Short communication

Seroprevalence of SARS-CoV-2 immunoglobulins in pregnant women and neonatal cord blood from a highly impacted region

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A R T I C L E I N F O	A B S T R A C T
	There is inadequate screening for SARS-COV-2 during pregnancy. We aimed to determine the impact of maternal and neonatal cord blood SARS-COV-2 antibodies and placental transfer ratios in a region with a low screening plan. We performed a blind study in one of the SARS-CoV-2 epicenters in South America. 32% of pregnant women were serological positive. Importantly, there is an efficient passive immunization of the fetus to SARS-CoV-2. We report high incidence of SARS-CoV-2 infection during pregnancy, which is higher than officially reported. Therefore the need of active immunization to enhance maternal protection and fetal passive immunization.

1. Introduction

Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2), the cause of the Coronavirus Diseases of 2019 (COVID-19), is responsible for the global pandemic of 2020 and 2021 with over 107 million confirmed cases and over 2.3 million deaths [1]. Ecuador is one of the most heavily impacted countries with an official report of 507,020 cases (2873 confirmed cases per 100,000) and 32,000 deaths (185.12 deaths/100,000) [2]. However, due to the lack of testing, these numbers are likely an underestimate.

Most of Ecuador's SARS-CoV-2 cases were concentrated in the Guayas and Los Rios provinces and their capitals: Guayaquil and Babahoyo [2]. The seroprevalence of SARS-CoV-2 for the rural population of the coastal Ecuador region was over 70% [3]. Unfortunately, there was no SARS-CoV-2 seroprevalence data from individual cities, such as Babahoyo. Thus, a major drawback of this data is the assumption that infections were uniform within cities; thus questioning the real extent of the infection in Ecuador.

An important population that deserves meticulous consideration during the COVID-19 pandemic is pregnant women. Current data suggest that maternal complications due to COVID-19 appear to be similar to reproductive aged non-pregnant women, although a subset of pregnant women may be at increased risk for admission to an intensive care unit, need for respiratory support, and death [4]. Additionally, there may be an increased risk of preterm birth, low birth weight, and cesarean deliveries although vertical transmission is considered rare [5–9].

Infections during pregnancy have consequences for both the mother and fetus [10,11]. Thus, monitoring infections during pregnancy is essential to generate understanding and establishing interventions pertaining to the protection of both mother and fetus. Unfortunately, surveillance of SARS-CoV-2 infections in pregnant women has been poorly implemented, especially in vulnerable countries, such as Ecuador.

The newborn's immunological protection heavily depends on transplacental delivery of maternally-derived antibodies. The extent to which maternal antibodies are produced in response to SARS-CoV-2 infection during pregnancy and the extent to. which they cross the placenta are critical in understanding the degree of passive protection that is afforded to the newborn [12]. To our knowledge, studies of transplacental transfer of maternal SARS-CoV-2–specific antibodies to newborns is limited to a few reports that are mainly located in the United States [12].

The objectives of this study were twofold: 1) determine the seroprevalence of SARS-CoV-2 specific antibodies in pregnant women in a location where regular screening is not implemented and 2) define the rate of transplacental antibody transfer. In this study, we report a high rate of SARS-CoV-2 infection during pregnancy and a highly effective transfer of maternal IgG antibodies to SARS-CoV-2 across the placenta and to the fetus. Our results can be extrapolated to suggest a potential benefit for vaccination during pregnancy.

2. Methods

2.1. Study population

This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines. This was retrospective study done with samples sent for tests that were originally not related to this study. A consent waiver was approved since samples were not collected for the study and the samples used were defined as dischargeable material.

The study included 100 serum samples collected from pregnant women at the time of delivery along with corresponding neonatal cord blood. Serum samples were collected for routine screening and blood typing. Neonatal cord blood samples were collected only for blood typing.

All samples were deidentified prior to testing. Clinical information and demographics were collected by reviewing electronic records that did not have any personal identifiers. Maternal illness was determined by the definition from the US Centers for Disease Control and Prevention (CDC): 1) asymptomatic: no symptoms related to shortness of breath radiographic evidence of pneumonia and normal oxygenation. 2) Symptomatic: shortness of breath or radiographic evidence of pneumonia with or without administration of supplement oxygen.

2.2. Antibody measurements

Levels of IgG and IgM SARS-CoV-2 specific nucleocapsid and spike antigens were determined using specific enzyme linked immunosorbent assay (ELISA) (DiaPro, Milano, Italy Cat number COV19G.CE and COV19 M.CE) according manufacturer's instructions.

2.3. Statistical analysis

IgM and IgG values of were plotted as continuous variables and expressed as the median (interquartile range) with error bars representing 95% confidence intervals. The relationship between the maternal and neonatal serologic data was determined using Pearson correlation and linear regression.

Maternal/fetal transfer ration was defined as fetal divided by maternal antibody levels:

$$TR = \frac{\text{Fetal} - \text{IgG (MFI)}}{\text{Maternal} - \text{IgG (MFI) per Beharier et al}}[13].$$

3. Results

3.1. Demographics

Patient clinical characteristics and demographics are summarized in Sup.Table 1. All serum specimens were collected from Babahoyo, Ecuador with a high incidence of COVID-19 infection but with low screening rate of pregnant women. Race and ethnicity were relatively homogeneous with 100% of participants being Hispanic women.

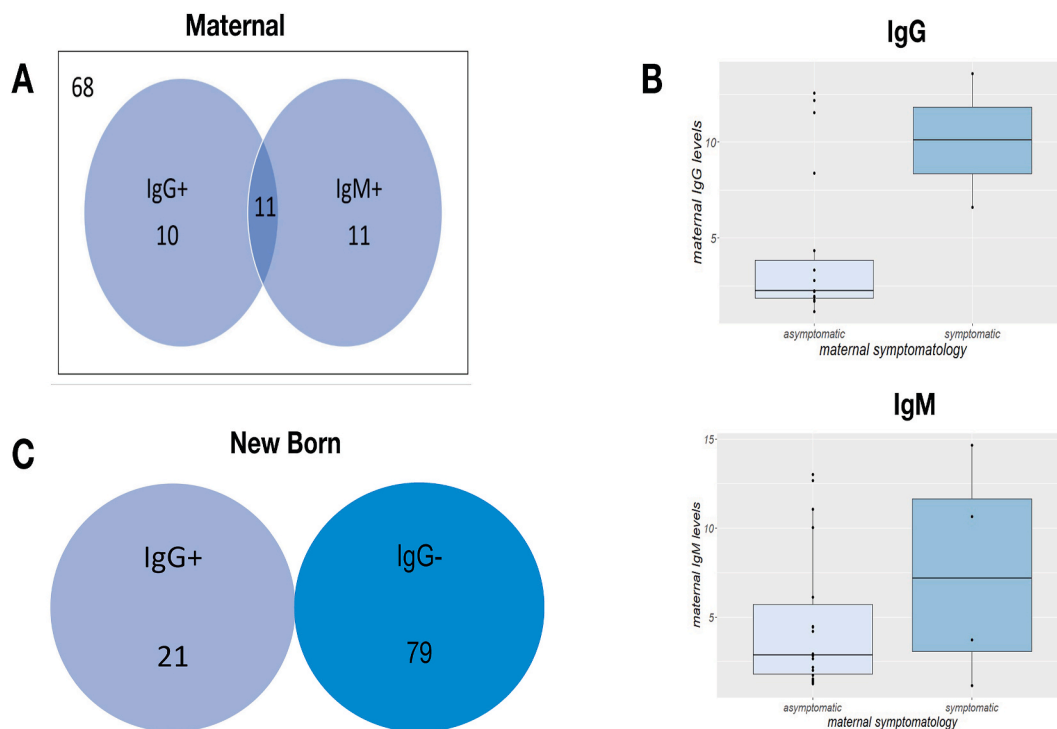### 3.2. Incidence of SARS-CoV-2 IgM and IgG in pregnant women

To determine the incidence of SARS-CoV-2 infection in pregnant women in a population where regular testing for SARS-CoV-2 was not

Table 1
Demographics.

Characteristics of Pregnant Women	Number (Percent)
Total	100 (100)
Age	**Average: 24.8**
16–19 yr	3 (3)
20–24 yr	21 (21)
25–34 yr	63 (63)
35–45 yr	13 (13)
Pregnancies	**Average: 2.19**
1	28 (28)
2	42 (42)
3	13 (13)
>4	17 (17)
Delivery	
Vaginal	18 (18)
C-Section	82 (82)
Blood Type	
O	75 (75)
A	20 (20)
B	5 (5)
RH Factor	
+	89 (95.7)
−	4 (4.3)
Covid Related Symptoms	
None	93 (94.89)
Mild	2 (2.04)
Severe	3 (3.06)
Pregnancy Complications	
No	100 (100)
Yes	0 (0)

Characteristics of Newborn Babies	Number (Percent)
Total	100 (100)
Gender	
Male	55 (55)
Female	45 (45)
Ballard	
<36	16 (16.16)
37–39	70 (70.71)
>40	13 (13.13)
Blood Type	
O	77 (77)
A	17 (17)
B	4 (4)
AB	2 (2)
RH Factor	
+	99 (99)
−	1 (1)
APGAR Scores (Average)	
1"	7.42
5"	8.42
10"	8.65
Birth Complications	
No	100 (100)
Yes	0 (0)
Birth Weight	
Low	2 (2.04)
Normal	96 (97.96)

implemented (NP-PCR), we evaluated the presence of SARS-CoV-2 nucleocapsid and spike protein IgG and IgM antibodies in blood samples from 100 patients that were collected at the time of delivery from September 3, 2020 to January 31, 2021 for reasons that were nonrelated to this study. Matched maternal-cord blood sera were available for all the 100 patients, which were also analyzed. The average age of the studied cohort was 24.8 years (range 16–41 years) and there were no reports of pregnancy complications (Sup Table 1).

From the 100 recruited pregnant women: 32 were seropositive for SARS-Cov-2; 22 were IgM and 2 were IgG seropositive (Fig. 1A); 11 were IgM positive but IgG negative, suggestive of a recent infection at the third trimester, close to the time of delivery; 10 were only IgG positive, suggestive of an infection early or before pregnancy; 11 were IgM and IgG positive (Fig. 1A).

Fig. 1. Incidence of SARS-CoV-2 Specific IgM and IgG in Pregnant Women. A. IgG and IgM sero-prevalence in pregnant women. 100 pregnant women were evaluated for IgG and IgM antibodies against Sars-CoV-2.68 patients were negative, 10 patients were IgG positive, 21 patients were IgG and IgM positive, and 11 were IgM only positive. **B. Correlation Between Seroprevalence and Symptoms**. Comparation of the IgG serology in symptomatic and asymptomatic pregnant women. IgG levels were higher in symptomatic pregnant women although it did not reach statistical significance. No differences were observed for IgM between symptomatic and asymptomatic pregnant women. **C. Assessment of Neonatal Cord Blood SARS-CoV-2 IgG Antibodies**: Determination of IgG antibodies to SARS-COV-2 in cord blood samples showed 21 serologic-positive and 79 negative. The 21 serologic positive samples correspond to the 21 positive mothers.

3.3. Correlation between symptoms and seroprevalence

A retrospective chart analysis of all 32 SARS-CoV-2 seropositive pregnant women showed that 12.5% (4/32) were symptomatic with COVID-19 like symptoms, whereas 87.5% (28/32) were asymptomatic.

Both asymptomatic and symptomatic pregnant women showed detectable IgG and/or IgM (Fig. 1B). Although not statistically significant, we did observe a correlative trend between IgG levels and symptomatic women (0.4136 p-value = 0.0623). No correlation was observed between symptoms and IgM levels (0.2379 p-value = 0.2863) (Fig. 1C).
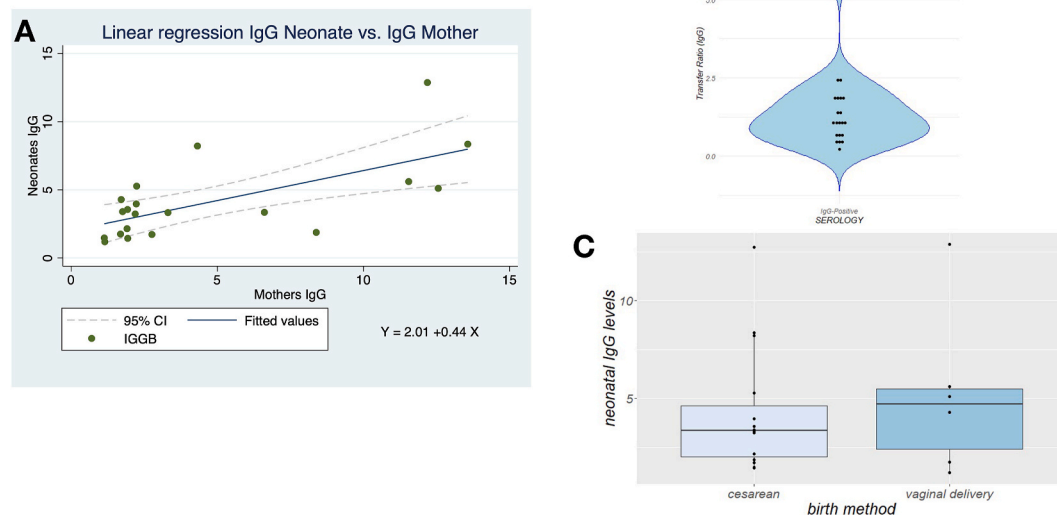
Fig. 2. Maternal Transfer of IgG Antibodies. A. Linear regression of maternal and newborn IgG to SARS-CoV-2. B. The Violin Plot was constructed using R's GGPlot2 library. The plotted values are the IgG Transfer Ratios of seropositive pairs of mothers and newborns (Newborn IgG/Mother IgG). The lowest value is 0.22, and the highest is 5.68. The mean is 1.41 and the standard deviation is 1.17, with most values located between 1 and 2. C. Impact of the Type of Delivery on Serologic Evaluation in the New Born.

3.4. Newborn passive immunization of IgG

To evaluate the degree of passive immunization to the fetus in SARS-CoV-2 infected pregnant women, we first analyzed the cord blood of the 21 identified SARS-CoV-2 IgG serology positive mothers (Fig. 1C). Twenty-one cord blood samples were IgG positive and positively correlated with the 21 IgG serology positive mothers (Pearson correlation coefficient = 0.4719 (p-value = 0.0308) and Fig. 2A). From the 11 IgM serologically positive mothers, the corresponding cord blood samples were IgG negative. We did not observe IgG in cord bloods obtained from serology-negative mothers (Fig. 1C).

3.5. Efficiency of IgG transfer from mother to fetus

Since we observed 100% transfer of IgG to COVID-19 specific nucleocapsid and spike protein from the mother to the infant, we evaluated whether the efficiency of transfer is similar between all the studied patients. We defined relative efficiency based on titer levels detected in the cord blood relative to the maternal titers. 5/21 samples showed relatively inefficient transfer of IgG SARS-CoV-2 antibodies from mothers to the cord blood (Sup.Table 2; Fig. 2B). Only 2/21 showed a high efficiency in antibody transfer (patient B073 and BO96) characterized by higher titers in the infant compared to the mother (Sup. Table 2; Fig. 2B). 14/21 samples showed similar levels of IgG to SARS-CoV-2 specific nucleocapsid and spike proteins between the mother and infant (Sup.Table 2 and Fig. 2A and B).

3.6. Type of delivery and seroprevalence

Since we aimed to study the passive immunization from mother to fetus, we evaluated the possibility of contamination at the time of delivery. We performed a retrospective chart analysis of all 32 serologically positive mothers and found that 24 women (75%) gave birth through cesarian delivery, whereas 8 women (25%) had vaginal delivery. More importantly, we did not observe significant differences between the IgG levels in the cord blood of neonates born from cesarian or vaginal delivery (p-value 0.6598 for IgG); confirming the presence of passive immunization from the mother to the fetus (Fig. 2C).

4. Discussion

We present for the first time a complete matching cohort with 32 IgG/IgM positive mothers and their respective neonates. We report two important clinical observations associated with SARS-CoV-2 infection during pregnancy. First, we show a high incidence of infected pregnant women in areas lacking appropriate screening programs. Second, we show data that suggests an efficient passive immunization of the fetus to SARS-CoV-2 during pregnancy.

Pregnant women are particularly vulnerable to the effects of socio-economic crisis that were generated by the pandemic. This vulnerability is evidenced in the high rate of pregnant women being infected, without their knowledge of the infection. From the 100 patients evaluated, none were diagnosed as SARS-CoV-2 positive by any test. Although, as determined in this study, 32% were serologically positive to SARS-CoV-2. Our study, shows a high incidence of SARS-CoV-2 infection in pregnant women, which is relevant considering the potential implications of the infection to future maternal and infant health [5,14].

Our findings demonstrate that pregnant women can efficiently respond to SARS-CoV-2 and do not develop major symptomatology; consequently, demonstrating the opposite of immune suppression. In addition, the rate of seroprevalence in the newborns demonstrate an active communication between the maternal immune system and fetus. A second aspect that is essential to consider is the role of the placenta during infections. Pregnant women represent an immunologically unique population, because their immune system is influenced by signals originating from the placenta [15,16]. We, and others, have shown that the placenta functions as an immune modulatory organ that regulates immune responses both at the implantation site and systemically. The placenta functions as an immunological barrier preventing viral transfer to the fetus, but allows transfer immunological components, such us immunoglobulins.

The presence of maternal IgG in the newborn provides an advantage for protection. In this study, although higher than other reports [17], we observe an efficient transfer of IgG specific to SARS-CoV-2 from the maternal side to the fetal side, which cannot be justified as contamination of maternal blood at the time of delivery. Seventy percent of the studied patients gave birth by cesarian section, which prevents potential contamination.

In conclusion, the risk of infection and developing other complications are high during pregnancy. The best prevention for protecting the mother from SARS-CoV-2 infection is vaccination; which, by the data shown here, will provide the fetus and neonate a strong and effective protection to SARS-CoV-2 infection through passive placenta transfer of SARS-CoV-2 specific antibodies.

High percentage of the patients underwent cesarean section. There were no differences on neonatal IgG levels observed between vaginal delivery or cesarean section.

Author contribution

Dr. Mor had full access to all the data in the study and takes full responsibility for the integrity of the data and accuracy of the data analysis.
Concept and design: Gil Mor and Hector Zambrano.
Acquisition analysis or interpretation of data: All Authors.
Drafting of the manuscript: Hector Zambrano, Anthony Maxwell, Kang Chen, Bernard Gonik, Gil Mor.
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Statistical analysis: Ketty Anchundia, Dalila Aviles[1], Rufo Andaluz[2], Nathaly Calderon EstefanieTorres[1], Nicolas Gonzalez-Granda.[1]

Table 2
Transfer ratio of IgG from maternal to fetal circulation.

IGG Mother	IGGBABY	TRANSFERRATIO	MOMCODE	BABYCODE
8.387	1.874	0.22344104	B039	RN039
1.937	1.437	0.741868869	B041	RN041
3.313	3.328	1.004527618	B043	RN043
1.152	1.188	1.03125	B056	RN074
12.56	5.107	0.40660828	B057	RN075
1.914	2.146	1.121212121	B058	RN076
2.193	3.227	1.471500228	B059	RN077
1.714	4.284	2.499416569	B060	RN078
1.931	3.554	1.840497152	B063	RN080
12.19	12.87	1.055783429	B064	RN081
11.55	5.604	0.485194805	B066	RN082
2.229	3.962	1.77747869	B069	RN083
1.135	1.467	1.292511013	B071	RN084
13.57	8.351	0.615401621	B072	RN085
2.242	12.73	5.677966102	B073	RN086
1.691	1.753	1.036664695	B074	RN087
2.242	5.268	2.349687779	B075	RN088
2.764	1.723	0.623371925	B083	RN090
6.613	3.348	0.506275518	B085	RN091
1.761	3.4	1.930721181	B092	RN095
4.322	8.213	1.900277649	B096	RN096

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Declaration of competing interest

No conflict of interest is reported from any of the authors.

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References

[1] W.H. Organization, WHO Coronavirus Disease (COVID-19) Dashboard | WHO Coronavirus Disease (COVID-19) Dashboard, 2020. Accessed, https://covid19.who.int/?gclid=CjwKCAiA65iBBhB-EiwAW253W_9m8duZ5h6WjUD6MkoaMkJs-FzK8Ca2SDmzvbzVhMC3vwXsmo6PHhoCYLcQAvD_BwE.

[2] W.H. Organization, Ecuador: WHO Coronavirus Disease (COVID-19) Dashboard | WHO Coronavirus Disease (COVID-19) Dashboard, 2021.

[3] O.H. Del Brutto, A.F. Costa, R.M. Mera, B.Y. Recalde, J.A. Bustos, H.H. García, SARS-CoV-2 in rural Latin America. A population-based study in coastal Ecuador, Clin. Infect. Dis. (2020).

[4] D. Sutton, T. Wen, A.P. Staniczenko, Y. Huang, M. Andrikopoulou, M. D'Alton, B. B. Feinberg, K. Fuchs, D. Goffman, C. Gyamfi-Bannerman, K. Kahe, R. Landau, J. A. Lasky, R. Miller, A.D. Ntoso, A. Panzer, J.J. Sheen, L.L. Simpson, A.M. Friedman, Clinical and demographic risk factors for COVID-19 during delivery hospitalizations in New York city, Am J Perinatol, 2021.

[5] S. Hayakawa, S. Komine-Aizawa, G.G. Mor, Covid-19 pandemic and pregnancy, J. Obstet. Gynaecol. Res. 46 (10) (2020) 1958–1966.

[6] A.J. Vivanti, C. Vauloup-Fellous, S. Prevot, V. Zupan, C. Suffee, J. Do Cao, A. Benachi, D. De Luca, Transplacental transmission of SARS-CoV-2 infection, Nat. Commun. 11 (1) (2020) 3572.

[7] L. Dong, J. Tian, S. He, C. Zhu, J. Wang, C. Liu, J. Yang, Possible vertical transmission of SARS-CoV-2 from an infected mother to her newborn, Jama 323 (18) (2020) 1846–1848.

[8] H. Zeng, C. Xu, J. Fan, Y. Tang, Q. Deng, W. Zhang, X. Long, Antibodies in infants born to mothers with COVID-19 pneumonia, Jama 323 (18) (2020) 1848–1849.

[9] B. Chmielewska, I. Barratt, R. Townsend, E. Kalafat, J. van der Meulen, I. Gurol-Urganci, P. O'Brien, E. Morris, T. Draycott, S. Thangaratinam, K. Le Doare, S. Ladhani, P. von Dadelszen, L. Magee, A. Khalil, Effects of the COVID-19 pandemic on maternal and perinatal outcomes: a systematic review and meta-analysis, Lancet Glob Health 9 (6) (2021) e759–e772.

[10] G. Mor, P. Aldo, A.B. Alvero, The unique immunological and microbial aspects of pregnancy, Nat. Rev. Immunol. 17 (8) (2017) 469–482.

[11] K. Racicot, J.Y. Kwon, P. Aldo, M. Silasi, G. Mor, Understanding the complexity of the immune system during pregnancy, Am. J. Reprod. Immunol. 72 (2) (2014) 107–116.

[12] D.D. Flannery, S. Gouma, M.B. Dhudasia, S. Mukhopadhyay, M.R. Pfeifer, E. C. Woodford, J.E. Triebwasser, J.S. Gerber, J.S. Morris, M.E. Weirick, C. M. McAllister, M.J. Bolton, C.P. Arevalo, E.M. Anderson, E.C. Goodwin, S. E. Hensley, K.M. Puopolo, Assessment of maternal and neonatal cord blood SARS-CoV-2 antibodies and placental transfer ratios, JAMA Pediatr (2021).

[13] O. Beharier, R. Plitman Mayo, T. Raz, K. Nahum Sacks, L. Schreiber, Y. Suissa-Cohen, R. Chen, R. Gomez-Tolub, E. Hadar, R. Gabbay-Benziv, Y. Jaffe Moshkovich, T. Biron-Shental, G. Shechter-Maor, S. Farladansky-Gershnabel, H. Yitzhak Sela, H. Benyamini-Raischer, N.D. Sela, D. Goldman-Wohl, Z. Shulman, A. Many, H. Barr, S. Yagel, M. Neeman, M. Kovo, Efficient maternal to neonatal transfer of antibodies against SARS-CoV-2 and BNT162b2 mRNA COVID-19 vaccine, J. Clin. Invest. (2021).

[14] J.M. Kubiak, E.A. Murphy, J. Yee, K.A. Cagino, R.L. Friedlander, S.M. Glynn, K. C. Matthews, M. Jurkiewicz, A.C. Sukhu, Z. Zhao, M. Prabhu, L.E. Riley, Y.J. Yang, Severe acute respiratory syndrome coronavirus 2 serology levels in pregnant women and their neonates, Am. J. Obstet. Gynecol. (2021).

[15] G. Mor, I. Cardenas, The immune system in pregnancy: a unique complexity, Am. J. Reprod. Immunol. 63 (6) (2010) 425–433.

[16] K. Racicot, G. Mor, Risks associated with viral infections during pregnancy, J. Clin. Invest. 127 (5) (2017) 1591–1599.

[17] A.G. Edlow, J.Z. Li, A.Y. Collier, C. Atyeo, K.E. James, A.A. Boatin, K.J. Gray, E. A. Bordt, L.L. Shook, L.M. Yonker, A. Fasano, K. Diouf, N. Croul, S. Devane, L. J. Yockey, R. Lima, J. Shui, J.D. Matute, P.H. Lerou, B.O. Akinwunmi, A. Schmidt, J. Feldman, B.M. Hauser, T.M. Caradonna, D. De la Flor, P. D'Avino, J. Regan, H. Corry, K. Coxen, J. Fajnzylber, D. Pepin, M.S. Seaman, D.H. Barouch, B. D. Walker, X.G. Yu, A.J. Kaimal, D.J. Roberts, G. Alter, 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 3 (12) (2020), e2030455.