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Maternal COVID-19 serological Changes. Comparison between seroconversion Rate in First and Third Trimester of Pregnancy and Subsequent Obstetric Complications: A Cohort Study

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Abstract: Pregnant women are especially vulnerable to respiratory diseases. We aimed to study seroconversion rate during pregnancy in a cohort of consecutive pregnancies tested in the first and third trimesters and to compare maternal and obstetric complications between women who seroconverted in the first *versus* the third trimester. This is an observational, cohort study carried out at Hospital Universitario de Torrejón, in Madrid, Spain, during the first peak of the COVID-19 pandemic. All consecutive singleton pregnancies with a viable fetus attending their 11-13 weeks scan between January 1st and May 15th, 2020, were included and monthly follow up until delivery. Antibodies against SARS-CoV-2 (IgA and IgG) were analyzed on stored serum samples obtained from the first and third trimester routine antenatal bloods in 470 pregnant women. Antibodies against SARS-CoV-2 were detected in 31 (6.6%) women in the first trimester and in 66 (14.0%) in the third trimester, including 48 (10.2%) that were negative in the first trimester (seroconversion during pregnancy). Although the rate of infection was significantly higher in the third *versus* the first trimester ($p = 0.003$), no significant differences in maternal or obstetric complications were observed in women testing positive in the first *versus* the third trimester.

Keywords: SARS-CoV-2; pregnancy; morbidity

1. Introduction

On December 31st, 2020, the health authorities notified the existence of a series of cases of pneumonia in the city of Wuhan. Since then, the disease called COVID-19, a severe acute respiratory syndrome caused by the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV2) [1], spread rapidly throughout most of the countries in the world. Currently, the gold standard for the diagnosis of active SARS-CoV2 infection is still rRT-PCR, a technique based on the detection of viral RNA in the nasopharynx. However, false negatives have been reported for this technique, mainly due to problems related to sample collection and/or detection methods [2]. Conversely, serological tests not only avoid these technical issues but, unlike rRT-PCR tests, they are also capable of detecting asymptomatic cases [2]. It was previously demonstrated that seasonal Influenza, Middle East Respiratory Syndrome (MERS) and SARS-CoV-1 infection during pregnancy have a different obstetric impact according to the trimester in which the infection is acquired [3–5]. Despite the increasing number of published studies, reported data is still insufficient to draw definite and unbiased conclusions regarding impact of SARS-CoV-2 infection on obstetric morbidity, or the clinical relevance of the time at which the infection occurs. Sequential serological tests performed in first and third trimester of pregnancy could be clinically useful to reliably identify time of infection

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and accurately determine the impact of the COVID-19 in the pregnancy according to the trimester in which the woman was infected.

In this study, we aimed to assess the immune status of a complete and consecutive cohort of pregnant women throughout the pregnancy (from the first to the third trimester) covering the first (March-June 2020) and second (June-December 2020) waves of the COVID-19 pandemic [6] in one of the hotspots of Madrid, Spain. In addition, we aimed to analyze the rates of obstetric complications in the group of women who seroconverted in the first trimester compared to those who seroconverted in the second or third trimesters of pregnancy.

2. Materials and Methods

2.1. Study design and population

This was a longitudinal, observational, ambispective study carried out between January 1st and December 25th, 2020, at Hospital Universitario de Torrejón (HUT), Madrid, Spain, as part of the PRECORSE study (Study for **PRE**gnancy **COR**onavirus **S**erologic **E**vidence), as previously described [7].

In our center, surplus of antenatal blood samples of all pregnant women is routinely frozen and stored at -80ºC degrees at the Biobank Network of the Region of Murcia, BIOBANC-MUR (reg. number B.0000859) for clinical and for research purposes. After the COVID-19 pandemic outbreak, all available stored serum samples collected from first trimester routine analysis between January 1st to May 15th, 2020, were identified. Samples corresponding to women who gave their written informed consent to participate in this study and fulfilled inclusion criteria (women over 18 years old, having singleton pregnancies with a non-malformed life fetus, and having their pregnancy care in our Obstetric Unit), were retrieved from the freezers and transferred on dry ice to Synlab laboratory in Madrid, Spain, for determination of anti-SARS-CoV2 immunoglobulin A (IgA) and immunoglobulin G (IgG). These women were followed up throughout their pregnancy according to the local protocol and, those testing positive in the first trimester were contacted and had monthly follow-ups in a specific clinic for maternal and fetal wellbeing and fetal biometry assessments. Surplus from their third trimester routine bloods was also tested for anti-SARS-CoV2 IgA and IgG. Maternal characteristics, medical and obstetric complications were prospectively recorded in all hospital appointments throughout the pregnancy, until the last pregnant woman gave birth on December 25th, 2020. Women included in the study were classified according to their serological status in the first and third trimester of pregnancy: those who had IgA or IgG anti SARS-CoV2 positive in the first trimester of pregnancy ("positive serology 1T"), and those who had IgA or IgG anti SARS-CoV2 positive in their third trimester, with a prior negative serology in the first trimester ("positive serology 3T").

All participants were unvaccinated against SARS-CoV2, and it was their first known SARS-CoV2 infection.

The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement was used for reporting the results.

2.2. Laboratory analysis and interpretation

Determination of anti-SARS-CoV2 IgA and IgG was performed by Enzyme-Linked Immunosorbent Assay (ELISA), providing semi-quantitative (extinction of the control patient sample/extinction of calibrator) serology results against the S1 domain of the spike protein of SARS-CoV-2 in serum samples (Anti-SARS-CoV-2 ELISA IgG and Anti-SARS-CoV-2 ELISA IgA, Euroimmunn Medizinische Labordiagnostika AG, Lubeck, Germany). IgA and IgG were considered positive, indeterminate, and negative when results were >1.1, 0.8 to 1.1 and <0.8, respectively, as recommended by the manufacturer (supplementary Table A1).

For anti-SARS-CoV2 IgG, sensitivity and specificity reported by manufacturers is 83.3% and 95.0% respectively in confirmed COVID-19 cases and, 70.8% and 96.6% respectively in suspected

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COVID-19 cases [8]. Overall sensibility and specificity reported for anti-SARS-CoV2 IgA are 86.7% and 82.7 %, respectively [9].

2.3. Statistical analysis and data management

Data were expressed as median (interquartile range) for continuous variables and in proportions (absolute and relative frequencies) for categorical variables. Mann-Whitney test and Fisher's exact test were used for comparing outcome groups for continuous and categorical data, respectively. Level of significance was set at 0.05. The statistical software package R was used for data analyses [10] and table1 package [11].

2.4. Ethical considerations

Approval from the local Research Ethics Committee Committee (Comité Ético de Investigación con Medicamentos de los Hospitales Universitarios Torrevieja y Elche-Vinalopó, Nº Reg: 2020.028) was obtained prior to the start of the study. Signed informed consent was obtained from all participants.

3. Results

3.1. Results from the first trimester of pregnancy.

Surplus of routine first trimester blood samples from 503 pregnant women were identified between January 1st and May 15th, 2020, in Hospital Universitario Torrejón in Madrid. 480 of the women were eligible, agreed, gave their consent to participate in the study and had their blood samples tested for anti-SARS-CoV2 specific antibodies. 10 of these were excluded due to insufficient sample for analysis (n=4) or lost to follow up very early in their pregnancy (n=6).

Finally, blood samples from 470 women were obtained (Table 1), including 31 (6.6%) samples which tested positive for SARS-CoV2 antibodies, either IgA and/or IgG.

3.2. Results from the third trimester of pregnancy.

Of the 470 women with results from the first trimester testing, seven had an early miscarriage (including 1 positive case in the first trimester), four a late miscarriage, three terminated the pregnancy and 54 were lost to follow up (including 3 positive cases in the first trimester). Therefore, 402 samples were available for anti-SARS-CoV2 specific antibodies testing in the third trimester, including 27 cases that were positive in the first trimester. 66 (16.4%) of the 402 third trimester samples tested positive, including 18 that had a positive result in the first trimester and 336 (83.6%) tested negative, including 9 that had a positive result in the first trimester. Therefore, seroconversion during pregnancy occurred in 48 cases with complete follow up (48, 12.8%, of the 375 negative pregnancies in the first trimester) (Figures 1 and 2), being statistically significantly higher than the seroconversion rate in the first trimester ($p = 0.003$).

3.3. Persistence of antibodies among the pregnancy.

From the 31 women with positive serology in the first trimester, 27 had their third trimester blood samples tested for COVID-19 (1 had an early miscarriage and 3 were lost to follow up). 18 (66.7 %) of the 27 cases with complete follow-up still had a positive anti-SARS-CoV2 serology in the third trimester, while 9 (33.3%) had negative both, IgG and IgA anti-SARS-CoV2 (supplementary Table A2).

3.4. Maternal morbidity.

Among the positive cases, there were no differences in baselines characteristics between women who seroconverted in the first trimester (n= 31) and those who did so in the third trimester (n= 48) (Table 1).

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No statistically significant differences were observed in maternal or obstetric morbidity (gestational hypertension, preeclampsia, gestational diabetes, fetal growth disorders, fetal anomalies, and other obstetrics complications as cholestasis, Rh isoimmunization, preterm birth and shortened cervix) according to the trimester of seroconversion (Table 2).

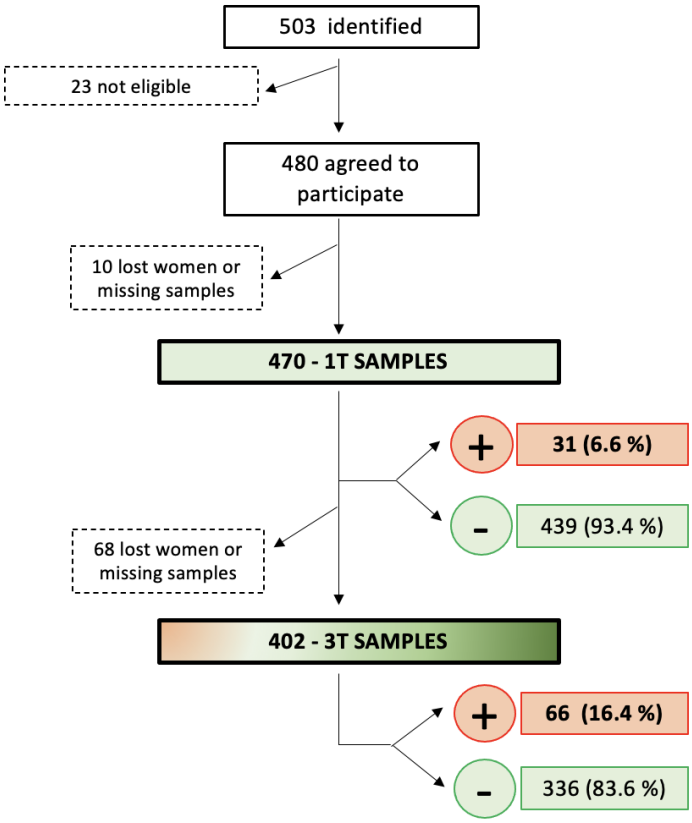

Figure 1. Patients' flowchart, study process and COVID-19 seroprevalence rate in first and third trimester of pregnancy.

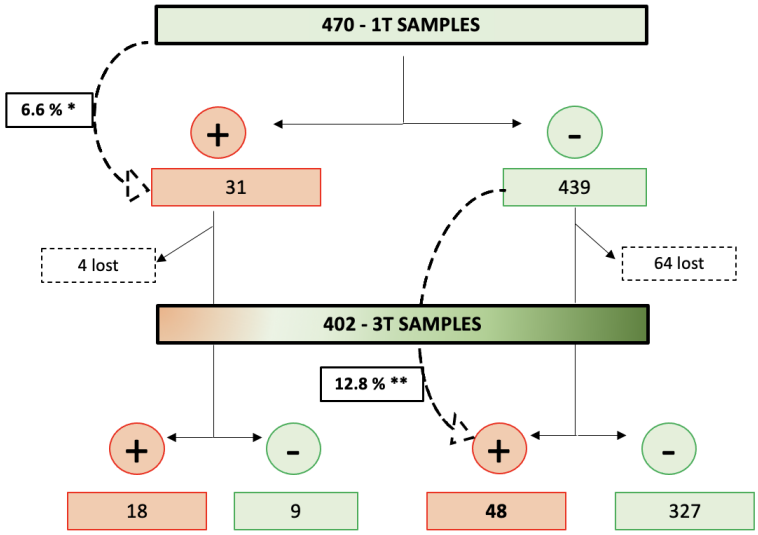

Figure 2. COVID-19 seroconversion rate throughout the pregnancy. * First trimester seroconversion rate.** Third trimester seroconversion rate.

Table 1. Maternal baseline characteristics.

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VARIABLE	POSITIVE SEROLOGY 1T (n=31)	POSITIVE SEROLOGY 3T (n= 48)	p value
Gestational age (weeks)	39.6 [38.9; 40.0] Missing: 2 (6.5%)	40.0 [38.6; 40.5] Missing: 0 (0%)	0.285
Maternal age (years)	35.0 [29.0; 38.0]	33.5 [29.8; 36.3]	0.398
Body mass index (kg/m²)	22.9 [21.5; 26.4]	25.3 [22.8; 28.6]	0.110
Nulliparus	17 (54.8 %)	20 (41.7 %)	0.356
Race			
Black	1 (3.2 %)	2 (4.2 %)	1.000
Non-hispanic white	22 (71.0 %)	37 (77.1 %)	0.601
Hispanic / latin	7 (22.6 %)	6 (12.5 %)	0.352
Asian	0	1 (2.1%)	1.000
North - african	1 (3.2 %)	1 (2.1%)	1.000
Other	0	1 (2.1%)	1.000
Blood type			
A Positive	12 (38.7 %)	15 (31.3 %)	0.628
A Negative	1 (3.2 %)	1 (2.1%)	1.000
O Positive	15 (48.4 %)	21 (43.8 %)	0.818
O Negative	1 (3.2 %)	3 (6.3 %)	1.000
B Positive	2 (6.5 %)	6 (12.5 %)	0.470
B Negative	0	0	1.000
AB Positive	0	1 (2.1%)	1.000
AB Negative	0	1 (2.1%)	1.000
Active smoking	0	5 (10.4 %)	0.151
Chronic medical pathology			
None	24 (77.4 %)	33 (68.8 %)	0.451
Hypertensive disorders	1 (3.2 %)	0	0.392
Diabetes Mellitus	0	0	1,000
Autoinmune or Inmunological disorders	2 (6.5 %)	0	0.151
Respiratory disease	1 (3.2 %)	2 (4.2%)	1.000
Others	7 (22.6 %)	15 (31.3 %)	0.451

1T: first trimester; 3T: third trimester.

Table 2. Obstetric complications according to serology group. .

	POSITIVE SEROLOGY 1T (n 30 *)	POSITIVE SEROLOGY 3T (n 48)	p value
None	23 (76.7 %)	33 (68.8 %)	0.606
Gestational Hypertension	1 (3.4 %)	0	0.385
Preeclampsia	0	0	1.000
Gestational Diabetes	3 (10.0 %)	3 (6.3 %)	0.670
Preterm birth	1 (3.3 %)	2 (4.2 %)	1.000
Other (Cholestasis, Rh isoinmunization, shortened cervix, plaquetopenia…)	0	1 (2.1%)	1.000
Small for gestational age	0	4 (8.3 %)	0.156
Intrauterine growth restriction	1 (3.3 %)	3 (6.3 %)	1.000

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Fetal anomalies	1 (3.3 %)	2 (4.2 %)	1.000

* n= 30, as one woman was excluded from the positive serology 1T group (initially n= 31) due to early miscarriage in her first trimester of pregnancy. 1T: first trimester; 3T: third trimester; Rh: Rhesus.

4. Discussion

4.1. Main findings of the study

The main finding of this study is that, during the first COVID-19 pandemic peak the seroconversion rate in the third trimester (12.8 %) was double than that in the first trimester (6.6 %). However, obstetric, or maternal complications did not differ between groups. Besides, we demonstrated that about two thirds (18/27) of the women with a positive serology in the first trimester, remained positive in the third trimester, showing that immunity may last for several months.

4.2. Comparison with previous studies

Only a few studies have evaluated the seroprevalence of SARS-CoV-2 infection at different stages of pregnancy during the 2020 outbreak of COVID-19 in Spain. The reported prevalence of positive serological tests in pregnant women in our country varied from 15% in the first trimester [12], to 20% in the third trimester and delivery [12–15], although the geographical location and time within the pandemic were different from our study and therefore, difficult to compare. The first explanation for the higher seroconversion rate in the third trimester found in our cohort may be due to maternal immunological changes that increase predisposition to infection along the second and third trimesters of pregnancy [5,16,17]. However, we have previously demonstrated that in our region, the rate of infection between pregnant women was similar to that reported in the general population [7,18]. Therefore, we believe that this finding would be better explained by the end of social isolation and other preventive measures that occurred during the summer period and lead to the second wave of the COVID-19 pandemic, rather than biological susceptibility.

Regarding obstetric and maternal morbidity, we expected similar effect in pregnancy as that reported in the literature in previous pandemics (MERS, SARS-CoV-1 and Influenzae), with higher rate of miscarriage if the infection happened in first trimester of pregnancy and more cases of IUGR in late pregnancy infection [3–5]. Many studies have found higher rates of obstetrics complications in SARS-CoV2 infection, such as preterm birth, premature rupture of membranes, low birth weight and stillbirth [19–22], but some others have not found any significant differences in obstetric complications [23–25].

The current knowledge about COVID-19 infection in different trimesters of pregnancy is still limited. In an attempt to compare obstetric morbidity according to the trimester of the infection, increasing evidence has been published, describing a higher incidence of adverse fetal outcome (including stillbirth, perinatal and neonatal death) and preterm birth in those pregnant women who were infected by SARS-CoV2 in their first trimester, while only lower fetal growth percentile and higher rate of small for gestational age (SGA) fetus has been described in pregnancies infected in the third trimester [22,26].

However, there is insufficient data assessing immunological status throughout pregnancy and, normally, only acute infection by rRT-PCR SARS-CoV2 has been assessed at a single time point. This could be leading to a selection bias, as the majority of the SARS-CoV2 infected population is actually asymptomatic and therefore no rRT-PCR will have been performed [5]. Mascio et al [22] analyzed 388 pregnancies that had a positive rRT-PCR SARS-CoV2 test during pregnancy, describing how perinatal outcomes (stillbirth, perinatal and neonatal death and preterm birth) were significantly worse with decreasing gestational age at the time of infection. In a retrospective study evaluating 882 positive pregnant women by rRT-PCR SARS-CoV2, including 85 diagnosed in the first trimester, reported that gestational age at the time of infection was the best predictor for gestational age at delivery [26]. To the best of our knowledge, only one study has been conducted to assess serology during both the first and third trimesters while examining the potential association between

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antibodies and pregnancy outcomes [27]. In this study, which involved 528 women, the authors performed serological assessments during the initial 11-13 screening visit and again upon admission for delivery, yet they did not discover any significant association between serological status and major obstetric complications. In contrast, our study conducted a third-trimester analysis at 35-36 weeks, which likely provides a more comprehensive evaluation of newly emerging complications.

Regarding serology testing for SARS-CoV-2 infection across different trimesters of pregnancy, there is a smaller-scale study involving 149 women who were assessed for anti-SARS-CoV-2 IgG antibodies during the first and second trimesters, as well as at birth [28]. The outcomes from this study were comparable to our own findings. They reported a seroprevalence rate of 12.1% during the first trimester and 16.1% during the second trimester. Notably, 71.4% of the women who tested positive during the first trimester remained positive at the time of delivery, which is similar to the 66.7% observed in our cohort. However, it's important to note that this study did not evaluate obstetric morbidity.

The high virulence of SARS-CoV-2, coupled with the ongoing debate about its potential adverse effects on pregnancy, underscores the importance of continued scientific research. Researchers should remain committed to exploring existing data, to be better prepared for potential future viral threats. Serological screening stands as a valuable tool that can provide higher-quality evidence regarding the disease's natural progression, severity, and prognosis based on the timing of infection, enhancing the clinical management of infected women.

4.3. Strengths and limitations

The main strength of our study is the longitudinal follow-up of a consecutive sample of pregnant women who were in their first trimester of pregnancy during the COVID-19 first outbreak in one of the most severely affected countries in Europe at that time. This has allowed us to analyze two blood samples, corresponding to the first and third trimesters, coinciding with the first and second waves of the pandemic. Testing these samples for anti-SARS-CoV2 immunoglobulins at these two different moments of the pregnancy, was a unique opportunity to improve our knowledge on the immune response in pregnancy, impact of social security measures on COVID-19 incidence and the relevance of the moment of the infection for pregnancy outcome.

We consider that the main limitation of our study relates to the small sample size which might be responsible for the lack of significant differences in the results and has prevented us to perform any subgroup analysis. Additionally, we did not record individual measures to prevent infection, therefore we have assumed that women were compliant with governmental restrictions.

5. Conclusions

COVID-19 seroconversion rate was higher in third than in first trimester of pregnancy, with most of the women infected during their first trimester remaining positive throughout gestation. Implementations of SARS-CoV2 serological test as part of Obstetric control in every trimester rutinary analysis can detect asymptomatic cases and reflect an accurate COVID-19 seroconversion rate. Although no differences were found neither in maternal nor obstetric complications according to the trimester of infection, larger studies are still needed.

6. Patents

This section is not mandatory but may be added if there are patents resulting from the work reported in this manuscript.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Figure S1: title; Table S1: title; Video S1: title.

Author Contributions: Conceptualization: MNR, AAP, IFB, MMG; Data curation: MNR, AAP; Formal analysis: MNR, AAP, MRF; Investigation: MNR, AAP, IFB, LGG, CGG, BS, MMG; Methodology: MNR, AAP, MRF, MST, DTLP, MMG; Project administration: MMG, BS; Supervision: AAP, IFB, MMG, BS; Validation: MNR, AAP, IFB,

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MRF, DTLP, MMG, BS; Writing – original draft: MNR, AAP, MMG; Writing – review & editing: MNR, AAP, IFB, BS, MMG.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institu-tional Review Board (Comité Ético de Investigación con Medicamentos de los Hospitales Universitarios Torrevieja y Elche-Vinalopó, Nº Reg: 2020.028, June 29th, 2020) was obtained prior to the start of the study. Signed informed consent was obtained from all participants.**T**he Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement was used for reporting the results.

Informed Consent Statement: Written informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to data protection regulations.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A1.

Table A1. Serological and clinical characteristics of the 48 women with positive COVID third trimester serology with prior negative testing in first trimester of pregnancy. Classification was performed as follows: presence of IgG anti-SARS-CoV2 was classified as 'past infection'; isolated IgA with no COVID-19 related symptoms was considered as 'exposure to viral infection' and isolated IgA and either medical history of COVID-19 related symptoms, positive contact prior to blood extraction or a positive result for an additional SARS-CoV2 test, were considered 'recent infection'.

INTERPRETATION 1T	NO SYMPTOMS	MILD SYMPTOMS	SEVERE SYMPTOMS	IgA 3T	IgG 3T	rRT-PCR	POSITIVE ADDITIONAL TEST	INTERPRETATION 3T
Negative	X			+	-			EXPOSURE
Negative	X			+	+/-			EXPOSURE
Negative	X			+	-			EXPOSURE
Negative	X			+	-			EXPOSURE
Negative	X			+	-			EXPOSURE
Negative	X			+	-			EXPOSURE
Negative	X			+	-			EXPOSURE
Negative	X			+	+/-			EXPOSURE
Negative	X			+	-			EXPOSURE
Negative	X			+	-			EXPOSURE
Negative	X			+	-			EXPOSURE
Negative	X			+	-			EXPOSURE
Negative	X			+	-			EXPOSURE
Negative	X			+	-			EXPOSURE
Negative	X			+	-			EXPOSURE
Negative	X			+	-			EXPOSURE
Negative	X			+	-			EXPOSURE

Negative	X			+	-			EXPOSURE
Negative	X			+	-			EXPOSURE
Negative	X			+	-			EXPOSURE
Negative	X			+				EXPOSURE
Negative	X			+	-			EXPOSURE
Negative	X			+	-			EXPOSURE
Negative	X			+	+		X (SEM 39)	RECENT INFECTION
Negative	X			+	+			PAST INFECTION
Negative	X			+	+			PAST INFECTION
Negative	X			-	+			PAST INFECTION
Negative		X		+/-	+		X	PAST INFECTION
Negative	X			-	+			PAST INFECTION
Negative		X		+	-		X	PAST INFECTION
Negative	X			+	+			PAST INFECTION
Negative			X	+	+			PAST INFECTION
Negative		X		+	+			PAST INFECTION
Negative		X		+	+		X	PAST INFECTION
Negative		X		+	+			PAST INFECTION
Negative		X		+/-	+/-		X	PAST INFECTION
Negative	X			-	+			PAST INFECTION
Negative		X		+	+/-			PAST INFECTION
Negative		X		+	+		X	PAST INFECTION
Negative	X			+	+			PAST INFECTION
Negative		X		+	-			PAST INFECTION
Negative	X			+	+			PAST INFECTION
Negative		X		+	+	+		PAST INFECTION
Negative	X			+	+			PAST INFECTION
Negative	X			+	+			PAST INFECTION

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Negative	X	-/+	x	X	**PAST INFECTION**
Negative	X	+	+		**PAST INFECTION**
Negative	X	+	+		**PAST INFECTION**

Appendix A2.

Table A2. Pregnant women with persistent COVID-19 serology in third trimester of pregnancy.

IgA 1T	IgG 1T	POSITIVE CONTACT	NO SYMPTOMS	MILD SYMPTOMS	SEVERE SYMPTOMS	INTERPRETATION 1T	IgA 3T	IgG 3T	rRT-PCR	POSITIVE ADDITIONAL TEST	INTERPRETATION 3T
+	-		X			EXPOSURE	+	-			EXPOSURE
+	-		X			EXPOSURE	+	-		X	EXPOSURE
+	-		X			EXPOSURE	+	-			EXPOSURE
+	-		X			EXPOSURE	+	-			EXPOSURE
+	-		X			EXPOSURE	+	-			EXPOSURE
+	-			X		RECENT INFECTION	+	-			PAST INFECTION
+	-			X		RECENT INFECTION	+	-			PAST INFECTION
+	+		X			PAST INFECTION	+	+			PAST INFECTION
+	+			X		PAST INFECTION	+	+			PAST INFECTION
+	+			X		PAST INFECTION	+	+		X	PAST INFECTION
+	+			X		PAST INFECTION	+	+		X	PAST INFECTION
+	+			X		PAST INFECTION	+/-	+/-			PAST INFECTION
+	+	X		X		PAST INFECTION	+	+		X	PAST INFECTION
+	+			X		PAST INFECTION	+	+		X	PAST INFECTION
+	+		X			PAST INFECTION	+	+			PAST INFECTION
+	+		X			PAST INFECTION	+	+		X	PAST INFECTION
+	+		X			PAST INFECTION	+	+			PAST INFECTION
+	+			X		PAST INFECTION	+	-		X	PAST INFECTION

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