

This is the peer reviewed version of the following article:

IGG ANTIBODY LEVELS AGAINST THE SARS-COV-2 SPIKE PROTEIN IN  
MOTHER-CHILD DYADS AFTER COVID-19 VACCINATION

Muñoz-Gómez, M.J., Martín-Vicente, M., Vigil-Vázquez, S. et al. IgG antibody levels against the SARS-CoV-2 spike protein in mother–child dyads after COVID-19 vaccination. *Infection* 52, 813–824 (2024).  
<https://doi.org/10.1007/s15010-023-02111-x>.

which has been published in final form at:

<https://doi.org/10.1007/s15010-023-02111-x>

# Title page

**Type of manuscript:** Original article

**Title:** IgG antibody levels against the SARS-CoV-2 spike protein in mother-child dyads after COVID-19 vaccination

**Running head:** Maternal-child IgG antibody response to COVID-19 vaccine

**Authors:** María José Muñoz-Gómez <sup>1,2 (‡)</sup>, María Martín-Vicente <sup>1, (‡)</sup>, Sara Vigil-Vázquez <sup>3, (‡)</sup>, Itziar Carrasco <sup>3</sup>, Alicia Hernanz Lobo <sup>2,4,5</sup>, Vicente Mas <sup>6</sup>, Mónica Vázquez <sup>6</sup>, Angela Manzanares <sup>5</sup>, Olga Cano <sup>6</sup>, Clara Zamora <sup>7</sup>, Roberto Alonso <sup>8</sup>, Daniel Sepulveda-Crespo <sup>1,2</sup>, Laura Tarancon-Diez <sup>9</sup>, María Ángeles Muñoz-Fernández <sup>9,10,11</sup>, Mar Muñoz-Chapuli <sup>7</sup>, Salvador Resino <sup>1,2 (\* ¥)</sup>, María Luisa Navarro <sup>2,4,5,12 (¥)</sup>, Isidoro Martínez <sup>1,2 (\* ¥)</sup>

(‡) M.M.V., S.V.V., and M.J.M.G. contributed equally to this study; (¥) S.R., M.L.N, and I.M. contributed equally to this study; (\*) Corresponding author.

## Authors' affiliations:

(1) Unidad de Infección Viral e Inmunidad, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain.

(2) Centro de Investigación Biomédica en Red en Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III, Madrid, Spain.

(3) Servicio de Neonatología, Hospital General Universitario Gregorio Marañón, Madrid, Spain

(4) Grupo de Investigación en Infectología Pediátrica, Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain.

(5) Servicio de Pediatría, Hospital General Universitario Gregorio Marañón, Madrid, Spain

(6) Unidad de Biología Viral, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Madrid, Spain.

(7) Servicio de Obstetricia y Ginecología, Hospital General Universitario Gregorio Marañón, Madrid, Spain

(8) Departamento de Microbiología Clínica y Enfermedades Infecciosas, Hospital General Universitario Gregorio Marañón, Madrid, Spain.

(9) Laboratorio de Inmunobiología Molecular, Sección de Inmunología. Hospital General Universitario Gregorio Marañón, Madrid, Spain.

(10) Spanish HIV-HGM BioBank, Madrid, Spain.

(11) Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Instituto de Salud Carlos III, Madrid, Spain.

(12) Universidad Complutense de Madrid, Madrid, Spain.

## Correspondence and requests for reprints:

**Corresponding author:** Salvador Resino; Centro Nacional de Microbiología, Instituto de Salud Carlos III (Campus Majadahonda); Carretera Majadahonda- Pozuelo, Km 2.2; 28220 Majadahonda (Madrid); Phone: +34918223266. E-mail: [sresino@isciii.es](mailto:sresino@isciii.es)

**Alternative corresponding author:** Isidoro Martínez; Centro Nacional de Microbiología, Instituto de Salud Carlos III (Campus Majadahonda); Carretera Majadahonda- Pozuelo, Km 2.2; 28220 Majadahonda (Madrid); Phone: +34918223272. E-mail: [imago@isciii.es](mailto:imago@isciii.es)

## Emails:

María José Muñoz-Gómez: [munozgomez.mjose@gmail.com](mailto:munozgomez.mjose@gmail.com)

María Martín-Vicente: [maria.mvicente92@gmail.com](mailto:maria.mvicente92@gmail.com)

Sara Vigil Vázquez: [saravv90@gmail.com](mailto:saravv90@gmail.com)

Itziar Carrasco: [itziiaarcg@gmail.com](mailto:itziiaarcg@gmail.com)

Alicia Hernanz Lobo: [h.lobo.alicia@gmail.com](mailto:h.lobo.alicia@gmail.com)

Vicente Mas: [vmass@isciii.es](mailto:vmass@isciii.es)

Mónica Vázquez: [mvazquez@isciii.es](mailto:mvazquez@isciii.es)

Angela Manzanares: [angela.manzacas@gmail.com](mailto:angela.manzacas@gmail.com)

Olga Cano: [olgacm@isciii.es](mailto:olgacm@isciii.es)

Clara Zamora: [clara.zdp@gmail.com](mailto:clara.zdp@gmail.com); [clara.zamora@salud.madrid.org](mailto:clara.zamora@salud.madrid.org)

Roberto Alonso: [roberto.alonso@salud.madrid.org](mailto:roberto.alonso@salud.madrid.org)

Daniel Sepúlveda-Crespo: [danisecre@hotmail.com](mailto:danisecre@hotmail.com)

Laura Tarancón-Díez: [ltarancondiez@gmail.com](mailto:ltarancondiez@gmail.com)

María Ángeles Muñoz-Fernández: [mmunoz.hgugm@gmail.com](mailto:mmunoz.hgugm@gmail.com)

Mar Muñoz-Chapuli: [mar.mchg@gmail.com](mailto:mar.mchg@gmail.com); [mar.munozchapuli@salud.madrid.org](mailto:mar.munozchapuli@salud.madrid.org)

Salvador Resino: [sresino@isciii.es](mailto:sresino@isciii.es)

María Luisa Navarro: [marisa.navarro.gomez@gmail.com](mailto:marisa.navarro.gomez@gmail.com)

Isidoro Martínez: [imago@isciii.es](mailto:imago@isciii.es)

**Character count of Title:** 108

**Word count of Text:** 3277

**Character count of Running Head:** 57

**Count of References:** 61

**Word count of Abstract:** 270

**Count of Tables:** 2

**Word count of Keywords:** 6

**Count of Figures:** 4

## Abstract

**Purpose:** We aimed to assess IgG antibodies against the SARS-CoV-2 spike protein (anti-SARS-CoV-2 S IgG) in vaccinated mothers and their infants at delivery and 2-3 months of age.

**Methods:** We conducted a prospective study on mothers who received at least one dose of the COVID-19 vaccine (Pfizer-BNT162b2, Moderna mRNA-1273, or Oxford-AstraZeneca ChAdOx1-S) during pregnancy and on their infants. The baseline was at the time of delivery (n=93), and the end of follow-up was 2 to 3 months post-partum (n=53). Serum anti-SARS-CoV-2 S IgG titers and ACE2 binding inhibition levels were quantified by immunoassays.

**Results:** Mothers and infants had high anti-SARS-CoV-2 S IgG titers against the B.1 lineage at birth. However, while antibody titers were maintained at 2-3 months post-partum in mothers, they decreased significantly in infants ( $p<0.001$ ). Positive and significant correlations were found between anti-SARS-CoV-2 S IgG titers and ACE2 binding inhibition levels in mothers and infants at birth and 2-3 months post-partum ( $r>0.8$ ,  $p<0.001$ ). Anti-S antibodies were also quantified for the Omicron variant at 2-3 months post-partum. The antibody titers against Omicron were significantly lower in mothers and infants than those against B.1 ( $p<0.001$ ). Again, a positive correlation was observed for Omicron between IgG titers and ACE2 binding inhibition both in mothers ( $r=0.818$ ,  $p<0.001$ ) and infants ( $r=0.386$ ,  $p<0.005$ ). Previous SARS-CoV-2 infection and COVID-19 vaccination near delivery positively impacted anti-SARS-CoV-2 S IgG levels.

**Conclusions:** COVID-19 mRNA vaccines induce high anti-SARS-CoV-2 S titers in pregnant women, which can inhibit the binding of ACE2 to protein S and are efficiently transferred to the fetus. However, there was a rapid decrease in antibody levels at 2 to 3 months post-partum, particularly in infants.

## Keywords

SARS-CoV-2; spike glycoprotein; antibody; vaccine, pregnant; infant

## **Introduction**

The Coronavirus Disease 2019 (COVID-19) pandemic continues to cause high morbidity and mortality worldwide as new, highly transmissible Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) variants emerge. Vaccination is the best option to avoid severe COVID-19 pathology [1]. Several vaccine formulations are being administered globally, although mRNA-based vaccines (BNT162b2 from Pfizer-BioNTech and mRNA-1273 from Moderna) are the most widely distributed in Western countries. These vaccines induce specific neutralizing antibodies against the “spike” or S glycoprotein of SARS-CoV-2, which mediates attachment to the angiotensin-converting enzyme 2 (ACE2) receptor on host cells [2, 3].

Pregnant women are at an increased risk of COVID-19-related intensive care admission, mortality, preterm delivery, pregnancy loss, and stillbirth [4-10]. These findings may be related to the significant physiological and immunologic changes during normal pregnancy to ensure fetal growth [11]. Additionally, although COVID-19 is usually mild in infants, severe illness and hospitalization can happen in this population [12, 13]. Therefore, protecting the mother-infant dyad against COVID-19 is crucial, and pregnant women should get vaccinated and stay up-to-date with their COVID-19 shots [14].

Although pregnant women were excluded from early vaccine clinical trials for safety and ethical reasons, current data show that vaccination with mRNA vaccines for COVID-19 is safe and prevents severe COVID-19 in them [15-19]. Additionally, vaccine-induced antibodies in mothers may protect their infants through transplacental antibody transfer and breast milk [20-28]. Several reports have indicated that COVID-19 vaccination of pregnant women induces even higher anti-S IgG titers than natural infection. Furthermore, these antibodies are efficiently transferred to the fetus [18, 29-34], particularly following booster vaccination [35]. As a result, neutralizing IgG levels against the S protein have been reported to be higher in infants born to vaccinated mothers than in those born to SARS-CoV-2-infected mothers [36], encouraging vaccination of pregnant women. Nevertheless, some reports have noted that maternal and infant IgG antibody levels decrease over time [34, 36]. Therefore, additional studies are necessary to confirm the magnitude, duration, and efficacy of the vaccine-induced humoral immune response.

## **Objective**

This study aimed to analyze the IgG antibody response against the SARS-CoV-2 spike protein, the primary target of neutralizing antibodies, in vaccinated mothers and their infants at delivery and 2-3 months of age.

# Materials and Methods

## Study design

We conducted a prospective study on pregnant women who were vaccinated with two doses of the COVID-19 vaccine, or one dose if they had previously been infected with SARS-CoV-2, at least one month before delivery. Some women were also vaccinated within 2-3 months after delivery. The study included their infants. All participants were from the Hospital General Universitario Gregorio Marañón (HGUGM) in Madrid, Spain, and were recruited within the GESNEO-COVID Cohort [37, 38].

The baseline for the study was at the time of delivery, between September 2021 and December 2021. At this point, we collected 93 serum samples from mothers and babies. The end of the follow-up period was 2 to 3 months post-partum, between December 2021 and March 2022. At this time, serum samples were only available from 53 mothers and infants, those who returned to the HGUGM pediatric care facility 2-3 months after delivery.

The HGUGM Ethics Committee approved the study (Ref.: IRB 00006051), which was conducted in accordance with the Declaration of Helsinki. All participants provided their informed consent before enrollment.

## Clinical data and samples

Epidemiological and clinical data from mothers and babies were collected from the hospital's electronic medical records. Serum samples from mothers were obtained via venipuncture at the time of delivery and again 2-3 months later. Serum samples from babies were collected either from heel blood (concurrent with blood collection for metabolic tests) or via venipuncture within 48 hours of life. All samples were received, processed, and stored in the laboratory of the HGUGM Microbiology Service.

## Previous SARS-CoV-2 infection

Serum samples from mothers and infants at delivery and 2-3 months post-partum were tested for SARS-CoV-2 infection by detecting antibodies (IgM/IgA/IgG) against the SARS-CoV-2 N protein using a commercial enzyme-linked immunosorbent assay (ELISA) (Platelia SARS-CoV-2 Total Ab, Bio-Rad Laboratories Inc., Hercules, California, USA). A sample was considered positive when the optical density ratio between the test and control samples included in the kit was greater than 1 (ratio  $\geq 1.0$ ). The sensitivity and specificity of this cut-off are 94.7% and 97.5%, respectively [39]. Previous infections were differentiated from recent infections based on anti-N antibody data (IgM/IgA/IgG) in the series of available samples. When a sample was positive for anti-N antibodies at delivery, it was considered positive. If a mother or infant had a negative sample at delivery but tested positive at 2-3 months after delivery, this indicated an infection during the follow-up period.

## Immunoassay for anti-SARS-CoV-2 S IgG quantification

A complete description of the materials and protocols for antibody quantification is given in Martin-Vicente et al. [21].

Briefly, the plasmid p $\alpha$ H, encoding the S protein ectodomain (residues 1-1208) of SARS-CoV-2 2019-nCoV (GenBank: MN908947) stabilized in the prefusion conformation, was kindly provided by Dr. Jason McLellan (University of Texas at Austin, USA) [40]. Mutagenesis was performed to create a HexaPro construct that enabled high-yield production of a stabilized prefusion spike protein [41]. The ectodomain of this protein includes the following substitutions: glycine at residue 614 (D614G), a "GSAS" substitution at the furin cleavage site (residues 682–685), and proline at residues 817, 892, 899, 942, 986, and 987. Throughout this text, we will be referred to this protein as B.1. The SARS-CoV-2 S Omicron (B.1.1.529) HexaPro construct contains the natural cleavage site "RRAR" (residues 682-685) and the following Omicron specific mutations: A67V,  $\Delta$ 69-70, T95I, G142D/ $\Delta$ 143-145,  $\Delta$ 211/L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, and L981F. Additionally, a plasmid encoding the ACE2 SARS-CoV-2 cell receptor (residues 1-165) was also constructed and fused to a StrepTag.

Antibody titers to the S protein were determined in an ELISA assay by incubating serial 1:3 dilutions of serum samples, starting from a 1:50 dilution and ending at a 1:36450 dilution, with the purified S protein ectodomain. One phase exponential decay least-squares fit curves, and the area under the curve (AUC) were calculated using GraphPad Prism 9.0. Antibody inhibition of the ACE2-S protein interaction was tested by ELISA. A pool of sera from individuals negative for anti-S antibodies, collected in 2016, was used as a control. After subtracting the background, the percentage of inhibition was calculated as  $[1 - (\text{OD}_{493} \text{ test serum} / \text{OD}_{493} \text{ control serum})] \times 100 \%$ .

### **Statistical analysis**

Figures were generated with GraphPad Prism 9.0 (GraphPad Software, Inc., San Diego, CA, USA). The Y axis of the figures was transformed to a logarithmic scale (log<sub>10</sub>) to compress it.

Statistical analysis was performed with IBM SPSS Statistics 25.0 (SPSS INC, Armonk, NY, USA) and Stata 15.0 (StataCorp, Texas, USA). The significance level was set at  $p < 0.05$  (two-tailed). The Kolmogorov-Smirnov test was used for evaluating data normality, finding that the antibody level variables had a non-normal distribution. Data were presented as follows: absolute counts (percentages) for categorical variables and median (interquartile range, IQR=P<sub>25</sub>th; P<sub>75</sub>th) for continuous variables. The Mann-Whitney U test was used to calculate differences between independent groups, and the Wilcoxon test for dependent groups. Correlation analysis was performed using the Spearman test. Generalized Linear Models (GLM) with a gamma distribution (log-link) were used to assess the association between various factors, such as previous SARS-CoV-2 infection and the timing of COVID-19 vaccination, and anti-SARS-CoV-2 S IgG levels. The GLM with a gamma distribution and log-link is an alternative way of analyzing right-skewed continuous variables. This test provides the arithmetic mean ratio (AMR) and the 95% confidence interval (95% CI). GLMs were also adjusted by the participant's characteristics, including age at delivery, comorbidities (hypothyroidism, diabetes, hypertension, preeclampsia, and others), and gestational age.

# Results

## Patient characteristics

The clinical characteristics of mother-child dyads after COVID-19 vaccination are shown in **Table 1**. Among the mothers, 11.8% (11/93) tested positive for anti-N antibodies (IgM/IgA/IgG) at the time of delivery. At 2-3 months after delivery, 39.6% (21/53) tested positive for anti-N antibodies. Among these, 18.8% (10/53) became positive during the follow-up period. As for the infants, 20.7% (11/53) tested positive for anti-N antibodies (IgM/IgA/IgG) at 2-3 months after delivery, with 11.3% (6/53) acquiring positivity during the follow-up period. All SARS-CoV-2 infections were asymptomatic or had mild symptoms; none required hospital admission.

**Table 1.** Clinical characteristics of mothers and their infants.

<b>Parameters</b>	<b>Values</b>
<b>A) Mothers</b>	
<b>No.</b>	93
<b>Age (years)</b>	34.8 (32.2; 39.1)
<b>Country of birth</b>	
<b>Spain</b>	74 (79.6%)
<b>Latin American Countries</b>	15 (16.1%)
<b>Other</b>	4 (4.3%)
<b>Comorbidities</b>	
<b>Hypertension</b>	5 (5.4%)
<b>Diabetes</b>	8 (8.6%)
<b>Hypothyroidism</b>	8 (8.6%)
<b>Preeclampsia</b>	6 (6.5%)
<b>Other</b>	3 (3.2%)
<b>SARS-CoV-2 Infection (+)</b>	
<b>Pre-perinatal (pre-pregnancy and pregnancy)</b>	11 (11.8%)
<b>Postnatal (n=53)</b>	21 (39.6%)
<b>B) Newborns</b>	
<b>No.</b>	93
<b>Gestational age (weeks)</b>	39.7 (38.3; 40.4)
<b>Mode of delivery</b>	
<b>Vaginal</b>	81 (87.1%)
<b>Cesarean</b>	12 (12.9%)
<b>Sex (male)</b>	53 (57.0%)
<b>Breastfeeding</b>	88 (94.6%)
<b>Condition at birth</b>	
<b>APGAR1</b>	9 (9; 9)
<b>APGAR5</b>	10 (10; 10)
<b>Weight (gr.)</b>	3.300 (3.000; 3.500)
<b>Height (cm)</b>	50 (49; 51)
<b>Head circumference (cm)</b>	34 (33; 35)
<b>SARS-CoV-2 Infection (+)</b>	
<b>Postnatal (n=53)</b>	11 (20.7%)

**Statistics:** Values are expressed as the median (Q1; Q3) and absolute count (percentage).

**Abbreviations:** SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; APGAR, Appearance, Pulse, Grimace, Activity, and Respiration; APGAR1, 1-minute APGAR; APGAR5, 5-minute APGAR.

**Table 2** provides detailed information about COVID-19 vaccination among the mothers in the study. All vaccine antigens used were based on the Wuhan-Hu-1 strain. In the first dose, 66 (71%) received the Pfizer BNT162b2 mRNA vaccine, 26 (27.9%) received the Moderna mRNA-1273 vaccine, and 1 (1.1%) received the Oxford-AstraZeneca ChAdOx1-S vaccine. Additionally, 79 (84.9%) of the mothers received a second dose before delivery, and 6 (11.3%) received a third dose at 2-3 months post-partum (before sample collection).

**Table 2.** Characteristics of COVID-19 vaccination.

Parameters	Values
<b>No.</b>	93
<b>SARS-CoV-2 vaccine</b>	
<b>First dose</b>	
<b>Pfizer-BioNTech (BNT162b2)</b>	66 (71.0%)
<b>Moderna (mRNA-1273)</b>	26 (27.9%)
<b>Oxford-AstraZeneca (ChAdOx1-S)</b>	1 (1.1%)
<b>Second dose (n =79)</b>	
<b>Pfizer-BioNTech (BNT162b2)</b>	57 (72.2%)
<b>Moderna (mRNA-1273)</b>	22 (27.8%)
<b>Third dose (n=6)</b>	
<b>Pfizer-BioNTech (BNT162b2)</b>	4 (66.7%)
<b>Moderna (mRNA-1273)</b>	2 (33.3%)
<b>SARS-CoV-2 vaccine before pregnancy (n=6)</b>	
<b>One dose</b>	
<b>Pfizer-BioNTech (BNT162b2)</b>	2 (66.7%)
<b>Moderna (mRNA-1273)</b>	1 (33.3%)
<b>Two doses</b>	
<b>First dose</b>	
<b>Pfizer-BioNTech (BNT162b2)</b>	2 (66.7%)
<b>Moderna (mRNA-1273)</b>	1 (33.3%)
<b>Second dose</b>	
<b>Pfizer-BioNTech (BNT162b2)</b>	2 (66.7%)
<b>Moderna (mRNA-1273)</b>	1 (33.3%)
<b>SARS-CoV-2 vaccine during pregnancy</b>	
<b>One dose</b>	
<b>Pfizer-BioNTech (BNT162b2)</b>	10 (71.4%)
<b>Moderna (mRNA-1273)</b>	4 (28.6%)
<b>Two doses</b>	
<b>First dose</b>	
<b>Pfizer-BioNTech (BNT162b2)</b>	56 (70.9%)
<b>Moderna (mRNA-1273)</b>	22 (27.8%)
<b>Oxford-AstraZeneca (ChAdOx1-S)</b>	1 (1.3%)
<b>Second dose</b>	
<b>Pfizer-BioNTech (BNT162b2)</b>	57 (72.2%)
<b>Moderna (mRNA-1273)</b>	22 (27.8%)

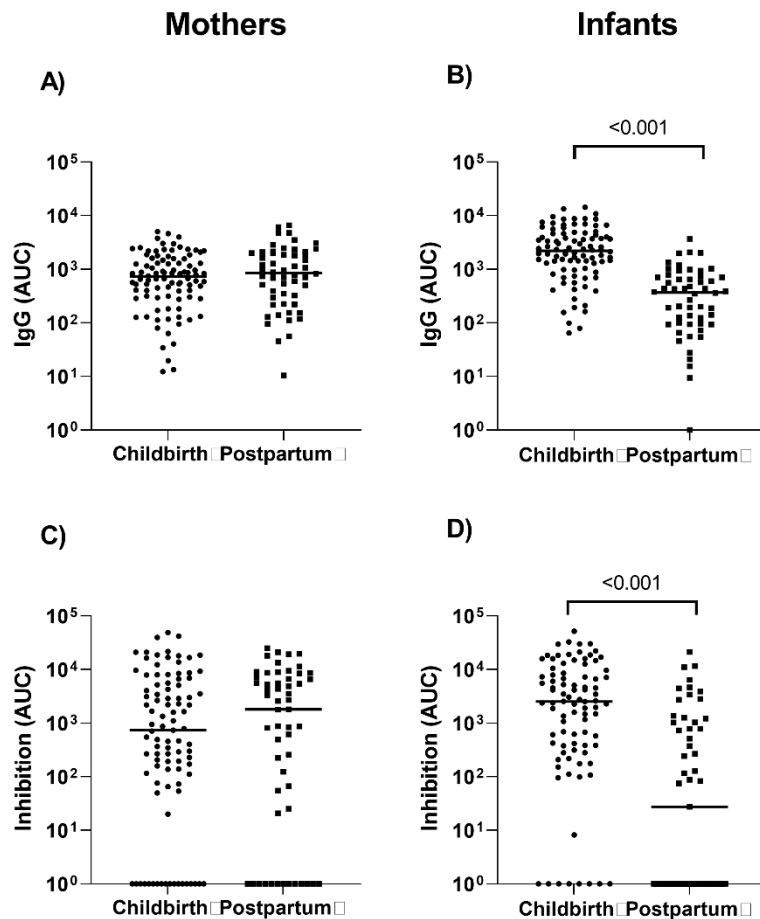
<b>SARS-CoV-2 vaccine after childbirth (n=53)</b>	
<b>One dose</b>	7 (13.2%)
<b>Pfizer-BioNTech (BNT162b2)</b>	5 (71.4%)
<b>Moderna (mRNA-1273)</b>	2 (28.6%)
<b>Two doses</b>	40 (75.5%)
<b>First dose</b>	
<b>Pfizer-BioNTech (BNT162b2)</b>	30 (75.0%)
<b>Moderna (mRNA-1273)</b>	9 (22.5%)
<b>Oxford-AstraZeneca (ChAdOx1-S)</b>	1 (2.5%)
<b>Second dose</b>	
<b>Pfizer-BioNTech (BNT162b2)</b>	31 (77.5%)
<b>Moderna (mRNA-1273)</b>	9 (22.5%)
<b>Three doses</b>	6 (11.3%)
<b>First dose</b>	
<b>Pfizer-BioNTech (BNT162b2)</b>	5 (83.3%)
<b>Moderna (mRNA-1273)</b>	1 (16.7%)
<b>Second dose</b>	
<b>Pfizer-BioNTech (BNT162b2)</b>	5 (83.3%)
<b>Moderna (mRNA-1273)</b>	1 (16.7%)
<b>Third dose</b>	
<b>Pfizer-BioNTech (BNT162b2)</b>	4 (66.7%)
<b>Moderna (mRNA-1273)</b>	2 (33.3%)

**Statistics:** Values are expressed as absolute count (percentage).

**Abbreviations:** SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

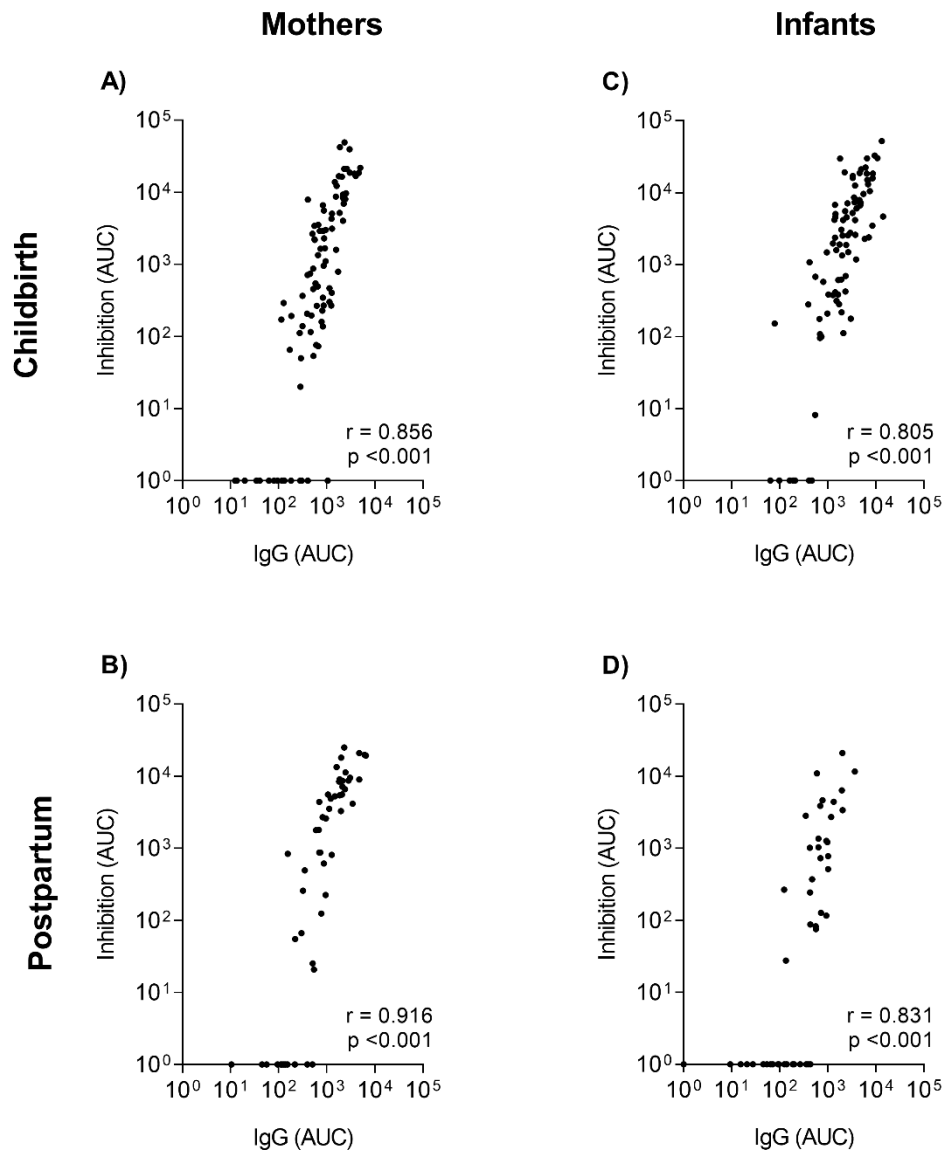
### **Serum anti-SARS-CoV-2 S antibodies in mothers and infants against the B.1 lineage**

Similar high anti-SARS-CoV-2 S IgG titers against the B.1 variant were observed at birth and 2-3 months post-partum in mothers (**Figure 1A**). However, the IgG antibody titers decreased significantly in infants at 2-3 months post-partum ( $p<0.001$ ; **Figure 1B**). As a result, the median titer of infants was three times higher than that of the mothers at childbirth ( $p<0.001$ ; **Supplementary Figure 1A**), but it decreased to 2.3 times lower at 2-3 months post-partum ( $p<0.001$ ; **Supplementary Figure 1B**). A similar pattern of results was observed when evaluating the antibody capacity to inhibit the binding of ACE2 to S (**Figures 1C & 1D**, **Supplementary Figures 1C & 1D**).



**Figure 1.** (A-B), IgG antibody levels against SARS-CoV-2 S protein (B.1 lineage) at childbirth and 2-3 months post-partum in maternal serum (A) and infant serum (B). (C-D), Inhibition of ACE-S interaction titers (B.1 lineage) at childbirth and 2-3 months post-partum in maternal serum (C) and infant serum (D). **Statistics:** Differences were calculated by the Wilcoxon test, and only p-values  $<0.05$  are shown. Medians are represented by a horizontal bar. **Abbreviations:** AUC, Area under the curve; IgG, anti-SARS-CoV-2 S IgG

These findings agree with the high correlation observed between IgG antibody titers and the capacity to inhibit the ACE2-S interaction in all cases (Figure 2). Additionally, a high correlation was observed between IgG antibody titers of mothers and infants, particularly at delivery ( $r=0.860$ ,  $p<0.001$ ) (Supplementary Figure 2). Furthermore, the average ratio of IgG antibody levels between infant and maternal sera was 3.7, indicating quantitative transplacental transfer of antibodies from mother to fetus.



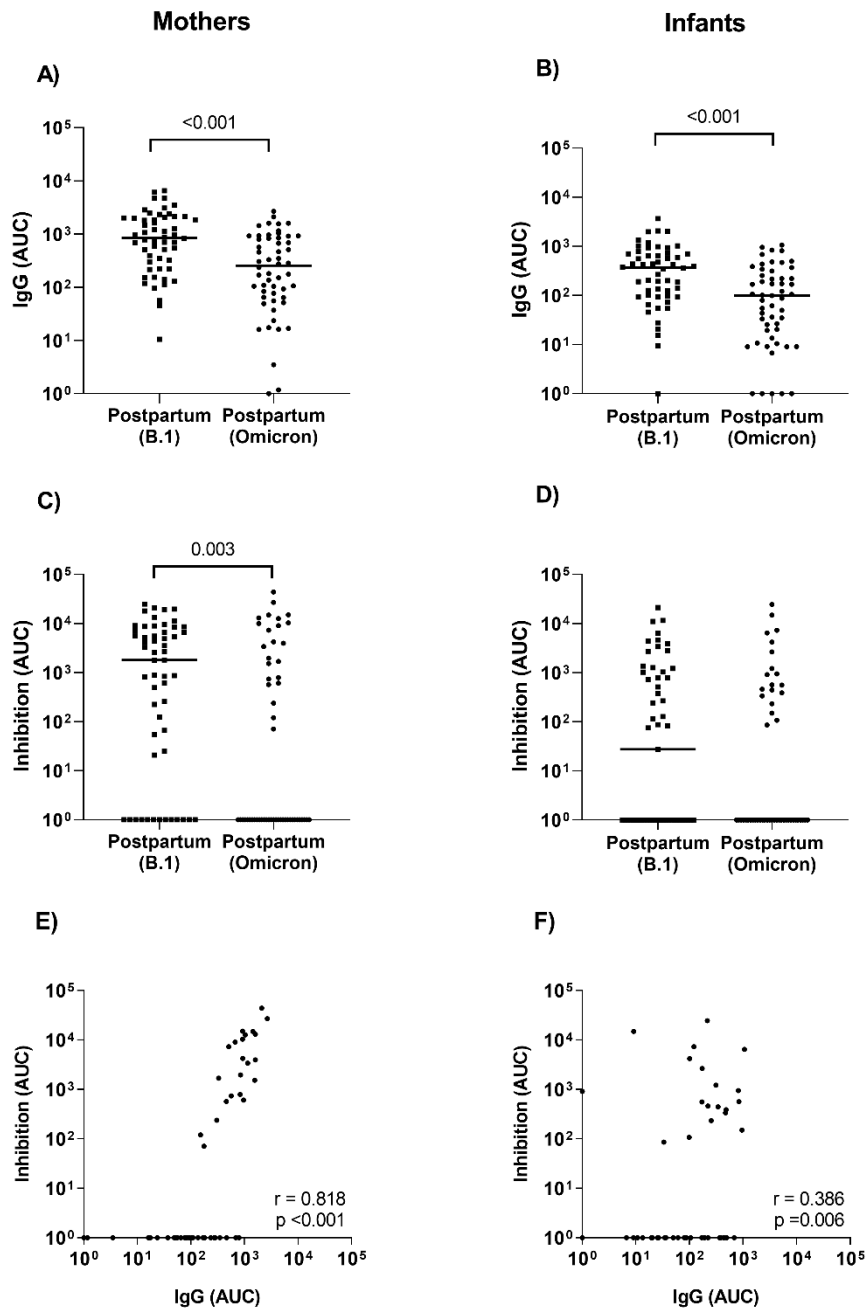
**Figure 2.** Correlation between IgG antibody levels against SARS-CoV-2 S protein (B.1 lineage) and its capacity to inhibit the ACE2 receptor-S protein interaction in maternal (A, B) and infant (C, D) serum at childbirth (A, C) and 2-3 months post-partum (B, D). **Statistics:** Correlation analysis was performed using the Spearman test. **Abbreviations:** AUC, the area under the curve; IgG, anti-SARS-CoV-2 S IgG; ACE2, angiotensin-converting enzyme 2.

### Serum anti-SARS-CoV-2 S antibodies in mothers and infants against the Omicron variant

IgG antibody levels against the S protein of the Omicron variant were determined only at 2-3 months post-partum and compared to the levels against the B.1 lineage (**Figure 3**). The median IgG antibody titers were 3.3 times lower against Omicron than against B.1 in mothers ( $p < 0.001$ ; **Figure 3A**) and 3.7 in infants ( $p < 0.001$ ; **Figure 3B**). The median inhibition titers of the ACE2-S interaction were also lower against Omicron than against the B.1 lineage in mothers ( $p < 0.003$ ; **Figure 3C**). Infants also had lower values against Omicron than the B.1 lineage (**Figure 3D**), but statistically significant differences were not achieved.

A good correlation was observed between IgG antibody titers and inhibition of the ACE2-S interaction, particularly in mothers ( $r = 0.818$ ,  $p < 0.001$ ; **Figure 3E**). As observed with the B.1 lineage, IgG levels against Omicron were lower in infants than in mothers (2.6 times,  $p < 0.02$ ; **Supplementary Figure 3A**). However, the differences between the inhibition titers of mothers and infants were not

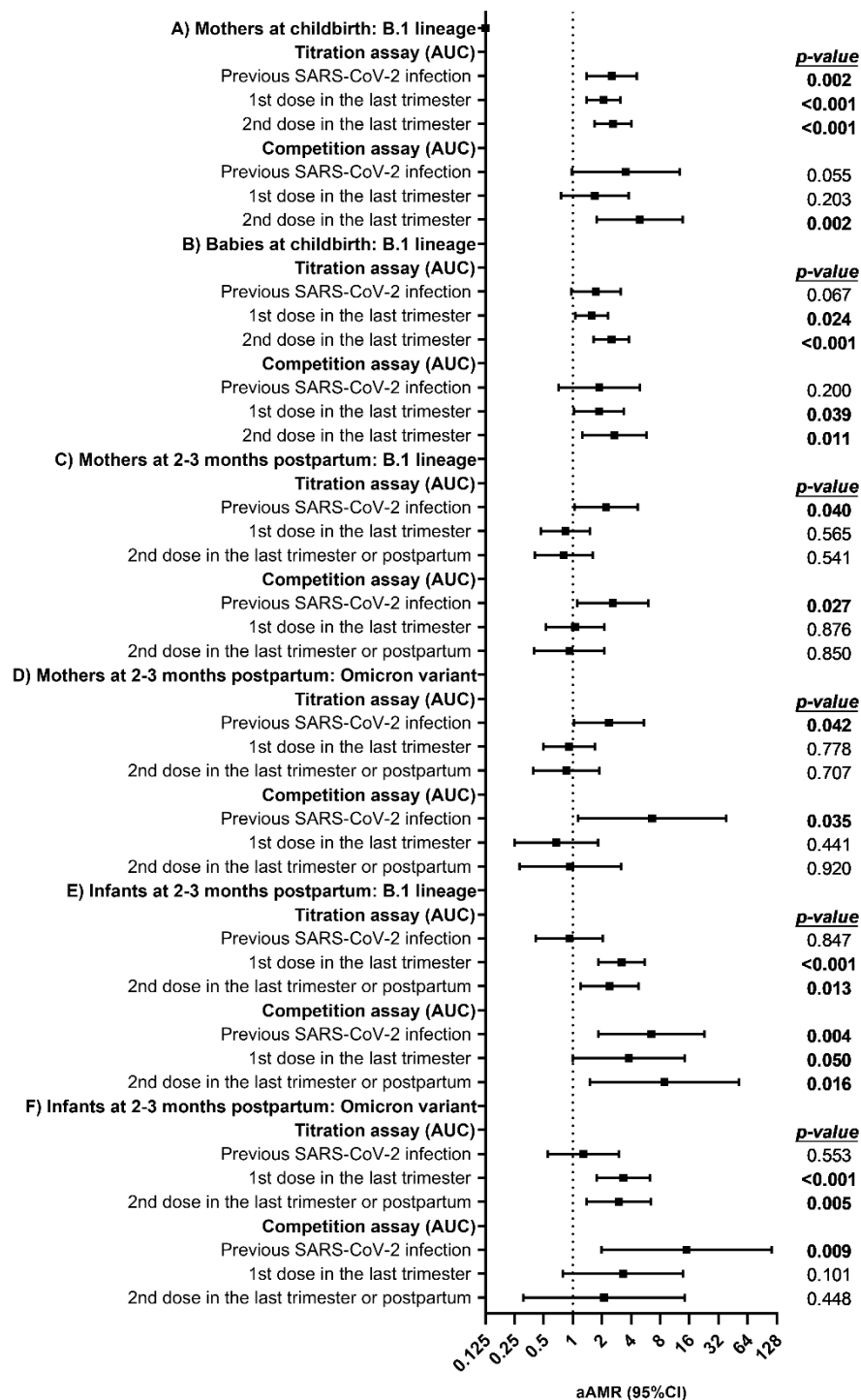
significant, and many individuals without antibodies with detectable inhibition capacity were found (**Supplementary Figure 3B**). A positive correlation was observed between IgG titers against Omicron of mothers and infants (**Supplementary Figure 3C**). Similar results were obtained for the inhibition titers (**Supplementary Figure 3D**).



**Figure 3.** Comparison of IgG antibody levels against SARS-CoV-2 S protein (A, B) and ACE2-S inhibition titers (C, D) between B.1 and Omicron variants in maternal (A, C) and infant serum (B, D) at 2-3 months post-partum. Correlation between anti-SARS-CoV-2 S IgG titers and inhibition titers to the Omicron variant in mother (E) and infant (F) serum. **Statistics:** Differences were calculated by the Mann-Whitney U test. Only p-values <0.05 are shown, and medians are represented by a horizontal bar. Correlation analysis was performed using the Spearman test. **Abbreviations:** AUC, the area under the curve; IgG, anti-SARS-CoV-2 S IgG.

### Factors associated with anti-SARS-CoV-2 S IgG levels

The influence of previous SARS-CoV-2 infection and the timing of COVID-19 vaccination on anti-SARS-CoV-2 S IgG levels is shown in **Figure 4** (full description provided in **Supplementary Table 1**).



**Figure 4.** Impact of previous SARS-CoV-2 infection and the timing of COVID-19 vaccination during pregnancy or the follow-up on anti-SARS-CoV-2 S IgG levels. **Statistic:** The statistics were performed using Generalized Linear Models with gamma distribution and logarithmic link. Values are expressed as the adjusted arithmetic mean ratio (aAMR) and 95% confidence interval (95%CI). Significant differences are shown in bold. **Abbreviations:** protein S, protein spike; COVID-19, coronavirus disease 2019; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; AUC, Area under the Curve.

For mothers at birth (**Figure 4, section A**), those with a history of previous SARS-CoV-2 infection (adjusted AMR (aAMR)=2.52; p=0.002) or those who received the 1<sup>st</sup> or 2<sup>nd</sup> dose of the COVID-19 vaccine during the last trimester of pregnancy (aAMR=2.08 (p<0.001) and aAMR=2.6 (p<0.001), respectively) exhibited higher IgG anti-SARS-CoV-2 S titers against the B.1 lineage. Furthermore, the 2<sup>nd</sup> dose of the COVID-19 vaccine during the last trimester of pregnancy was associated with increased levels of ACE2 binding inhibition (aAMR=4.91; p=0.002).

For babies at childbirth (**Figure 4, section B**), factors linked to higher levels of anti-SARS-CoV-2 S IgG response against the B.1 included receiving the 1<sup>st</sup> dose of the COVID-19 vaccine during the last trimester of pregnancy (aAMR=1.57 (p=0.024) for IgG levels and aAMR=1.87 (p=0.039) for ACE2 binding inhibition) and receiving the 2<sup>nd</sup> dose of the COVID-19 vaccine during the last trimester of pregnancy (aAMR=2.51 (p<0.001) for IgG levels and aAMR=2.69 (p=0.011) for inhibition of ACE2 binding).

For mothers at 2-3 months post-partum (**Figure 4, section C & D**), a history of previous SARS-CoV-2 infection was associated with higher titers of anti-SARS-CoV-2 S IgG (aAMR=2.2; p=0.040) and increased ACE2 binding inhibition levels (aAMR=2.59; p=0.027) against the B.1 lineage (**Figure 4, section C**). Similarly, for the Omicron variant (**Figure 4, section D**), a previous SARS-CoV-2 infection was also associated with elevated titers of anti-SARS-CoV-2 S IgG (aAMR=2.36; p=0.042) and higher ACE2 binding inhibition levels (aAMR=6.61; p=0.035).

For infants at 2-3 months post-partum and the B.1 lineage (**Figure 4, section E**), receiving the 1<sup>st</sup> dose of the COVID-19 vaccine during the last trimester of pregnancy (aAMR=3.19; p<0.001) and the 2<sup>nd</sup> dose of the COVID-19 vaccine during the last trimester of pregnancy or 2-3 months post-partum (aAMR=2.4; p=0.013) were associated with higher levels of anti-SARS-CoV-2 S IgG against the B.1 lineage. Furthermore, previous SARS-CoV-2 infection (aAMR=6.48; p=0.004), receiving the 1<sup>st</sup> dose of COVID-19 vaccine during the last trimester of pregnancy (aAMR=3.78; p<0.05), and the 2<sup>nd</sup> dose of COVID-19 vaccine in the last trimester of pregnancy or 2-3 months post-partum (aAMR=8.85; p=0.016) were linked to increased ACE2 binding inhibition levels against the B.1 lineage.

For infants at 2-3 months post-partum and the Omicron variant (**Figure 4, section F**), receiving the 1<sup>st</sup> dose of the COVID-19 vaccine during the last trimester of pregnancy (aAMR=3.32; p<0.001) and the 2<sup>nd</sup> dose of the COVID-19 vaccine during the last trimester of pregnancy or 2-3 months post-partum (aAMR=2.99; p=0.005) were associated with higher levels of anti-SARS-CoV-2 S IgG against the Omicron variant. Furthermore, a history of previous SARS-CoV-2 infection was linked to elevated levels of ACE2 binding inhibition against the Omicron variant (aAMR=14.97; p=0.009).

## Discussion

Pregnant women and their babies are vulnerable to SARS-CoV-2 infection [42]. Therefore, studies on the immune response after vaccination, including the analysis of antibody levels, their transplacental transfer to the fetus, and the durability and amplitude of the anti-SARS-CoV-2 S IgG response, are relevant for better protecting both the mothers and newborns. This study addressed these issues in pregnant women who received monovalent COVID-19 mRNA vaccines based on the Wuhan-Hu-1 strain. The main findings were as follows: i) at delivery, most mothers had high levels of anti-SARS-CoV S antibodies against the B.1 lineage, which were efficiently transferred to the fetus; ii) these antibodies were capable of inhibiting the ACE2-S interaction; iii) while anti-SARS-CoV-2 S antibody levels remained high after 2-3 months post-partum in mothers, they decreased significantly in infants; iv) anti-SARS-CoV-2 S antibody levels against the Omicron variant were substantially lower than those against the B.1 lineage; v) previous infection with SARS-CoV-2 or COVID-19 vaccination near delivery positively influenced anti-SARS-CoV-2 S antibody levels.

Recent studies have shown that COVID-19 vaccination induces even higher levels of anti-SARS-CoV-2 S antibodies than natural infection and that these antibodies are efficiently transferred to the fetus in pregnant women [19, 34, 43, 44]. Anti-SARS-CoV-2 S antibodies have been reported to correlate with the inhibition of the ACE2-S interaction and neutralizing activity [18, 21]. The present study also demonstrated a high correlation between anti-S IgG titers and ACE2-S inhibition in both mothers and infants at delivery and 2-3 months post-partum. These findings support the observation that COVID-19 vaccination during pregnancy protects both mothers and infants against severe infections [23, 45]. In addition, the infections observed in mothers and infants 2-3 months after birth, during the period when the highly contagious Omicron variant was circulating in the country, were asymptomatic or mild (data not shown).

Our study observed a median IgG transfer ratio of approximately three, which is higher than what is typically seen after SARS-CoV-2 infection [21]. Due to efficient transplacental transfer, anti-SARS-CoV-2 S antibody levels in infants correlated very well with maternal antibody levels, as previously seen after SARS-CoV-2 infection [46-48] and vaccination [31, 49, 50]. However, despite mothers maintaining high antibody titers 2-3 months post-partum, median antibody levels in infants decreased sixfold, suggesting that infants may become more vulnerable to SARS-CoV-2 infection after this period [51]. Consequently, Burns et al. [51] reported that antibody-mediated protection against SARS-CoV-2 completely waned in infants by six months of age. Furthermore, although mothers maintained high antibody levels 2-3 months after delivery, the effectiveness of the vaccine decreased 5-6 months after the second dose, highlighting the need for a booster dose [52].

Given that the highest levels of anti-SARS-CoV-2 S at birth correlate with extended infection-free periods in infants [51], it is imperative to develop an optimized vaccination schedule to increase antibody levels in pregnant women and newborns. Some studies have reported that the majority of maternal IgGs are transferred to the fetus during the last four weeks of pregnancy [44, 53, 54]. This aligns with our findings, which show higher antibody levels in infants born to mothers vaccinated during the last trimester of pregnancy. Therefore, optimizing vaccination schedules, considering the number of vaccine doses and the administration period, particularly in the last trimester of pregnancy, can increase the antibody levels acquired by infants, as observed in this study and others [34, 44, 49, 55, 56]. Vaccination should be performed even after SARS-CoV-2 infection, as combined immune stimulation (infection and vaccination) leads to increased antibody titers, as observed in this study and elsewhere [55, 57, 58].

Finally, we found substantially lower antibody levels against the Omicron variant than against the B.1 lineage. The high number of mutations in the Omicron S protein could explain the lower levels of antibodies that recognize this variant and its resistance to neutralization by vaccine-induced antibodies [59]. The decreased immunity against new variants not fully covered by the current vaccine is expected to increase vulnerability to SARS-CoV-2 infection, particularly in infants. Consequently, Burns et al. [51] observed an abrupt increase in infant-infected cases during the emergence of Omicron. Other studies also found reduced neutralizing antibody titers against the Delta variant in

vaccinated women and their newborns' serum [44, 60]. These results highlight the importance of updating the vaccine formulation according to the circulating variants.

### **Study limitations**

This study has several limitations that must be taken into account. First, the sample size is small, limiting statistical power and increasing the risk of false positive results. Second, this study had a prospective design that introduced some unavoidable biases, such as the loss of follow-up for some samples at 2-3 months. This occurred because some infants were examined in other hospitals or primary care centers. Third, the study did not include a control group of non-pregnant vaccinated women. Fourth, although we analyzed IgM/IgA/IgG levels in the breast milk of vaccinated pregnant women, we found low IgG levels in only a few mothers and negligible IgM and IgG values in all samples (data not shown), which precluded further analysis. Probably, a more sensitive assay would have detected IgG in more pregnant women. A positive correlation between serum and breast milk in vaccinated women has been observed for anti-S IgG antibody levels. However, median anti-S IgG antibody levels were more than 200 times lower in milk than in serum [25, 61].

### **Conclusions**

COVID-19 mRNA vaccines generate high anti-SARS-CoV-2 S titers in pregnant women, which are efficiently transferred to the fetus. These antibodies inhibit ACE2-S interaction. However, it is crucial to carefully design immunization schedules and formulations due to the rapid decrease in antibody levels, especially in infants. Factors to consider were the number of doses, the administration period, previous SARS-CoV-2 infection, and circulating variants.

## **List of abbreviations**

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

Coronavirus Disease 2019 (COVID-19)

Angiotensin-converting enzyme 2 (ACE2)

Immunoglobulin G (IgG)

Anti-SARS-CoV-2 S IgG, IgG against the SARS-CoV-2 spike protein

Hospital General Universitario Gregorio Marañón (HGUGM)

Enzyme-linked immunosorbent assay (ELISA)

Area under the curve (AUC)

Interquartile range (IQR)

Generalized Linear Models (GLM)

Arithmetic mean ratio (AMR)

Adjusted arithmetic mean ratio (aAMR)

95% confidence interval (95%CI)

## **Declarations**

### **Ethics approval and consent to participate**

The HGUGM Ethics Committee approved the study (Ref.: IRB 00006051), which was conducted following the Declaration of Helsinki. All participants gave their informed consent before enrollment.

### **Consent for publication**

Not applicable

### **Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

### **Competing interests**

The authors declare that they have no competing interests.

The funding sources played no role in the study's design, collection, analysis, interpretation of the data, or manuscript writing.

### **Funding**

This study was supported by grants from Instituto de Salud Carlos III (ISCII; grant numbers COV20\_00808). The study was also funded by the Centro de Investigación Biomédica en Red (CIBER) de Enfermedades Infecciosas (CB21/13/00044 and CB21/13/00077) and de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN) (CB22/01/00041). LTD is supported by the Instituto de Salud Carlos III (ISCIII) under grant agreement “CD20/00025” through the Sara Borrell Program. DSC is a ‘Sara Borrell’ researcher supported by ISCIII (grant number CD20CIII/00001).

### **Acknowledgments**

This study would not have been possible without the collaboration of all the patients, medical and nursery staff, and data managers who participated in the project.

### **Author contributions**

Data curation: MJMG, MMV, SVV, IC, AHL, LTD, MMC, CZ, RA, MMC, MLN, and IM.

Investigation: MJMG, MMV, SVV, IC, AHL, VM, MV, AM, OC, SR, MMF, MLN, and IM.

Data analysis and interpretation: MJMG, SR, and IM.

Supervision and visualization: MLN, SR, and IM.

Funding acquisition: SR, MAMF, and MLN.

Drafting the article: MJMG, SR, and IM.

Critical revision of the article: MMV, DSC, VM, and MLN.

All authors have read and approved the final manuscript.

### **Authors' information**

Not applicable.

## References

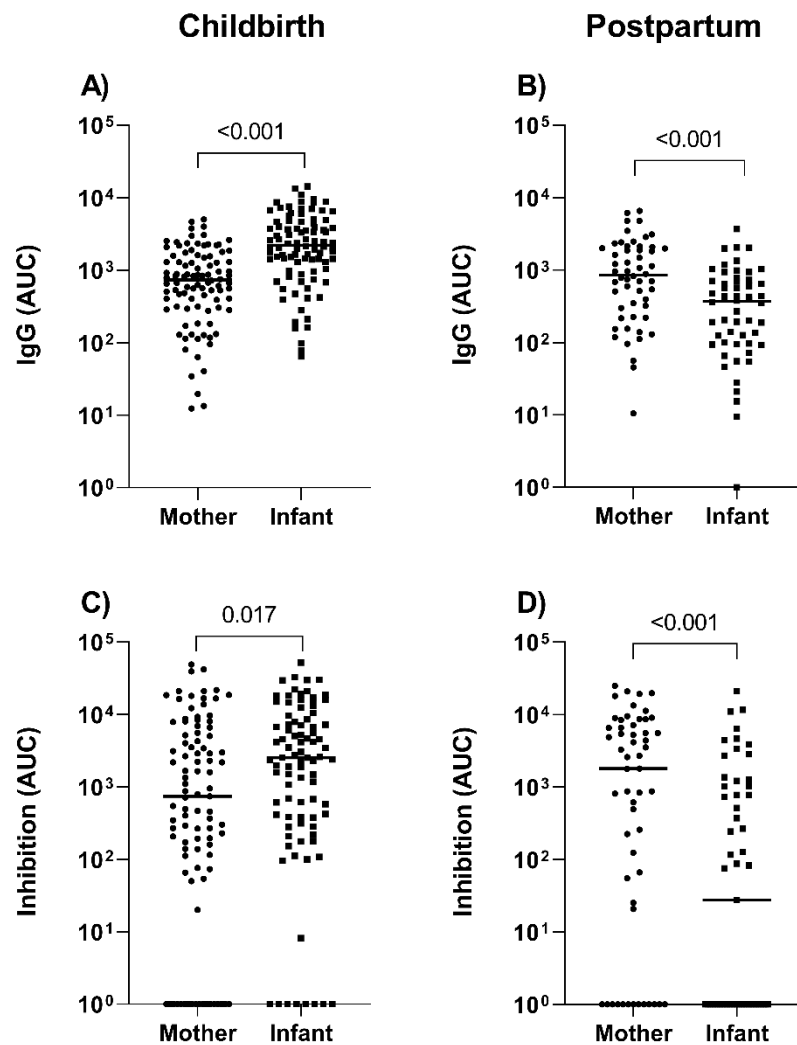
1. Korang SK, von Rohden E, Veroniki AA, Ong G, Ngalamika O, Siddiqui F, et al. Vaccines to prevent COVID-19: A living systematic review with Trial Sequential Analysis and network meta-analysis of randomized clinical trials. *PLoS One*. 2022;17(1):e0260733. <https://doi.org/10.1371/journal.pone.0260733>.
2. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Velesler D. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell*. 2020;181(2):281-92 e6. <https://doi.org/10.1016/j.cell.2020.02.058>.
3. Premkumar L, Segovia-Chumbez B, Jadi R, Martinez DR, Raut R, Markmann A, et al. The receptor binding domain of the viral spike protein is an immunodominant and highly specific target of antibodies in SARS-CoV-2 patients. *Sci Immunol*. 2020;5(48). <https://doi.org/10.1126/sciimmunol.abc8413>.
4. Chmielewska B, Barratt I, Townsend R, Kalafat E, van der Meulen J, Gurol-Urganci I, et al. Effects of the COVID-19 pandemic on maternal and perinatal outcomes: a systematic review and meta-analysis. *Lancet Glob Health*. 2021. [https://doi.org/10.1016/S2214-109X\(21\)00079-6](https://doi.org/10.1016/S2214-109X(21)00079-6).
5. Zambrano LD, Ellington S, Strid P, Galang RR, Oduyebo T, Tong VT, et al. Update: Characteristics of Symptomatic Women of Reproductive Age with Laboratory-Confirmed SARS-CoV-2 Infection by Pregnancy Status - United States, January 22-October 3, 2020. *MMWR Morb Mortal Wkly Rep*. 2020;69(44):1641-7. <https://doi.org/10.15585/mmwr.mm6944e3>.
6. DeSisto CL, Wallace B, Simeone RM, Polen K, Ko JY, Meaney-Delman D, et al. Risk for Stillbirth Among Women With and Without COVID-19 at Delivery Hospitalization - United States, March 2020-September 2021. *MMWR Morb Mortal Wkly Rep*. 2021;70(47):1640-5. <https://doi.org/10.15585/mmwr.mm7047e1>.
7. Woodworth KR, Olsen EO, Neelam V, Lewis EL, Galang RR, Oduyebo T, et al. Birth and Infant Outcomes Following Laboratory-Confirmed SARS-CoV-2 Infection in Pregnancy - SET-NET, 16 Jurisdictions, March 29-October 14, 2020. *MMWR Morb Mortal Wkly Rep*. 2020;69(44):1635-40. <https://doi.org/10.15585/mmwr.mm6944e2>.
8. Metz TD, Clifton RG, Hughes BL, Sandoval G, Saade GR, Grobman WA, et al. Disease Severity and Perinatal Outcomes of Pregnant Patients With Coronavirus Disease 2019 (COVID-19). *Obstet Gynecol*. 2021;137(4):571-80. <https://doi.org/10.1097/AOG.0000000000004339>.
9. Metz TD, Clifton RG, Hughes BL, Sandoval GJ, Grobman WA, Saade GR, et al. Association of SARS-CoV-2 Infection With Serious Maternal Morbidity and Mortality From Obstetric Complications. *JAMA*. 2022;327(8):748-59. <https://doi.org/10.1001/jama.2022.1190>.
10. Schwartz DA, Avvad-Portari E, Babal P, Baldewijns M, Blomberg M, Bouachba A, et al. Placental Tissue Destruction and Insufficiency From COVID-19 Causes Stillbirth and Neonatal Death From Hypoxic-Ischemic Injury. *Arch Pathol Lab Med*. 2022;146(6):660-76. <https://doi.org/10.5858/arpa.2022-0029-SA>.
11. Mor G, Aldo P, Alvero AB. The unique immunological and microbial aspects of pregnancy. *Nat Rev Immunol*. 2017;17(8):469-82. <https://doi.org/10.1038/nri.2017.64>.
12. Marks KJ, Whitaker M, Agathis NT, Anglin O, Milucky J, Patel K, et al. Hospitalization of Infants and Children Aged 0-4 Years with Laboratory-Confirmed COVID-19 - COVID-NET, 14 States, March 2020-February 2022. *MMWR Morb Mortal Wkly Rep*. 2022;71(11):429-36. <https://doi.org/10.15585/mmwr.mm7111e2>.
13. Hobbs CV, Woodworth K, Young CC, Jackson AM, Newhams MM, Dapul H, et al. Frequency, Characteristics and Complications of COVID-19 in Hospitalized Infants. *Pediatr Infect Dis J*. 2022;41(3):e81-e6. <https://doi.org/10.1097/INF.0000000000003435>.
14. Centers for Disease Control and Prevention. Pregnant and Recently Pregnant People. At Increased Risk for Severe Illness from COVID-19. 2022.
15. Butt AA, Chemaitelly H, Al Khal A, Coyle PV, Saleh H, Kaleeckal AH, et al. SARS-CoV-2 vaccine effectiveness in preventing confirmed infection in pregnant women. *J Clin Invest*. 2021;131(23). <https://doi.org/10.1172/JCI153662>.

16. Dagan N, Barda N, Biron-Shental T, Makov-Assif M, Key C, Kohane IS, et al. Effectiveness of the BNT162b2 mRNA COVID-19 vaccine in pregnancy. *Nat Med.* 2021;27(10):1693-5. <https://doi.org/10.1038/s41591-021-01490-8>.
17. Shimabukuro TT, Kim SY, Myers TR, Moro PL, Oduyebo T, Panagiotakopoulos L, et al. Preliminary Findings of mRNA Covid-19 Vaccine Safety in Pregnant Persons. *N Engl J Med.* 2021;384(24):2273-82. <https://doi.org/10.1056/NEJMoa2104983>.
18. Collier AY, McMahan K, Yu J, Tostanoski LH, Aguayo R, Ansel J, et al. Immunogenicity of COVID-19 mRNA Vaccines in Pregnant and Lactating Women. *JAMA.* 2021;325(23):2370-80. <https://doi.org/10.1001/jama.2021.7563>.
19. Gray KJ, Bordt EA, Atyeo C, Deriso E, Akinwunmi B, Young N, et al. Coronavirus disease 2019 vaccine response in pregnant and lactating women: a cohort study. *Am J Obstet Gynecol.* 2021;225(3):303 e1- e17. <https://doi.org/10.1016/j.ajog.2021.03.023>.
20. Halasa NB, Olson SM, Staat MA, Newhams MM, Price AM, Boom JA, et al. Effectiveness of Maternal Vaccination with mRNA COVID-19 Vaccine During Pregnancy Against COVID-19-Associated Hospitalization in Infants Aged <6 Months - 17 States, July 2021-January 2022. *MMWR Morb Mortal Wkly Rep.* 2022;71(7):264-70. <https://doi.org/10.15585/mmwr.mm7107e3>.
21. Martin-Vicente M, Carrasco I, Munoz-Gomez MJ, Lobo AH, Mas V, Vigil-Vazquez S, et al. Antibody levels to SARS-CoV-2 spike protein in mothers and children from delivery to six months later. *Birth.* 2022. <https://doi.org/10.1111/birt.12667>.
22. Carlsen EO, Magnus MC, Oakley L, Fell DB, Greve-Isdahl M, Kinge JM, et al. Association of COVID-19 Vaccination During Pregnancy With Incidence of SARS-CoV-2 Infection in Infants. *JAMA Intern Med.* 2022. <https://doi.org/10.1001/jamainternmed.2022.2442>.
23. Halasa NB, Olson SM, Staat MA, Newhams MM, Price AM, Pannaraj PS, et al. Maternal Vaccination and Risk of Hospitalization for Covid-19 among Infants. *N Engl J Med.* 2022. <https://doi.org/10.1056/NEJMoa2204399>.
24. Atyeo CG, Shook LL, Brigida S, De Guzman RM, Demidkin S, Muir C, et al. Maternal immune response and placental antibody transfer after COVID-19 vaccination across trimester and platforms. *Nat Commun.* 2022;13(1):3571. <https://doi.org/10.1038/s41467-022-31169-8>.
25. Pietrasanta C, Darwich A, Ronchi A, Crippa B, Spada E, Mosca F, et al. Humoral response to anti-SARS-CoV-2 vaccine in breastfeeding mothers and mother-to-infant antibody transfer through breast milk. *NPJ Vaccines.* 2022;7(1):63. <https://doi.org/10.1038/s41541-022-00499-5>.
26. Fox A, Marino J, Amanat F, Krammer F, Hahn-Holbrook J, Zolla-Pazner S, et al. Robust and Specific Secretory IgA Against SARS-CoV-2 Detected in Human Milk. *iScience.* 2020;23(11):101735. <https://doi.org/10.1016/j.isci.2020.101735>.
27. Perl SH, Uzan-Yulzari A, Klainer H, Asiskovich L, Youngster M, Rinott E, et al. SARS-CoV-2-Specific Antibodies in Breast Milk After COVID-19 Vaccination of Breastfeeding Women. *JAMA.* 2021;325(19):2013-4. <https://doi.org/10.1001/jama.2021.5782>.
28. Goncalves J, Juliano AM, Charepe N, Alenquer M, Athayde D, Ferreira F, et al. Secretory IgA and T cells targeting SARS-CoV-2 spike protein are transferred to the breastmilk upon mRNA vaccination. *Cell Rep Med.* 2021;2(12):100468. <https://doi.org/10.1016/j.xcrm.2021.100468>.
29. Dude CM, Joseph NT, Forrest AD, Verkerke HP, Cheedarla N, Govindaraj S, et al. Antibody response, neutralizing potency, and transplacental antibody transfer following SARS-CoV-2 infection versus mRNA-1273, BNT162b2 COVID-19 vaccination in pregnancy. *Int J Gynaecol Obstet.* 2023;162(1):154-62. <https://doi.org/10.1002/ijgo.14648>.
30. Gray KJ, Bordt EA, Atyeo C, Deriso E, Akinwunmi B, Young N, et al. COVID-19 vaccine response in pregnant and lactating women: a cohort study. *medRxiv.* 2021. <https://doi.org/10.1101/2021.03.07.21253094>.
31. Nir O, Schwartz A, Toussia-Cohen S, Leibovitch L, Strauss T, Asraf K, et al. Maternal-neonatal transfer of SARS-CoV-2 immunoglobulin G antibodies among parturient women treated with BNT162b2 messenger RNA vaccine during pregnancy. *Am J Obstet Gynecol MFM.* 2022;4(1):100492. <https://doi.org/10.1016/j.ajogmf.2021.100492>.

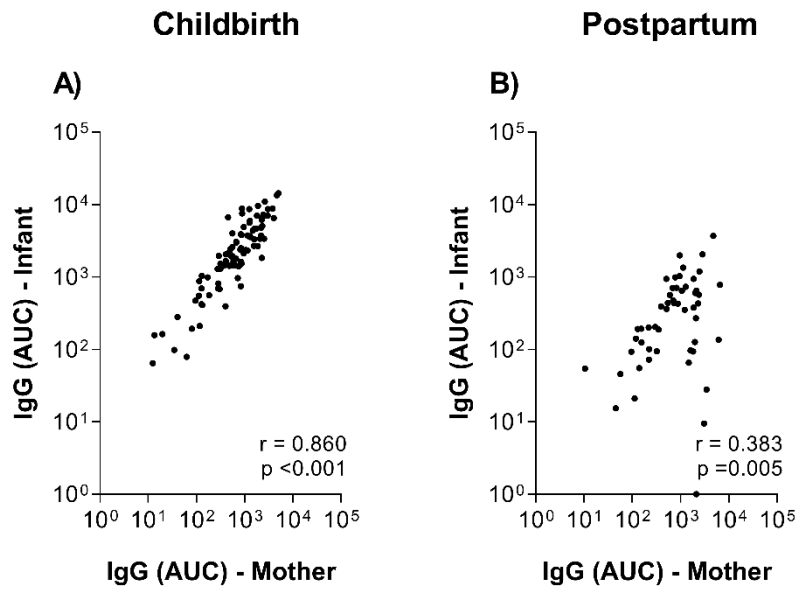
32. Prabhu M, Murphy EA, Sukhu AC, Yee J, Singh S, Eng D, et al. Antibody Response to Coronavirus Disease 2019 (COVID-19) Messenger RNA Vaccination in Pregnant Women and Transplacental Passage Into Cord Blood. *Obstet Gynecol.* 2021;138(2):278-80. <https://doi.org/10.1097/AOG.0000000000004438>.
33. Mithal LB, Otero S, Shanes ED, Goldstein JA, Miller ES. Cord blood antibodies following maternal coronavirus disease 2019 vaccination during pregnancy. *Am J Obstet Gynecol.* 2021;225(2):192-4. <https://doi.org/10.1016/j.ajog.2021.03.035>.
34. Otero S, Miller ES, Sunderraj A, Shanes ED, Sakowicz A, Goldstein JA, et al. Maternal Antibody Response and Transplacental Transfer Following Severe Acute Respiratory Syndrome Coronavirus 2 Infection or Vaccination in Pregnancy. *Clin Infect Dis.* 2023;76(2):220-8. <https://doi.org/10.1093/cid/ciac793>.
35. Munoz FM, Posavad CM, Richardson BA, Badell ML, Bunge KE, Mulligan MJ, et al. COVID-19 booster vaccination during pregnancy enhances maternal binding and neutralizing antibody responses and transplacental antibody transfer to the newborn. *Vaccine.* 2023;41(36):5296-303. <https://doi.org/10.1016/j.vaccine.2023.06.032>.
36. Shook LL, Atyeo CG, Yonker LM, Fasano A, Gray KJ, Alter G, et al. Durability of Anti-Spike Antibodies in Infants After Maternal COVID-19 Vaccination or Natural Infection. *JAMA.* 2022;327(11):1087-9. <https://doi.org/10.1001/jama.2022.1206>.
37. Carrasco I, Munoz-Chapuli M, Vigil-Vazquez S, Aguilera-Alonso D, Hernandez C, Sanchez-Sanchez C, et al. SARS-CoV-2 infection in pregnant women and newborns in a Spanish cohort (GESNEO-COVID) during the first wave. *BMC Pregnancy Childbirth.* 2021;21(1):326. <https://doi.org/10.1186/s12884-021-03784-8>.
38. Vazquez-Alejo E, Tarancon-Diez L, Carrasco I, Vigil-Vazquez S, Munoz-Chapuli M, Rincon-Lopez E, et al. SARS-CoV2 Infection During Pregnancy Causes Persistent Immune Abnormalities in Women Without Affecting the Newborns. *Front Immunol.* 2022;13:947549. <https://doi.org/10.3389/fimmu.2022.947549>.
39. Tre-Hardy M, Wilmet A, Beukinga I, Favresse J, Dogne JM, Douxfils J, et al. Analytical and clinical validation of an ELISA for specific SARS-CoV-2 IgG, IgA, and IgM antibodies. *J Med Virol.* 2021;93(2):803-11. <https://doi.org/10.1002/jmv.26303>.
40. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science.* 2020;367(6483):1260-3. <https://doi.org/10.1126/science.abb2507>.
41. Hsieh CL, Goldsmith JA, Schaub JM, DiVenere AM, Kuo HC, Javanmardi K, et al. Structure-based design of prefusion-stabilized SARS-CoV-2 spikes. *Science.* 2020;369(6510):1501-5. <https://doi.org/10.1126/science.abd0826>.
42. Jamieson DJ, Rasmussen SA. An update on COVID-19 and pregnancy. *Am J Obstet Gynecol.* 2022;226(2):177-86. <https://doi.org/10.1016/j.ajog.2021.08.054>.
43. Falsaperla R, Leone G, Familiari M, Ruggieri M. COVID-19 vaccination in pregnant and lactating women: a systematic review. *Expert Rev Vaccines.* 2021;20(12):1619-28. <https://doi.org/10.1080/14760584.2021.1986390>.
44. Matsui Y, Li L, Prahl M, Cassidy AG, Ozarslan N, Golan Y, et al. Neutralizing antibody activity against SARS-CoV-2 variants in gestational age-matched mother-infant dyads after infection or vaccination. *JCI Insight.* 2022;7(12). <https://doi.org/10.1172/jci.insight.157354>.
45. Theiler RN, Wick M, Mehta R, Weaver AL, Virk A, Swift M. Pregnancy and birth outcomes after SARS-CoV-2 vaccination in pregnancy. *Am J Obstet Gynecol MFM.* 2021;3(6):100467. <https://doi.org/10.1016/j.ajogmf.2021.100467>.
46. Flannery DD, Gouma S, Dhudasia MB, Mukhopadhyay S, Pfeifer MR, Woodford EC, et al. Assessment of Maternal and Neonatal Cord Blood SARS-CoV-2 Antibodies and Placental Transfer Ratios. *JAMA Pediatr.* 2021;175(6):594-600. <https://doi.org/10.1001/jamapediatrics.2021.0038>.
47. Joseph NT, Dude CM, Verkerke HP, Irby LS, Dunlop AL, Patel RM, et al. Maternal Antibody Response, Neutralizing Potency, and Placental Antibody Transfer After Severe Acute Respiratory

- Syndrome Coronavirus 2 (SARS-CoV-2) Infection. *Obstet Gynecol.* 2021;138(2):189-97. <https://doi.org/10.1097/AOG.0000000000004440>.
48. Rathberger K, Hausler S, Wellmann S, Weigl M, Langhammer F, Bazzano MV, et al. SARS-CoV-2 in pregnancy and possible transfer of immunity: assessment of peripartur maternal and neonatal antibody levels and a longitudinal follow-up. *J Perinat Med.* 2021;49(6):702-8. <https://doi.org/10.1515/jpm-2021-0166>.
49. Rottenstreich A, Zarbiv G, Oiknine-Djian E, Vorontsov O, Zigron R, Kleinstern G, et al. Timing of SARS-CoV-2 vaccination during the third trimester of pregnancy and transplacental antibody transfer: a prospective cohort study. *Clin Microbiol Infect.* 2022;28(3):419-25. <https://doi.org/10.1016/j.cmi.2021.10.003>.
50. Popescu DE, Citu C, Jura AMC, Lungu N, Navolan D, Craina M, et al. The Benefits of Vaccination against SARS-CoV-2 during Pregnancy in Favor of the Mother/Newborn Dyad. *Vaccines (Basel).* 2022;10(6). <https://doi.org/10.3390/vaccines10060848>.
51. Burns MD, Muir C, Atyeo C, Davis JP, Demidkin S, Akinwunmi B, et al. Relationship between Anti-Spike Antibodies and Risk of SARS-CoV-2 Infection in Infants Born to COVID-19 Vaccinated Mothers. *Vaccines (Basel).* 2022;10(10). <https://doi.org/10.3390/vaccines10101696>.
52. Kalafat E, Magee LA, von Dadelszen P, Heath P, Khalil A. COVID-19 booster doses in pregnancy and global vaccine equity. *Lancet.* 2022;399(10328):907-8. [https://doi.org/10.1016/S0140-6736\(22\)00166-0](https://doi.org/10.1016/S0140-6736(22)00166-0).
53. Moore KM, Suthar MS. Comprehensive analysis of COVID-19 during pregnancy. *Biochem Biophys Res Commun.* 2021;538:180-6. <https://doi.org/10.1016/j.bbrc.2020.12.064>.
54. Palmeira P, Quinello C, Silveira-Lessa AL, Zago CA, Carneiro-Sampaio M. IgG placental transfer in healthy and pathological pregnancies. *Clin Dev Immunol.* 2012;2012:985646. <https://doi.org/10.1155/2012/985646>.
55. Yang YJ, Murphy EA, Singh S, Sukhu AC, Wolfe I, Adurty S, et al. Association of Gestational Age at Coronavirus Disease 2019 (COVID-19) Vaccination, History of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection, and a Vaccine Booster Dose With Maternal and Umbilical Cord Antibody Levels at Delivery. *Obstet Gynecol.* 2022;139(3):373-80. <https://doi.org/10.1097/AOG.0000000000004693>.
56. Kugelman N, Nahshon C, Shaked-Mishan P, Cohen N, Lahav Sher M, Barsha H, et al. Third trimester messenger RNA COVID-19 booster vaccination upsurge maternal and neonatal SARS-CoV-2 immunoglobulin G antibody levels at birth. *Eur J Obstet Gynecol Reprod Biol.* 2022;274:148-54. <https://doi.org/10.1016/j.ejogrb.2022.05.029>.
57. Cortes-Sarabia K, Gutierrez-Torres M, Mendoza-Renteria EM, Leyva-Vazquez MA, Vences-Velazquez A, Hernandez-Sotelo D, et al. Variation in the Humoral Immune Response Induced by the Administration of the BNT162b2 Pfizer/BioNTech Vaccine: A Systematic Review. *Vaccines (Basel).* 2022;10(6). <https://doi.org/10.3390/vaccines10060909>.
58. Krammer F, Srivastava K, Alshammary H, Amoako AA, Awawda MH, Beach KF, et al. Antibody Responses in Seropositive Persons after a Single Dose of SARS-CoV-2 mRNA Vaccine. *N Engl J Med.* 2021;384(14):1372-4. <https://doi.org/10.1056/NEJMc2101667>.
59. Hoffmann M, Kruger N, Schulz S, Cossmann A, Rocha C, Kempf A, et al. The Omicron variant is highly resistant against antibody-mediated neutralization: Implications for control of the COVID-19 pandemic. *Cell.* 2022;185(3):447-56 e11. <https://doi.org/10.1016/j.cell.2021.12.032>.
60. Shen CJ, Fu YC, Lin YP, Shen CF, Sun DJ, Chen HY, et al. Evaluation of Transplacental Antibody Transfer in SARS-CoV-2-Immunized Pregnant Women. *Vaccines (Basel).* 2022;10(1). <https://doi.org/10.3390/vaccines10010101>.
61. Esteve-Palau E, Gonzalez-Cuevas A, Guerrero ME, Garcia-Terol C, Alvarez MC, Garcia G, et al. Quantification and Progress Over Time of Specific Antibodies Against Severe Acute Respiratory Syndrome Coronavirus 2 in Breast Milk of Lactating Women Vaccinated With BNT162b2 Pfizer-BioNTech Coronavirus Disease 2019 Vaccine (LacCOVID). *Open Forum Infect Dis.* 2022;9(6):ofac239. <https://doi.org/10.1093/ofid/ofac239>.

## Supplementary Materials

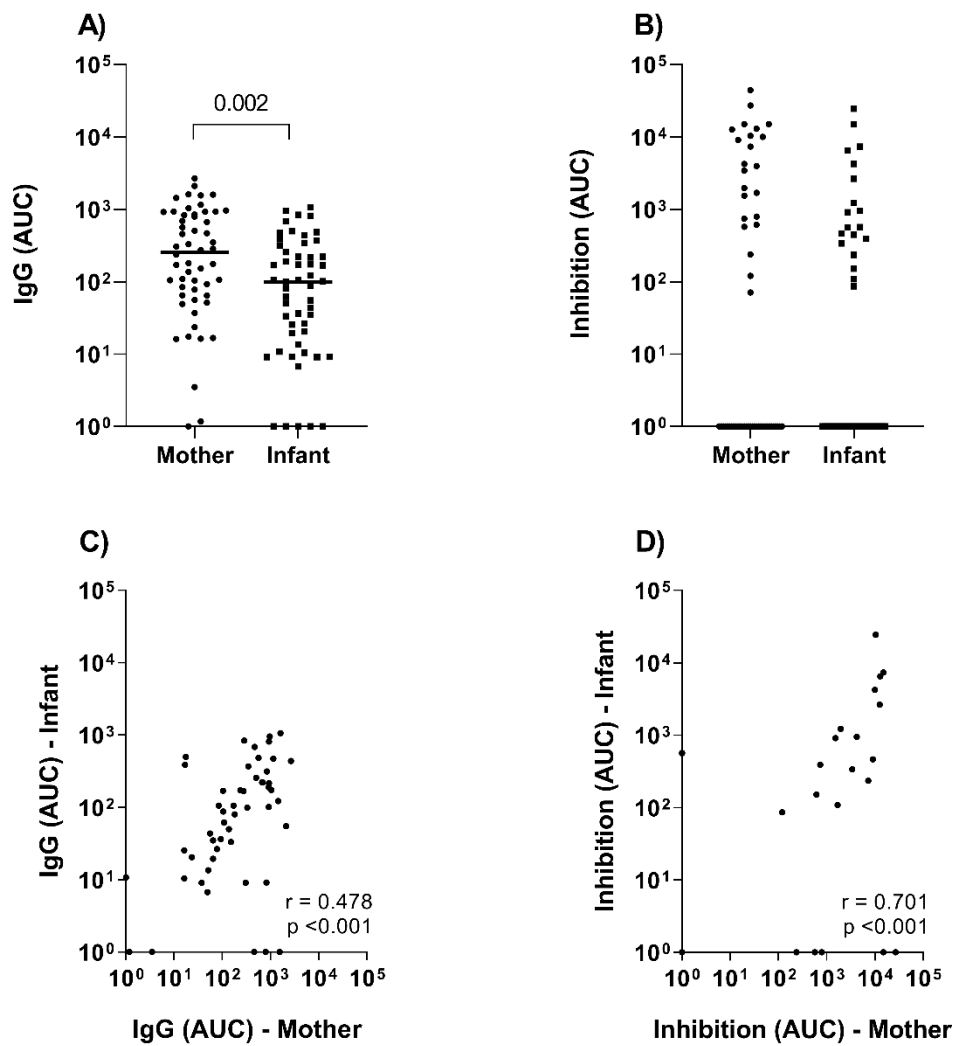


**Supplementary Figure 1.** Comparison of serum IgG antibody levels against the SARS-CoV-2 S protein (B.1 lineage) (A & B) and ACE2-S interaction inhibition titers (C & D) between mothers and infants at childbirth (A & C) and at 2-3 months postpartum (B & D). **Statistics:** Differences were calculated by the Mann-Whitney U test, and only p-values <0.05 are shown. Medians are represented by a horizontal bar. **Abbreviations:** AUC, Area under the ROC curve; IgG, anti-SARS-CoV-2 S IgG; ACE2, angiotensin-converting enzyme 2.



**Supplementary Figure 2.** Correlation between serum IgG antibody levels against the SARS-CoV-2 S protein (B.1 lineage) between mothers and infants at childbirth (A) and at 2-3 months postpartum (B). **Statistics:** Correlation analysis was performed using the Spearman test. **Abbreviations:** AUC, the area under the ROC curve; IgG, anti-SARS-CoV-2 S IgG.

### Postpartum (Omicron)



**Supplementary Figure 3.** Comparison of serum IgG antibody levels against the SARS-CoV-2 S protein (Omicron variant) (A & C) and ACE2-S interaction inhibition titers (B & D) between mothers and infants at 2-3 months postpartum. Correlation between serum IgG antibody levels (C) and ACE2-S interaction inhibition titers (D) between mothers and infants at 2-3 months postpartum (Omicron variant). **Statistics:** Differences were calculated by the Mann-Whitney U test, and only p-values  $< 0.05$  are shown. Medians are represented by a horizontal bar. Correlation analysis was performed using the Spearman test. **Abbreviations:** AUC, Area under the ROC curve; IgG, anti-SARS-CoV-2 S IgG; ACE2, angiotensin-converting enzyme 2.

**Supplementary Table 1.** Impact of previous SARS-CoV-2 infection and the timing of COVID-19 vaccination during pregnancy or the follow-up on anti-SARS-CoV-2 S IgG levels.

<b>A) Mothers in childbirth: B.1 lineage</b>	<b>Unadjusted analysis</b>		<b>Adjusted analysis</b>	
	<b>AMR (95%CI)</b>	<b>p</b>	<b>aAMR (95%CI)</b>	<b>p</b>
<b>Titration assay (AUC)</b>				
Previous SARS-CoV-2 infection	2.52 (1.39 - 4.58)	<b>0.002</b>	2.52 (1.39 - 4.58)	<b>0.002</b>
1 <sup>st</sup> dose in the last trimester	2.08 (1.39 - 3.12)	<b>&lt;0.001</b>	2.08 (1.39 - 3.12)	<b>&lt;0.001</b>
2 <sup>nd</sup> dose in the last trimester	2.39 (1.49 - 3.83)	<b>&lt;0.001</b>	2.6 (1.68 - 4.02)	<b>&lt;0.001</b>
<b>Competition assay (AUC)</b>				
Previous SARS-CoV-2 infection	3.52 (0.98 - 12.73)	0.055	3.52 (0.98 - 12.73)	0.055
1 <sup>st</sup> dose in the last trimester	1.61 (0.71 - 3.66)	0.259	1.69 (0.75 - 3.77)	0.203
2 <sup>nd</sup> dose in the last trimester	3.19 (1.16 - 8.78)	<b>0.025</b>	4.91 (1.77 - 13.65)	<b>0.002</b>
<b>B) Babies in childbirth: B.1 lineage</b>				
<b>Titration assay (AUC)</b>	<b>AMR (95%CI)</b>	<b>p</b>	<b>aAMR (95%CI)</b>	<b>p</b>
Previous SARS-CoV-2 infection	1.74 (0.96 - 3.13)	0.067	1.74 (0.96 - 3.13)	0.067
1 <sup>st</sup> dose in the last trimester	1.57 (1.06 - 2.32)	<b>0.024</b>	1.57 (1.06 - 2.32)	<b>0.024</b>
2 <sup>nd</sup> dose in the last trimester	2.3 (1.47 - 3.59)	<b>&lt;0.001</b>	2.51 (1.65 - 3.82)	<b>&lt;0.001</b>
<b>Competition assay (AUC)</b>				
Previous SARS-CoV-2 infection	1.88 (0.72 - 4.94)	0.200	1.88 (0.72 - 4.94)	0.200
1 <sup>st</sup> dose in the last trimester	1.87 (1.03 - 3.38)	<b>0.039</b>	1.87 (1.03 - 3.38)	<b>0.039</b>
2 <sup>nd</sup> dose in the last trimester	2.04 (0.9 - 4.65)	0.089	2.69 (1.26 - 5.76)	<b>0.011</b>
<b>C) Mothers at 2-3 months postpartum: B.1 lineage</b>				
<b>Titration assay (AUC)</b>	<b>AMR (95%CI)</b>	<b>p</b>	<b>aAMR (95%CI)</b>	<b>p</b>
Previous SARS-CoV-2 infection	2.2 (1.04 - 4.68)	<b>0.040</b>	2.2 (1.04 - 4.68)	<b>0.040</b>
1 <sup>st</sup> dose in the last trimester	0.84 (0.47 - 1.51)	0.565	0.84 (0.47 - 1.51)	0.565
2 <sup>nd</sup> dose in the last trimester or postpartum	0.71 (0.35 - 1.46)	0.350	0.81 (0.4 - 1.61)	0.541
<b>Competition assay (AUC)</b>				
Previous SARS-CoV-2 infection	2.29 (0.89 - 5.94)	0.087	2.59 (1.11 - 6.05)	<b>0.027</b>
1 <sup>st</sup> dose in the last trimester	1.04 (0.5 - 2.18)	0.913	1.06 (0.53 - 2.12)	0.876
2 <sup>nd</sup> dose in the last trimester or postpartum	0.8 (0.31 - 2.02)	0.631	0.92 (0.4 - 2.13)	0.850
<b>D) Mothers at 2-3 months postpartum: Omicron variant</b>				
<b>Titration assay (AUC)</b>	<b>AMR (95%CI)</b>	<b>p</b>	<b>aAMR (95%CI)</b>	<b>p</b>
Previous SARS-CoV-2 infection	2.39 (1.01 - 5.65)	<b>0.048</b>	2.36 (1.03 - 5.4)	<b>0.042</b>
1 <sup>st</sup> dose in the last trimester	0.88 (0.47 - 1.66)	0.689	0.92 (0.49 - 1.7)	0.778
2 <sup>nd</sup> dose in the last trimester or postpartum	0.7 (0.31 - 1.57)	0.387	0.86 (0.39 - 1.89)	0.707
<b>Competition assay (AUC)</b>				
Previous SARS-CoV-2 infection	7.12 (1.06 - 47.85)	<b>0.044</b>	6.61 (1.14 - 38.44)	<b>0.035</b>
1 <sup>st</sup> dose in the last trimester	0.53 (0.16 - 1.74)	0.296	0.68 (0.25 - 1.83)	0.441
2 <sup>nd</sup> dose in the last trimester or postpartum	0.63 (0.16 - 2.46)	0.508	0.94 (0.28 - 3.15)	0.920
<b>E) Infants at 2-3 months postpartum: B.1 lineage</b>				
<b>Titration assay (AUC)</b>	<b>AMR (95%CI)</b>	<b>p</b>	<b>aAMR (95%CI)</b>	<b>p</b>
Previous SARS-CoV-2 infection	0.97 (0.43 - 2.2)	0.936	0.93 (0.42 - 2.05)	0.847
1 <sup>st</sup> dose in the last trimester	3.35 (1.92 - 5.86)	<b>&lt;0.001</b>	3.19 (1.84 - 5.53)	<b>&lt;0.001</b>
2 <sup>nd</sup> dose in the last trimester or postpartum	2.61 (1.18 - 5.76)	<b>0.018</b>	2.4 (1.2 - 4.79)	<b>0.013</b>
<b>Competition assay (AUC)</b>				
Previous SARS-CoV-2 infection	4.26 (0.97 - 18.82)	0.056	6.48 (1.83 - 22.96)	<b>0.004</b>
1 <sup>st</sup> dose in the last trimester	3.7 (0.91 - 15.1)	0.068	3.78 (1 - 14.33)	<b>0.050</b>
2 <sup>nd</sup> dose in the last trimester or postpartum	4.87 (1.15 - 20.6)	<b>0.031</b>	8.85 (1.5 - 52.22)	<b>0.016</b>
<b>F) Infants at 2-3 months postpartum: Omicron variant</b>				

<b>Titration assay (AUC)</b>	<b>AMR (95%CI)</b>	<b><i>p</i></b>	<b>aAMR (95%CI)</b>	<b><i>p</i></b>
Previous SARS-CoV-2 infection	1.25 (0.51 - 3.04)	0.628	1.29 (0.56 - 3)	0.553
1 <sup>st</sup> dose in the last trimester	3.28 (1.74 - 6.2)	<b>&lt;0.001</b>	3.32 (1.77 - 6.25)	<b>&lt;0.001</b>
2 <sup>nd</sup> dose in the last trimester/postpartum	2.81 (1.21 - 6.54)	<b>0.016</b>	2.99 (1.39 - 6.4)	<b>0.005</b>
<b>Competition assay (AUC)</b>				
Previous SARS-CoV-2 infection	15.8 (1.94 - 128.66)	<b>0.010</b>	14.97 (1.98 - 113.17)	<b>0.009</b>
1 <sup>st</sup> dose in the last trimester	3.56 (0.84 - 15.18)	0.086	3.31 (0.79 - 13.79)	0.101
2 <sup>nd</sup> dose in the last trimester or postpartum	2.2 (0.31 - 15.75)	0.432	2.1 (0.31 - 14.3)	0.448

**Statistic:** The statistics were performed using GLMs with gamma distribution and logarithmic link. Values are expressed as the arithmetic mean ratio (AMR) and 95% confidence interval (95%CI). Significant differences are shown in bold.

**Abbreviations:** protein S, protein spike; COVID-19, coronavirus disease 2019; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; AUC, Area under the ROC Curve.