely the fact that the dogma for placenta-driven antibody transfer was still wildly incomplete as modern studies continue to find conflicting results that never seem to be comprehensive (Alter, 2021). Using her antibody profiling technology, Dr. Alter was able to explore the mechanisms behind placenta-driven antibody selection at a depth that was previously unimaginable.
The first substantial piece of literature declaring a dogma for vertical transmission was published in the 1960s. The dogma stated that the transfer across the placenta is highly selective for IgG antibodies, that there is a hierarchy of the IgG subclasses that are selected for transfer (IgG1 = IgG3 > IgG4 > IgG2), and that maternal and fetal antibody levels are directly correlated (Figure 6; Kohler & Farr, 1966). This dogma was upheld for ~30 years until a paradigm shift occurred through the findings of various studies in the 1990s that identified the neonatal FcRn, a Fc receptor that is highly expressed on the syncytiotrophoblasts in the placenta that appeared to play a specific role of transferring IgG antibodies from the mother to the fetus (Figure 7). Thus, it must be assumed that all IgGs will be equally represented in the cord blood as the FcRn binds to all IgGs indiscriminately (Story et al., 1994 and Dickinson et al., 1999). Although the role of the FcRn in trans-placental transfer of IgGs has been verified, its role as the primary receptor selecting for transfer across the placenta has been put into question as more recent studies.
have found that certain IgG1s are transferred preferentially to the fetus whereas others are transferred poorly (Fu et al., 2016). Therefore, Dr. Alter and her team sought to fill in this gap between the role of the FcRn in IgG transfer and the observed differential transfer of IgG1s across the placenta.

Dr. Alter and her colleagues began this investigation by looking at the function of antibodies that were transferred across the placenta following the administration of the pertussis, or whooping cough, vaccination (DTaP) that the majority of women receive during their pregnancy. As the DTaP is composed of four antigens, Dr. Alter was able to use those antigens to capture pertussis specific antibodies. The analyses of this experiment yielded significant results that suggest that the placenta was selectively filtering antigen specific IgGs based on their function as it was inducing natural killer (NK) cell activation at distinctively high levels (Jennewein et al., 2019). Dr. Alter and her team were also able to identify a very significant trend in the preferential transfer of glycosylated antibodies and suppression of agalactosylated – that is, antibodies have galactose sugar structures on their backend versus antibodies that do not have galactose backends respectively. To affirm these results, they performed a monoclonal antibody experiment including antibodies that had either very short (G0F PG128) or long (G2F PGT128) galactose structures. Monoclonal antibodies are laboratory-engineered molecules that serve a similar neutralizing function as the antibody it is emulating. The findings of this experiment showed that there was increased receptor binding to G2F PGT128 in the neonatal receptor FcRN as well as FcgRIII, a receptor that largely regulates NK cell activation (Figure 8A; Jennewein et al., 2019). Ultimately, the results of these Fc receptor analyses allowed Dr. Alter and her colleagues to claim that antibody
glycosylation plays a distinct role in the IgGs that are selected by the placenta and provide robust NK cell protection for the fetus (Figure 8B).

**Figure 8.** A. Galactosylation Selectively Enhances FcRn, FCGR3 Binding, and NK Cell Activation. A monoclonal antibody was glycoengineered to either be fully agalactosylated (G0F) or fully digalactosylated (G2F). The bar graphs show the binding levels of the glycovariant monoclonal antibody to distinct human Fc-receptors by ELISA. Data are presented as area under the curve (AUC) of four 5-fold dilutions or AUC of five 2-fold dilutions (FcRn). Error bars show the SD. B. FcR Expression in the Placenta: The model depicts the potentially involvement of FcRn and FCGR3A collaboration with the trophoblasts, on selective antibody capture and subclass and Fc-glycan sieving, aimed at selectively transferring NK cell-activating antibodies to newborns (Jennewein et al., 2019).
The COVID-19 pandemic introduces an additional important layer to understanding trans-placental antibody transfer as viral infections often change the kind of antibodies that are produced. Previous studies of malaria and HIV have also shown that the quality of antibody transfer is reduced during active infection which results in the transfer of fewer antibodies (Alter, 2021). With Dr. Alter as the experiment leader, Ateyo et al. investigated SARS-CoV-2 antibody transfer through an investigation of 56 maternal-fetal pairs (34 pregnant healthy controls and 22 pregnant patients who were COVID+ in the third trimester). The authors found that transfer of SARS-CoV-2 antigens (RBD, S, and N in the COVID+ patients to the fetuses was neutral if not decreased – the opposite of what would be expected in healthy individuals. This was particularly notable as the COVID+ patients were still transferring increased levels of antibodies to flu-specific antigens, so there was something specifically affecting the transfer of SARS-CoV-2 IgGs (Ateyo et al, 2021).

**Figure 9.** The effect of time on SARS-CoV-2-specific antibody transfer: The dot plots show the relative IgG titer for RBD, S, N and HA in maternal plasma and cord blood in which the mother was infected during the second or third trimester. Lines connect mother:cord dyads. The data is represented as the absorbance at 450 nm subtracted by the reference absorbance read at 570 nm. The data was background corrected using plasma that was negative for SARS-CoV-2 antibodies (Ateyo et al., 2021).
To investigate whether this decrease in COVID antibodies was consistent across mothers with second trimester SARS-CoV-2 infections as well, the authors introduced additional cohorts of second (n = 29) and third (n = 28) trimester COVID+ patients. Ateyo et al. found that the drastic changes in SARS-CoV-2 antibody transfer was not present in second trimester infections and higher antibody levels were noted (Figure 9). Therefore, the authors suggest that the shift in antibody profiles and subsequent transfer observed in the third trimester is influenced by COVID-induced inflammation. These changes are not present in second trimester infections because the inflammation associated with active infection has resolved, so the placenta is able to revert back to healthy function for the entirety of the third trimester when vertical transmission is most active (Ateyo et al., 2021). These results are significant as they indicate, contrary to most other viral infections, that SARS-COV-2 vaccination during the second trimester will likely lead to the most effective outcomes.

Further investigation of the mechanisms of maternal-fetal transfer for mothers with third trimester infection suggested that glycosylation would play a significant role in SARS-CoV-2 antibody response as well. Ateyo et al. found that the presence of fucose sugars on the glycosylated tails was a large determinant of which antibodies were transferred to the baby – antibodies that lacked fucose were selectively transferred and antibodies with fucose present were retained (Ateyo et al, 2021). Dr. Alter suggests that this change in selection is likely because afucosylated antibodies are better at binding to Fc receptors including the neonatal receptor. This finding suggests even greater implications as fucose plays a key role in NK cell activation through the same FcgRII receptor (Alter, 2021). In summary, Ateyo et al. explain, infection of SARS-CoV-2 in the
third trimester causes the antibodies that are produced to have different glycan profiles. This, in turn, results in the augmentation of specific Fc receptors associated with NK cell activation such as FcgRIII in the placenta so that, while there are less antibodies present in the cord overall, the antibodies that the fetus receives have the highest capacity for protection through a robust recruitment of NK cell activity.

Research Plan

Using antibody data extracted from specimens collected at the UMass Memorial Medical Center and UMass Chan Medical School, this thesis will conduct further analyses based on the early studies of SARS-CoV-2 in the obstetric population discussed above. After cord blood samples are compared to positive and negative controls, the interaction between preeclampsia and SARS-CoV-2 will be explored from an antibody perspective. Next, an investigation of seropositivity and PCR status led to analyses of antibody levels and serostatus relative to trimester of SARS-CoV-2 infection.
Methods

Study Populations

Three populations were included in this study: 144 pregnant women from the UMass Memorial Medical Center (UMMC) labor and delivery unit, 49 COVID+ hospitalized non-pregnant women from the UMass Chan Medical School (UMCMS) hospital, and 21 healthy female controls whose samples were collected prior to the pandemic.

Obstetric Population: As part of the CARES Study, the pregnant population observed in this experiment were collected at UMMC in the labor and delivery unit from April 2020 to August 2021. There were over 200 patients approached for the study and a total of 144 patients consented to participate in the study. Among the 144 patients enrolled, antibody data was available for 84 patients. The sample size of this dataset and within specific analyses was limited due to missing information across the various metrics examined (Figure 10). As such, racial and ethnic demographic information was only available for 80 of the 84 patients, but the distribution was as follows: 47.5% White/Caucasian, 32.5% Hispanic/Latina, 13.75% Black/African American, and 6.25% Asian/Asian American (Figure 11). These patients ranged from 21 years of age to 44 years of age. The labor and delivery unit at UMMC, is the main unit in the area that has the capacity to manage high-risk pregnancies. Therefore, the proportion of the patients with high-risk conditions such as preeclampsia at UMMC is greater than is representative of the obstetric population as a whole (0.6-1.2%; Society for Maternal-Fetal Medicine, 2013). Of the 84 CARES patients included in the study, 28 of them, or ~33%, were diagnosed with preeclampsia. Finally,
universal screening was implemented in the UMMC labor and delivery unit for the duration of the CARES study.

**Figure 10.** CARES Study flow chart including the sample sizes of all conducted analyses.

*Nonpregnant Population:* Originally collected as part of the Consolidated COVID-19 Clinical and Observational Pathogenesis and Epidemiology (COVID-COPE) Study, the non-pregnant population consists of hospitalized COVID-19 patients and serves as a positive control relative to the pregnant population in this study. Blood samples from 49 symptomatic women were collected between April 2020 - June 2020 at UMMC. The demographics of the group was less diverse than the pregnant group: 71% White/Caucasian, 14.3% Hispanic/Latino, 10.2% Black/African American, and 8.16% Asian/Asian American (Figure 11). As available specimens for women of reproductive
age were limited within this population, specimens from women of all ages were analyzed which resulted in an age range of 19-97 years old. The median age of the non-pregnant group was 75 years old.

Pre-Pandemic Population: The pre-pandemic population included 21 healthy women whose preserved samples were primarily collected at UMMC in 2016, but the collection dates ranged from July 2007-August 2019. This population was the least racially and ethnically diverse group with 88.8% of the population identifying as White/Caucasian, 7.4% of the population being Black/African American, and the last 3.7% being Asian/Asian American (Figure 11). All of the women included were 18+ years of age with the oldest age reported as 66 years.
Experimental Timeline

As SARS-CoV-2 is constantly evolving and two new variants, as well several vaccines, have emerged since the beginning of the CARES study, it is important to situate our study population within the timeline of variants and development of the vaccine. The majority of the data was collected prior to the establishment of the delta variant in the United States. As the delta variant was confirmed to be present in the United States in May 2021, it is likely that the Delta variant is only possibly represented in the last two months of the fifteen-month study. Similarly, SARS-CoV-2 vaccines were not made available to the public beyond healthcare workers and the elderly until early-mid 2021 across the United States. Therefore, none of the nonpregnant COVID patients whose samples were collected between April – June 2020 were vaccinated. With the additional uncertainty of the vaccine in pregnant women as the obstetric population was excluded from experimental trials, only four of the CARES women with antibody data available had received a vaccine prior to delivery even though samples were collected through August 2021. Finally, there were actively used treatment options for SARS-CoV-2 at the time of the study, but none of the participants received any COVID-specific treatments beyond medications for symptom management.

Laboratory Analyses

SARS-CoV-2 Enzyme-Linked ImmunoSorbent Assay (ELISA): The SARS-CoV-2 ELISA assay developed at the Ragon Institute as previously published (PMID: 32780998) was implemented in the Moormann lab at UMCMS. Receptor-binding domain
(RBD), Spike trimer subunits (S), and nucleocapsid protein (N) SARS-CoV-2 antigens utilized in the study were provided by MassBiologics in Mattapan, MA (PMID:32826914). SARS-CoV2 RBD antigens provided by the Ragon Institute in Cambridge, MA were also used. Briefly, 384-well MaxiSorp plates (Thermo-Fisher, Waltham, MA) were coated for 30 minutes at room temperature with 50 µL of antigen diluted in coating buffer at the final concentration of 1 µg/mL for N; 0.5 µg/mL for RBD; 2.5 µg/mL for S; or human plasma from a healthy donor at 1:20,000 dilution as HRP control. Plates were washed three times with wash buffer using the AQUAMax™ 2000 plate washer (Molecular Devices, San Jose, CA) and then blocked for 30 minutes at room temperature in 100 µL of blocking buffer. Plates were washed three times and 50 µL of samples diluted in 140 mM of NaCl, 50 mM of Tris-HCl, 0.05% of Tween and 1% BSA, were added (dilution of 1:100), as well as plate internal controls composed of High, Medium, Low (based on RBD-specific IgG) and Pre-pandemic pool in triplicate. Plates were sealed and incubated at 37°C for 30 minutes, then washed five times. Next, 50 µL of diluted HRP conjugated anti-human antibody was added to each well: 1:25000 for anti-IgG and anti-IgM; 1:10000 for anti-IgA (HRP-anti human antibodies from Bethyl Laboratories, Montgomery, Texas). The plate was incubated in the dark at room temperature for 30 minutes and then washed five times. Next, 40 µL of 1-Step Ultra TMB peroxidase substrate (Thermo-Fisher, Waltham, MA) was added to each well and developed at room temperature (3 minutes for IgG; 5 minutes for IgA and IgM). The reaction was stopped with 40 µL of 1M Sulfuric Acid (EMD Millipore, Burlington, MA). Absorbance was measured using an “End-Point” protocol at 450 nm and 570 nm on the SpectraMax iD5 ELISA plate reader (Molecular Devices, San Jose, CA) using the SoftMax Pro software version 7.1 (Molecular Devices, San Jose, CA). The 570 nm OD was subtracted from the 450 nm OD for the final OD value.
**Standard Curves:** CR3022 (PMID: 32245784) dilution curves were used to assess RBD and S proteins. The antibody (IgG, IgA or IgM) was diluted to a concentration of 2.5 μg/ml in the dilution buffer and a 12 two-fold serial dilution curve was generated in triplicate and plated. A pool of plasma from SARS-CoV-2 hospitalized patients (n=15) was used as a standard curve for the N protein with a 12 two-fold serial dilution. Finally, the 4-parameter logistic standard curve generated by the SoftMax Pro software was used.

**Statistical Analyses**

RBD IgG OD data was observed across various clinical factors and served as the primary dependent variable in the analyses of this experiment. To account for the limited sample sizes in each of the independent groups, only univariate analyses were used in this project. Beyond one Chi-squared test of independence, all of the parametric tests used in this experiment (ANOVAs and t-tests) have the following assumptions: random sampling, normally distributed data, and equal variances across factors. Random sampling was assumed for all of the included data and normality and homoscedasticity were tested using Shapiro-Wilk tests and Levene’s tests respectively. The Shapiro-Wilk tests found that none of the raw antibody data was normally distributed with substantial right skew present. Therefore, a common logarithm transformation was applied to all of the antibody data upon which several of the study groups properly fit a normal distribution.

**Analysis 1 – Nonpregnant and Pre-Pandemic Comparison (Figure 12):** The first analysis investigated RBD IgG levels across the three study populations. A one-way ANOVA was
determined to be the ideal parametric test because the independent variable, patient population status, was categorical and included three, non-repeated factors and the objective was to compare the central tendency value of the dependent variable (RBD IgG levels) for each group. Although the log transformation decreased the extent of the right skew, the RBD IgG data for all three groups still did not fit a normal distribution. Therefore, a Kruskal-Wallis (K-W) test, the non-parametric equivalent of an ANOVA was performed. The ANOVA was followed up with pairwise Wilcoxon analyses with an applied Bonferroni correction to adjust for alpha inflation were conducted.

Analysis 2 – CARES Preeclampsia and COVID Comparison (Figure 13): The second analysis sought to determine if preeclampsia status would influence antibody levels transferred to the fetus. Similar to the first analysis, a one-way ANOVA was chosen because the independent variable, patient preeclampsia and COVID status in this case, was categorical and included four, non-repeated factors. The goal of this analysis was also to determine if there was a difference in the central tendency value of the antibody levels for each group. Regardless of the log transformation, the IgG data was not normally distributed, so another K-W test was conducted. Bonferroni corrected Wilcoxon pairwise tests were then performed.

Analysis 3 – COVID PCR and Serostatus Status (Figure 14): To further investigate the relationship between COVID PCR status and serostatus, this analysis used IgG antibody levels in CARES patients to investigate rates of seropositivity relative to patient PCR status. The independent variable in this analysis was COVID PCR status – a categorical
variable with two, non-repeated factors. Therefore, a Wilcoxon test, the non-parametric equivalent of a t-test, was performed because the PCR status data did not meet the assumption of a normal distribution.

**Analysis 4 – COVID Serostatus and PCR Status by Trimester (Figure 15):** Given the results of the previous analysis, the fourth analysis investigated the subgroup of CARES patients who were PCR positive according to their seropositivity and trimester of infection. First trimester infections were excluded from this analysis due to the low sample size available (n = 3) and relatively low maternal-fetal antibody transfer in the first trimester found in previous studies (Arora et al., 2017 and Ateyo et al., 2021). As both serostatus and trimester of infection were categorical variables, a Chi-squared test of independence was performed to determine difference between the groups. All of the frequency values included were greater than 5, so the Yates correction was removed prior to running the test.

**Analysis 5 – Trimesters of SARS-CoV-2 Infection (Figure 16):** The last analysis further investigated the influence of trimesters of SARS-CoV-2 infection on antibody levels in the cord and sought to directly compare mean IgG levels between the second and third trimesters. Application of the log transformation was successful in creating normally distributed dataset and a Levene’s test confirmed that the data was homoscedastic. As the first trimester data was excluded, there were only two independent variables, and a t-test was performed.
Supplemental Analyses: RBD IgG OD levels were also evaluated across the demographics of race/ethnicity, and age. Antibody data for race/ethnicity was normally distributed and possessed homogenous variances, so an ANOVA test was performed (Supplemental Figure 1). The antibody data for age was not normally distributed, so a Kruskal-Wallis test was conducted (Supplemental Figure 2). IgG antibody differences were also explored across the sex of the neonate and symptomology at COVID diagnosis as previous studies have found trends of lower antibody levels in male neonates and patients who have pre-symptomatic or asymptomatic SARS-CoV infection (Bordt et al., 2021 and Hamady et al., 2021). Antibody data for the influence of neonate sex was not normally distributed, so a nonparametric Wilcoxon test was used (Supplemental Figure 3). The symptomology antibody data fit a normal distribution, so a parametric t-test was performed (Supplemental Figures 4).

Thresholds for Seropositivity

Two statistically relevant cut offs were considered and applied to the RBD IgG OD data: Maximum Specificity (Max Spec.) and 3 Standard Deviation (3 SD). The max spec. cut off was simply a representation of the largest value within the pre-pandemic control population. With this cut off, all of the negative controls would fall below the cut off which ultimately maximizes the specificity of the cut off value. The 3SD cut off, the standard for clinically validated diagnostic tests, is a representation of the value three standard deviations above the mean of the negative pre-pandemic control population. As is suggested by their names, the 3SD cut off is less specific than the max spec. cut off,
but it provides the benefit of a greater sensitivity. Ultimately, the three SD cut off was the main threshold used in this experiment as sensitivity was prioritized.

Relevant Clinical Definitions

COVID Status – UMMC: A patient was considered to be COVID positive in the UMMC labor and delivery unit if they had a positive PCR test result.

Serostatus: Seropositivity was determined by whether a patient’s IgG levels fell above or below the 3SD cut off. A patient was considered to be seropositive for SARS-CoV-2 if their RBD IgG OD antibody levels were higher than 0.484, the 3 SD cut off.

Preeclampsia Status: A patient was considered to have preeclampsia at UMMC if they met the following criteria: blood pressure above 140/90 and urine protein levels of 0.3 grams or greater in 24-hours. Severe features can be developed if metrics rise (see Supplemental Figure 1) with or without proteinuria, which commonly requires prompt delivery regardless of the patient’s gestational age.

Ethical Approval

CARES Study IRB approval: Study participants were approached and consented in the Massachusetts Memorial Medical Center (UMass Memorial) labor and delivery unit in Worcester, MA between April 2020 and August 2021. The Institutional Review Board
(IRB) at the UMCMS COVID-19 Analysis on Perinatal Specimens Related to Exposure (CARES) Study (H00020140).

COVID-COPE Study IRB approval: Study participants were enrolled at the University of Massachusetts Chan Medical School (UMass Chan) and the Massachusetts Memorial Medical Center (UMass Memorial) in Worcester, MA between April and August of 2020 under the Institutional Review Board (IRB) approved Consolidated COVID-19 Clinical and Observational Pathogenesis and Epidemiology (COVID-COPE) Study Protocol (H00020145). RTW included graduate and medical students and permission was obtained from the Students as Research Subjects (SAS) Ad Hoc Advisory Committee, a governing body which aims to protect the rights and interests of students at UMass Chan.
Results

Comparison between non-pregnant women and cord blood antibody levels

The purpose of the first analysis was to investigate RBD IgG levels across the three study populations. As expected, there was a significant difference between median RBD IgG OD levels extracted from the blood samples from healthy women prior to the pandemic, hospitalized nonpregnant women, and from umbilical cord of pregnant women part of the CARES study (KW $X^2_2 = 41.857, p = 8.145 \times 10^{-10}$). While the median RBD IgG OD level of the pre-pandemic group was statistically lower than the CARES ($p = 3.10 \times 10^{-8}$) and nonpregnant ($p = 1.20 \times 10^{-9}$) COVID-19 patients, there was not a significant difference between median RBD IgG OD levels of the CARES and

![Figure 12. Levels of RBD IgG OD across Healthy Pre-pandemic (n = 21), CARES Cord Blood (n = 84), and COVID+ Nonpregnant (n = 51) groups.](image)
nonpregnant groups (p = 0.580). The percent seropositive in each group using the 3 SD cutoff is as follows: 0% in the pre-pandemic group, ~39% in the nonpregnant group, and ~40% in the CARES group.

*CARES Preeclampsia and COVID PCR Comparisons*

Given parallels drawn between the inflammatory responses of SARS-CoV-2 and preeclampsia, preeclampsia status was included in our serological analysis of SARS-CoV-2. In order to determine if antibody levels transferred to the infant differed by PCR positivity or preeclampsia diagnosis of the mother, we stratified the pregnant women by these variables within the CARES study population. A significant difference between the RBD IgG OD levels of CARES pregnant women of varying preeclampsia and PCR COVID statuses* was observed (KW $X^2_3 = 11.289$, p = 0.0103). There was a distinct trend towards significant difference between the preeclampsia -/COVID + group and the preeclampsia -/COVID - group (0.078) as well as the preeclampsia -/COVID + group and the preeclampsia +/COVID - (p = 0.114). Preeclampsia was present in ~33% of the CARES patients (28/84) and only ~32% of those that were preeclampsia+ were also COVID+ (9/28). Therefore, there also did not appear to be a significant connection between COVID positivity and preeclampsia.
The objective of this analysis was to compare IgG antibody levels in CARES patients according to COVID PCR positivity and investigate rates of seropositivity relative to PCR status. As expected, there was a significant difference in median RBD IgG OD levels of neonates whose mothers were SARS-CoV-2 PCR positive versus negative (W = 1190, p = 0.00144). The proportion of seropositive patients in the PCR

*In this analysis, the antibodies extracted from the cord blood served as a surrogate of the mother’s serostatus.

**COVID PCR and Serostatus Status**

The objective of this analysis was to compare IgG antibody levels in CARES patients according to COVID PCR positivity and investigate rates of seropositivity relative to PCR status. As expected, there was a significant difference in median RBD IgG OD levels of neonates whose mothers were SARS-CoV-2 PCR positive versus negative (W = 1190, p = 0.00144). The proportion of seropositive patients in the PCR
negative and PCR positive groups is \( \sim 21\% \) and \( \sim 53\% \) respectively. Mothers who were PCR negative but had antibodies most likely had infections prior to becoming pregnant.

**Figure 14.** RBD IgG OD levels in neonates whose mothers were determined to be positive (n = 51) and negative (n = 33) for COVID through a SARS-CoV-2 PCR test.

**COVID Serostatus and PCR Status by Trimester**

Given the number of CARES patients who were PCR+ and seronegative, we sought to identify whether there was a correlation between serostatus in this subpopulation and the trimester in which they were exposed to SARS-CoV-2. Across patients who tested positive for SARS-CoV-2 using a PCR test and had trimester of infection data available, 46% of their neonates were considered to be seronegative and
54% were considered to be seropositive using the 3SD cutoff. Within those subgroups, ~56% of the seronegative group had mothers who had 3rd trimester COVID infections and ~67% of the seropositive group had mothers who had 2nd trimester COVID infections (Figure 15A&B). Trimester of infection was found to significantly influence the seropositivity of neonates whose mothers were PCR positive for COVID ($X^2 = 3.43$, p-value = 0.0635).

**Figure 15.** Trimester of COVID infection distribution across mothers who were PCR positive, but whose neonates were considered to be seronegative (A) versus mothers who were PCR positive and whose neonates were considered to be seropositive(B). Seropositivity was determined using the 3SD cutoff.
**SeroStatus by Trimesters of SARS-CoV-2 Infection**

Additional analyses on trimester of infection affirmed the previous results and found that there was a significant difference between mean RBD IgG OD levels in neonates whose mothers contracted SARS-CoV-2 in the second trimester versus the third trimester (Figure 16; $t_{45} = 2.765, p = 0.00822$). On average, mothers with second trimester COVID infections transferred the highest levels of RBD IgG OD to their babies. Mean antibody levels were ~48% lower in neonates whose mothers tested positive in the third trimester ($\bar{x}_3 = 0.639$) than mean levels in neonates whose moms had second semester infections ($\bar{x}_2 = 1.232$).

![Figure 16. RBD IgG OD levels in neonates whose mothers contracted in the second trimester (n = 25) and third trimester (n = 22).](image)
Discussion

Key Findings

In this study, the impact of SARS-CoV-2 during pregnancy was investigated through various antibody driven analyses. The major findings of this paper suggest a more positive outlook for the obstetric population than may have been originally assumed and provide insight into optimizing COVID vaccines during pregnancy. The first analysis found that there was no striking difference between the RBD IgG levels produced in the nonpregnant PCR+ individuals and the levels in the CARES cord blood (Figure 12). These results suggest that fetuses are actively receiving comparable amounts of antibodies relative to individuals who contracted COVID themselves.

Further analysis of antibody levels across CARES patients according to their COVID PCR and preeclampsia diagnosis found that preeclampsia status was not associated with the levels of RBD IgG transfer to the fetus (Figure 13). This is evident as the antibody levels in COVID PCR+ moms with diagnosed preeclampsia were not significantly different than those without preeclampsia. PCR- CARES patients have either never been exposed to SARS-CoV-2 or they were remotely infected prior to becoming pregnant. Therefore, if higher rates of seropositivity were detected in PCR - patients with a preeclampsia diagnosis, there would be a possible link between preeclampsia and any residual effects of previous infection. Overall, it appears that production and maternal-fetal antibody transfer is not significantly influenced by preeclampsia infections regardless of the strong associations found between the two conditions.
Furthermore, investigation of seropositivity in the COVID PCR+ CARES patients found that there was a strong correlation between trimester of maternal SARS-CoV-2 infection and IgG antibody levels present in the neonate with significantly highest RBD IgG levels in the cord after second trimester infections (Figure 15). These results were affirmed in trimester specific analysis of all CARES patients (Figure 16). While these findings stand out against results for other viral antigens, the higher IgG levels found in the cord of mothers with second trimester infections were expected and are consistent with recent literature specific to SARS-CoV-2 trans-placental antibody transfer and fetal RBD IgG levels (Ateyo et al., 2021). As Dr. Galit Alter detailed in her presentation, antibody production and maternal-transfer are drastically shifted in the third trimester infections which is likely due to the inflammation induced by active COVID-19 during the phase of pregnancy in which the most maternal-fetal transfer should take place. As was mentioned in the introduction, the results of this analysis have significant implications for the optimal COVID vaccination time during pregnancy. According to the results of this thesis, which are supported by significant literature, maternal-fetal transfer of SARS-CoV-2 antibodies across the placenta is most effective with second trimester infections and therefore medical professionals should seriously consider second trimester vaccination administration.

*Misclassification Bias for SARS-CoV-2*

As this paper was conducted using cross-sectional sampling, as opposed to repeated measures, it is important to discuss the possibility of misclassification due to the time dependent nature of SARS-CoV-2 manifestation in humans. The patients in this
study were classified according to two main factors: PCR status and serostatus. As the main diagnostic test used for SARS-CoV-2 throughout the course of the pandemic, PCR tests are highly effective tools that are used to detect viral RNA in the body. PCR tests are highly accurate in detecting active SARS-CoV-2 infections in pre-symptomatic, asymptomatic, and symptomatic individuals, but it is not a perfect diagnostic tool as it is dependent on the timing relative to exposure to the virus. In a 2021 study of SARS-CoV-2 PCR testing, Rabaan et al. found that time of specimen sampling along with the progression of the disease in the individual play a significant role in the effectiveness of PCR testing as a diagnostic tool (Rabaan et al., 2021). Since the disease progression and duration of viral shedding of SARS-CoV-2 is different for all people exposed to the virus, there is a high potential for misclassification of PCR status if patients are only tested once as was true of the CARES patients. For instance, it is possible that a CARES patient had asymptomatic COVID during their pregnancy, but their viral load declined to the point of being undetectable by the time they received their next PCR test resulting in a misclassification. It is also possible to have false negative results from PCR tests if the sample is collected before the viral load accumulates (Böger et al., 2021). Recent studies have found that peak viral load typically coincides with symptom onset and that viral kinetics can be influenced by factors such as disease severity, the presence of symptoms, and age (Néant et al., 2021 and Liu et al, 2020b).

Misclassification of serostatus is also possible. Limitations in classification of CARES patients according to serostatus would likely be the result of one of two reasons: the timing of collection as antibodies propagate and wane at slightly different rates across patients, or the fact that some people simply produce little to no antibodies. Similar to the
limitations of PCR classification, it is possible to sample patients too early or too late. A PCR+ CARES patient could have been classified as seronegative if their cord blood samples were collected prior to significant antibody production – this is possible for patients with third trimester infections. Conversely, patients who contracted COVID in the second trimester might have already experienced antibody waning to the extent of seronegative classification – especially considering previous studies that identified peak antibody levels at ~50 days PSO (Ortega et al., 2021). In a recent study of SARS-CoV-2 using convalescent plasma, Klein et al., supervised by Dr. Aaron Tobian at Johns Hopkins University, found that ~20% of the samples did not produce enough antibodies to be detectable. Although the exact mechanisms are unknown, these results support the hypothesis that SARS-CoV-2 is actively attempting to shut down antibody responses to infection (Klein et al., 2020, Kaneko et al., 2020, and Alter et al., 2021).

**Limitations**

There were several key limitations to this study. First, the sample size of patients with antibody data was smaller than was originally intended. As was illustrated in the experimental flowchart, the CARES study originally included 144 consented patients, but antibody data was only extracted from the cord serum of 84 of them. This was likely due to various complications such as insufficient amounts of blood collected, participation lost to a critical delivery, or issues resulting from specimen transfer across campuses. By effectively cutting the sample size of the study nearly in half, the scope of possible analyses was limited due to issues of statistical power. The lack of normally distributed
data can also be attributed to the low sample sizes, at least partially, through the central limit theorem.

Secondly, mother-fetus dyads were not included in this study as blood samples were only currently available for the umbilical cord blood and not maternal blood. This prevented us from being able to make claims about the actual quality of vertical transmission observed like Dr. Alter’s studies since the cord blood simply reflects the serostatus of the baby, leaving a gap of knowledge about the maternal serostatus.

As mentioned above, this thesis was also limited by the cross-sectional nature of the experiential model. While it was not possible in this experiment, a solution to these classification limitations would be to conduct repeated measures experiments. Access to multiple measures for each CARES participant would have helped account for any possible misclassifications as a result of sampling time.

Finally, the lack of diversity present in the patient population of this study limits the scope by which we can make suggestions based on the data – particularly in the nonpregnant and pre-pandemic populations. Given the low sample sizes representing patients of color in the nonpregnant and pre-pandemic populations, differences in race were not able to be reasonably tested. Thankfully, the primary dataset investigated in this study was the CARES study which was the most diverse and more representative of the U.S. and Massachusetts populations. Regardless, studies further representing the critical topics discussed in this paper in communities of color is essential in ensuring that healthcare workers are providing the most inclusive and best care to all of their patients.
COVID-19 Pandemic Updates

Since the conclusion of data collection for this study, SARS-CoV-2 has continued to evolve, and significant progress has been made in vaccine development and roll out across the globe. Various forms of treatment intervention have also been approved.

Five main variants of SARS-CoV-2 have been designated as a variant of concern (VOC) by the WHO since the beginning of the COVID-19 pandemic: alpha, beta, gamma, delta, omicron (Figure 17). According to WHO, a variant is designated a VOC if it increases transmissibility, increases virulence, and/or decreases the effectiveness of diagnostics, vaccines, or treatments (WHO, 2021c). As disease transmission appears to be increasing with each new variant, early studies on the most recent omicron variant have hopeful implications for clinical outcomes associated with the variant. In a preliminary study through the Kaiser Permanente health system, Lewnard et al. found that the mortality and ICU admission rates associated with the omicron variant are approximately ~91% and ~74% lower than the delta variant (Lewnard et al., 2022). Furthermore, in a report published on January 8th, 2022, the CDC announced omicron as the dominant variant and estimated that ~98.3% of COVID cases in the U.S. were the omicron variant (CDC, 2021a). While the initial findings on the omicron variant suggest less deadly clinical outcomes, the increased transmission is causing U.S. case numbers to
peak and straining ICU capacity. Given the trajectory of SARS-CoV-2 thus far, it is highly likely that there will be more variants to come.

<table>
<thead>
<tr>
<th>WHO label</th>
<th>Pango lineage*</th>
<th>GISAID clade</th>
<th>Nextstrain clade</th>
<th>Additional amino acid changes monitored*</th>
<th>Earliest documented samples</th>
<th>Date of designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>B.1.1.7</td>
<td>GRY</td>
<td>20I (V1)</td>
<td>+S:484K, +S:452R</td>
<td>United Kingdom, Sep-2020</td>
<td>18-Dec-2020</td>
</tr>
<tr>
<td>Gamma</td>
<td>P.1</td>
<td>GR/501Y.V3</td>
<td>20J (V3)</td>
<td>+S:681H</td>
<td>Brazil, Nov-2020</td>
<td>11-Jan-2021</td>
</tr>
</tbody>
</table>

Figure 17. WHO-designated SARS-CoV-2 variants of concern (WHO, 2021c).

Along with the emergence of new variants, various vaccines have been developed across the globe. In the U.S., three primary vaccines have been approved for administration with striking effectiveness: Pfizer (BioNTech - BNT162b2), Moderna (NIAID - mRNA-1273), and Johnson & Johnson (J&J; Janssen Pharmaceutical Companies - JNJ-78436735). The Pfizer and Moderna vaccines are both mRNA-based vaccines that have efficacy rates of >90% after two administered doses. The J&J vaccine is a viral vector vaccine that only requires one dose, but is less effective at preventing the virus with an efficacy rate of ~66% (CDC, 2021d). To date, most studies on vaccine efficacy in pregnant women have included only the Pfizer and Moderna vaccines with
great outcomes, although viral vector vaccines such as J&J have been previously administered to pregnant women during the Ebola outbreak without any adverse outcomes (CDC, 2021b). As vertical transmission is typically most effective in the third trimester, the majority of early vaccine trials in the obstetric population only included pregnant women vaccinated in the third trimester (Dagan et al., 2021). However, the results of this study were supported in a more recently published study of the Pfizer vaccine: Kugelman et al. found that vaccination in the second trimester of pregnancy induced a robust humoral response that is sustained throughout the pregnancy and provided the mother and fetus with prolonged safety against SARS-CoV-2 (Kugelman et al., 2022).

Although they have yet to be widely implemented, several treatments for SARS-CoV-2 have become available. Monoclonal antibodies, such as those used in Dr. Alter’s study of SARS-CoV-2 antibody transfer, have been found to successfully prevent severe or deadly COVID-19. In November 2020, a COVID monoclonal antibody product called REGEN-COV was approved by the U.S. Food and Drug Administration (FDA) for emergency use for treatment of COVID in high-risk patients (U.S. FDA, 2021). It is specifically intended to be used after exposure to SARS-CoV-2 to prevent mild-to-moderate cases from progressing to severe COVID. REGEN-COV contains two SARS-CoV-2 monoclonal antibodies, casirivimab and imdevimab, that are capable of reducing the viral load of SARS-CoV-2 and have been found to decrease the likelihood of hospitalization or death by ~70% (Mayer et al., 2021). As is true for vaccine trials, pregnant women are excluded from testing on treatments such as REGEN-COV. However, since the FDA officially considered pregnancy as a high-risk criterion for...
REGEN-COV in May 2021, several small-scale studies have found that the product yields the same promising results in pregnant women as in the test populations without any complications (Hirshberg et al., 2021 and Mayer et al., 2021). While COVID vaccines have been found to be effective in vertical transmission of antibodies in both the second and third trimesters, monoclonal antibody treatments could provide alternative defenses against severe SARS-CoV-2 infections – particularly unvaccinated mothers (Mayer et al., 2021). While the effects of monoclonal antibodies such as casirivimab and imdevimab have yet to be widely tested in pregnant women, the possibility that they provide the obstetric population is immense.
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Supplemental Figures

**Supplemental Figure 1.** RBD IgG OD levels across pregnant women of various races and ethnicities: Asian/Asian American (n = 5), Black/African American/African (n = 11), Hispanic/Latina (n = 26), and White/Caucasian (n = 38). All patients who identified within the Asian/Asian American, Black/African American/African, and White/Caucasian groups identified themselves as not being Hispanic/Latina.

**Supplemental Figure 2.** RBD IgG OD levels of neonates delivered from women of various age groups between 21-44: 21-26 (n = 19), 27-32 (n = 30), 33-38 (n = 24) and 39-44 (n = 6).
**Supplemental Figure 3.** Median RBD IgG OD levels in female (n = 47) versus male (n = 37) neonates

**Supplemental Figure 4.** RBD IgG OD levels in neonates whose mothers were asymptomatic (n = 7) and symptomatic (n = 43) at the time of their COVID diagnosis.