SARS-CoV-2 infection versus vaccination in pregnancy: Implications for maternal and infant immunity

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Abstract

Background: SARS-CoV-2 infection has been associated with adverse maternal and neonatal outcomes, yet uptake of SARS-CoV-2 vaccines during pregnancy and lactation has been slow. As a result, millions of pregnant and lactating women and their infants remain susceptible to the virus.

Methods: We measured Spike-specific immunoglobulin G (anti-S IgG) and A (anti-S IgA) in serum and breastmilk (BM) samples from 3 prospective mother-infant cohorts recruited in two academic medical centers. The primary aim was to determine the impact of maternal SARS-CoV-2 immunization *vs* infection and their timing on systemic and mucosal immunity.

Results: The study included 28 mothers infected with SARS-CoV-2 in late pregnancy (INF), 11 uninfected mothers who received 2 doses of the BNT162b2 vaccine in the latter half of pregnancy (VAX-P) and 12 uninfected mothers who received 2 doses of BNT162b2 during lactation (VAX-L). VAX dyads had significantly higher serum anti-S IgG compared to INF dyads ($p<.0001$), while INF mothers had higher BM:serum anti-S IgA ratios compared to VAX mothers ($p=.0001$). Median IgG placental transfer ratios were significantly higher in VAX-P compared to INF mothers ($p<0.0001$). There was a significant positive correlation between maternal and neonatal serum anti-S IgG after vaccination ($r=0.68$, $p=0.013$), but not infection.

Conclusions: BNT161b2 vaccination in late pregnancy or lactation enhances systemic immunity through serum anti-S Ig, while SARS-CoV-2 infection induces mucosal over systemic immunity more efficiently through BM Ig production. Next generation vaccines boosting mucosal immunity could provide additional protection to the mother-infant dyad. Future studies should focus on identifying the optimal timing of primary and/or booster maternal vaccination for maximal benefit.

Keywords: breastmilk, COVID-19, newborn, pregnancy, SARS-CoV-2 vaccination

Background

Pregnant women are vulnerable to infectious diseases owing to a distinct maternal-fetal immune tolerance physiology [1]. The balancing act between host self-defense against infection and immune acceptance of paternal-fetal antigens increases their vulnerability to infectious diseases compared to their non-pregnant counterparts [2]. A study from the Centers for Disease Control and Prevention (CDC) suggests that pregnant women with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection are more likely to require intensive care or mechanical ventilation than nonpregnant women of reproductive age [3], while two large retrospective multicenter studies in the US found that women with COVID-19 giving birth had higher rates of mortality, sepsis, mechanical ventilation, ICU admission, and preterm birth than women without COVID-19 [4-6]. COVID-19 diagnosis in the 2^{nd} *vs* 3^{rd} trimester of pregnancy differentially affected the immune response at delivery, suggesting that the timing of immune stimulation is an important parameter [7]. More specifically, women infected during the 3^{rd} trimester exhibited cytokine signatures that clinically correlated with a high incidence of late pregnancy- and postpartum-related complications, particularly, preeclampsia and fetal growth restriction [8]. Despite poor maternal perinatal outcomes, vertical SARS-CoV-2 infection is rare [9] and the prevalence of neonatal SARS-CoV-2 infection is low compared to other age groups, likely due to placental protective mechanisms limiting viral entry [10]. However, neonates remain susceptible to horizontally transmitted infection owing to their distinct immune system [11]. Transplacental antibody (Ab) transfer, starting around 28 weeks of gestation, is the main form of passive immunization for young infants, with protection persisting for the first 3-5 months of life. Reduced transplacental Ab transfer ratios have been reported after natural SARS-CoV-2 infection compared to other viral infections such as influenza [12], along with short-lived durability of vertically acquired IgG titers after birth [13]. Considering the above, infants born

preterm -especially before 28 weeks of gestation- or whose mothers contracted SARS-CoV-2 around the time of delivery, may lack protective IgG antibodies and remain at risk for horizontally transmitted postnatal infection. An important route of passive protection for the infant after birth is *via* consumption of breastmilk (BM). Indeed, BM contains IgA produced by plasma cells and memory B cells migrated from maternal respiratory and gut mucosal sites to the lactating breast, thus conferring neonatal mucosal protection [14]. This mechanism of maternal mucosal protection *via* BM was also suggested after maternal SARS-CoV-2 infection [15]. Immunological outcomes after maternal SARS-CoV-2 vaccination during pregnancy and lactation are sparse but urgently needed to inform maternal immunization practices. We present data on vaccine- *vs* natural infection-induced anti-S Ab titers in the serum and BM of pregnant and lactating mothers and their newborns. Our primary goal was to determine relative Ab presence in maternal serum and BM after maternal perinatal SARS-CoV-2 infection or vaccination, and how their timing may affect systemic and mucosal Ab transfer from the mother to the newborn.

Methods

Study Design

We designed a prospective study including 3 convenience cohorts of mothers and their infants: 1) pregnant women infected with SARS-CoV-2 during the third trimester (INF) as evidenced by a positive molecular test at the time of delivery, 2) pregnant women vaccinated with two doses of BNT162b2 mRNA vaccine during the latter half of pregnancy (VAX-P), and 3) lactating women who received two doses of BNT162b2 mRNA vaccine after delivery (VAX-L) **(Figure 1**). INF and VAX-P mothers were followed at Policlinico Umberto I Hospital, Sapienza University of Rome, Italy, from October 2020 to December 2021, while VAX-L mothers were recruited at

Bambino Gesù Children's Hospital from February to April 2021. The study protocol was conducted in conformity with the Declaration of Helsinki for medical research involving human subjects and was approved by the Ethical Committee of Policlinico Umberto I Hospital in Rome, Italy (Reference number 6206).

Sample collection

Maternal peripheral blood as well as neonatal (cord and peripheral) blood samples were collected as shown in **Figure 1**. Each newborn born to an INF mother routinely had peripheral blood drawn at 48 hours of life (2d) as part of the hospital protocol, while healthy non-exposed newborns born to VAX-P mothers were not routinely phlebotomized after birth, so cord blood was collected instead. Serum specimens were collected from INF mothers at 2 days after delivery (median of 5 days after infection) and 60 days after infection, from VAX-P mothers 60 days post-second vaccine dose and from VAX-L mothers 10 days post-second vaccine dose. Cord blood was collected from the umbilical vein after delivery and peripheral blood was collected by venipuncture into serum separator tubes. Blood was centrifuged at 1400 rpm for 5 minutes at room temperature. In clinically stable mothers who were willing to pump milk, BM was collected after nipple disinfection using a manual sterile pump. Serum and BM samples were aliquoted into cryogenic vials and stored at −80°C until further analysis.

Antibody assays

Total and SARS-CoV-2 anti-S human IgG and IgA antibodies were evaluated on serum and BM samples using the anti-SARS-CoV-2 ELISA commercial kit (EUROIMMUN Medizinische Labor Diagnostika AG, Lübeck, Germany). All serum samples were diluted 1:100 according to the manufacturer's instructions [15]. Values were then normalized for comparison with a calibrator. Results were evaluated by calculating the ratio between the extinction of samples and

the extinction of the calibrator. Results are reported as the ratio between OD samples and OD calibrator.

Statistical analysis

Demographics were summarized with descriptive statistics (median and IQR or min-max for continuous values). Immunological and clinical variables were compared between the different cohorts and study times. Values were compared by the non-parametric two-tailed Mann–Whitney U-test. A p-value < 0.05 was considered statistically significant. Statistical analyses were performed with GraphPad Prism 8.0 (GraphPad Software) and IBM Statistical Package for Social Science software version 25.0 (SPSS Inc. Chicago, IL, USA).

Results

We analyzed serum and BM specimens from mothers infected with SARS-CoV-2 during pregnancy (INF) and mothers who were uninfected and vaccinated with 2 doses of BNT162b2 mRNA vaccine either during pregnancy (VAX-P) or postpartum, during lactation (VAX-L) (**Figure 1**). Samples from INF mothers were analyzed at two time points: 2 days (INF_2d) and 2 months (INF_2mo) after delivery. Relevant demographic and pregnancy characteristics of infected and vaccinated mothers are provided in **Table 1**. Among pregnant participants, the mean gestational age at the first vaccine dose was 26 weeks, with 5 women (45%) receiving their first vaccine dose in the second trimester and 6 (55%) in the third trimester. The exact timing of serum and BM sampling in relation to infection or vaccination (2^{nd} dose) is summarized in **Table 1**.

Anti-S IgG in maternal serum was significantly higher in vaccinated *vs* infected mothers (**Figure 2, Table 2**). Inter-individual variability in IgG concentrations was greater in infected, compared to vaccinated women (**Figure 2A**). In INF mothers, positivity for SARS-CoV-2 was detected at

the time of delivery, when 43% of them had no detectable IgG in the serum. Anti-S IgG significantly increased two months later, reflecting the normal kinetics of the immune response to a recent infection. Mothers vaccinated in the post-partum period had significantly higher serum anti-S IgG levels compared to those vaccinated during pregnancy (**Figure 2A**). Similarly, anti-S IgA levels in maternal serum were significantly higher in VAX-L compared to all other cohorts ($p<0.0001$) **(Figure 2B)**. The high levels of IgG and IgA in VAX-L mothers may be explained by the short interval between vaccination and sampling.

Neonates of mothers vaccinated in the late 2nd or early 3rd trimester of pregnancy [median time from vaccination to sample collection = 56 days (IQR= 45.5)] demonstrated a significant elevation in serum anti-S IgG levels compared to neonates born to mothers with SARS-CoV-2 infection in the late 3rd trimester [median time from infection to sample collection= 5 days (IQR=5.75) for INF_2d and 67 days (IQR 5.75) for INF_2mo] (**Figure 2C**). In contrast, neonatal anti-S IgA levels were undetectable in all cohorts (**Figure 2D**). Three infants born to INF mothers had confirmed SARS-CoV-2 infection either by nasopharyngeal molecular testing or serology, one of which was possibly vertically- and 2 postnatally-acquired and demonstrated high anti-S IgG and IgA levels at 2 months of age, likely resulting from their own immune reaction to the virus. In agreement with the observation that only IgG are transported through the placenta during the last months of pregnancy [16], in the VAX-P cohort we found a positive correlation between neonatal and maternal serum anti-S IgG, but not IgA (**Figure 2E-F**). As neonates of INF mothers did not have detectable serum anti-S IgG at 2d and detection of transplacentally-transferred Ab as not expected at 2 months, no correlation was evaluated between INF_2d and INF_2mo.

BM anti-S IgG levels remained low and unchanged across time after SARS-CoV-2 infection, but were significantly higher in women vaccinated either in pregnancy or during lactation (**Figure 3A**). As expected, in all cohorts BM anti-S IgA was more abundant than IgG (**Figure 3B**). BM anti-S IgA were significantly higher in VAX-L *vs* VAX-P or INF_2mo mothers (**Figure 3B**). IgG and IgA BM:maternal serum ratios were higher in INF_2d compared to all other cohorts (**Figure 3C-D**). There was a significant correlation between the levels of serum and BM anti-S IgG in both cohorts of vaccinated mothers, while no correlation was found between serum and BM anti-S IgA (**Figure 3E-H**).

Discussion

Neonates of mothers vaccinated during pregnancy demonstrated a significant elevation in serum anti-S IgG levels compared to neonates born to mothers experiencing SARS-CoV-2 infection during late pregnancy. This finding could reflect a favorable transplacental Ab transfer ratio after vaccination or be the consequence of higher levels of maternal serum IgG and a longer time for placental transfer in the vaccinated group. Others have also shown that vaccine-induced humoral responses (Spike and RBD IgA, IgG and IgM) in the sera and BM of pregnant and lactating women were statistically significantly greater than those induced by natural infection [17] suggesting that vaccination during pregnancy may confer enhanced immunity compared to natural infection. Transfer of anti-S IgG across the placenta increases with time lapsed from vaccination [18, 19] and infection [20, 21], a finding corroborated by our data. In addition to enhanced transplacental Ab transfer, early vs. late third-trimester maternal SARS-CoV-2 immunization has been associated with increased neonatal neutralizing Ab levels [22], providing a functional readout of SARS-CoV-2 immunity. The advantage of earlier rather than later immunization in pregnancy has been well documented when examining influenza and pertussis

immunization [23, 24]. Serum anti-S IgA levels were significantly elevated in mothers vaccinated postpartum compared to mothers vaccinated during pregnancy who had almost undetectable levels. Since the intervention (vaccination) was the same, we hypothesize that this observation may be explained by the unique kinetics of IgA, which rapidly declines after SARS-CoV-2 vaccination, compared to IgG which decays at a slower rate [25].

BM anti-S IgG levels revealed a similar pattern to serum anti-S IgG, except that the magnitude of the Ab response was much lower in BM. This finding is to be expected as only a fraction of systemic antibodies reach the BM either by active transport or transudation [26]. BM IgA primarily derives from mucosal plasma cells and memory B cells migrated to the mammary gland, and locally produced IgA is transported to the milk by transcytosis [27]. Early presence of anti-S IgA in the BM of INF mothers, who also had significantly higher BM:serum Ab ratios compared to the vaccinated groups, indicates that natural infection more efficiently induces mucosal immune responses [28, 29], compared to BNT162b2 mRNA vaccination which primarily drives serum Ab production and activates systemic immunity [30]. Anti-S IgA in the BM of vaccinated mothers derives from vaccine-generated memory B cells and plasma cells migrated to the inflammatory environment of the lactating breast [31, 32].

After infection or vaccination, antibodies transferred via the BM are important in the protection against respiratory infections during early life, and especially viral infections [33, 34]. Systems serology profiling of matched serum and colostrum samples of lactating mothers infected with SARS-CoV-2 during pregnancy revealed preferential transfer of IgA and IgM in BM with limited IgG transfer [35]. In a prospective cohort of pregnant women infected with SARS-CoV-2 in late pregnancy, BM was shown to contain not only anti-S IgA, but also immune complexes composed of the viral Spike bound to maternal anti-S IgA that may have actively triggered the

infant's local mucosal immune response [15]. The kinetics and duration of the SARS-CoV-2 Ab response in human BM may differ between infected and vaccinated mothers. More specifically, infection was associated with a highly variable IgA-dominant anti-RBD response that was sustained through at least 90 days, while vaccination was associated with an IgG-dominant response that declined overtime [36]. Even though most studies to date involve mRNA COVID vaccines, it is conceivable that anti-SARS-CoV-2 IgA and IgG levels in human milk after vaccination may be dependent on vaccine type and previous SARS-CoV-2 exposure [37].

Our study has some limitations including a small sample size, study of a single vaccine type and no long-term follow-up of infants to measure duration of protection. Moreover, mucosal immunity in newborn saliva was not evaluated. Future studies should evaluate short- and long-term clinical outcomes of maternal vaccination in addition to Ab concentrations to assess clinical benefits from these antibodies and identify correlates of immune protection conferred by mRNA vaccines. Alongside systemic immunity, mucosal immunity should also be investigated in the context of the immune response to vaccines given in infancy. More studies are needed to understand the durability of Ab transfer following both maternal infection and vaccination to guide vaccine design and deployment in the future for protection of the neonate.

Clinical considerations for vaccination against SARS-CoV-2 in pregnancy

Immunization during pregnancy confers antigen-specific immunity not only to the mother but also to her offspring. At this time, infant immunization vs SARS-CoV-2 is complicated by: a) the low incidence of clinically evident COVID-19 disease in exposed newborns [38] making vaccine efficacy clinical trials harder to conduct, b) the relatively low risk of short-term serious direct COVID-19 effects in neonates [38], necessitating a very low risk and considerable benefit from vaccination, and c) the distinct neonatal immune system characterized by suboptimal responses

to most early-life vaccines necessitating booster doses during the first year of life for protection [11]. Given high risk of SARS-CoV-2-related direct harms in pregnant women and the indirect harms on their offspring [38, 39], maternal immunization vs SARS-CoV-2 is a critical prevention strategy with significant benefits for the mother-infant dyad. With rising cumulative rates of SARS-CoV-2 infection and increasing prevalence of variants, positions by medical and scientific communities have evolved to recommend COVID-19 vaccination during pregnancy. Information from universal surveillance systems and national registries [40, 41] shows that COVID-19 vaccination during pregnancy is not associated with increased pregnancy or delivery complications. A large retrospective population-based Israeli cohort of pregnant women showed that vaccination with BNT162b2 mRNA during the 2nd or 3rd trimester was associated with significantly lower risk for SARS-CoV-2 infection compared to no vaccination during the 28-70 days of follow up after the 1st vaccine dose (adjusted hazard ratio of 0.22 [95% CI, 0.11-0.43]) [42]. Randomized clinical trials evaluating the safety, tolerability, and immunogenicity of mRNA-based SARS-CoV-2 vaccines in pregnant women are now underway (NCT04754594). Until such data becomes available, population-derived statistics can be utilized to infer benefit-risk ratios for pregnant women.

Our study which included cohorts of women during pregnancy and postpartum provides immunological data supporting vaccination of mothers during pregnancy or after delivery. Vaccine receipt by the early third trimester of pregnancy a) prevents severe infection and its sequela in the mother by conferring robust systemic immunity and b) protects the newborn via transplacental Ab transfer. Vaccine receipt after delivery and during the lactation period a) prevents severe infection and its sequela in the mother by conferring robust systemic immunity and b) may contribute to neonatal mucosal immunity via BM Ab transfer or production by

mammary gland plasma cells. Our results support the notion that vaccination is of substantial benefit during pregnancy, given the risk SARS-CoV-2 infection poses to both mother and infant. A combination of vaccination before and after delivery may comprise an optimal strategy to maximize maternal immunization benefit to the offspring. Future efforts should focus on development of vaccine technologies that also robustly activate mucosal immunity.

Conclusions

Our study demonstrated the efficient transfer of SARS-CoV-2 anti-S IgG across the placenta in women vaccinated with the BNT162b2 mRNA vaccine during the latter half of pregnancy, to their neonates, with a strong positive correlation between maternal serum and cord blood Ab concentrations. Despite its strong systemic antibody response, BNT162b2 had a small effect on mucosal immunity *via* BM IgA, a gap that should be addressed by next generation vaccines. A 2-dose vaccination during the 2nd and 3rd trimester of pregnancy followed by a booster dose post-partum may represent a safe strategy for preventing perinatal COVID-19 disease and conferring both systemic IgG- and mucosal IgA-mediated immunity *via* BM provision to the infant.

NOTES

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Conflict of Interest: VP participated in a lecture on COVID vaccination in children and an honoraria payment was made to their university. They also participated in the National Greek Advisory Committee for Immunization Practices. CP was a steering committee member for clinical trials #MN42988 and MN42989 by La Roche Ltd. AA received an NIAID DSSA IOF grant for Comprehensive Analysis of the Neonatal Immune System via High-throughout Proteomics and salary support from the Mueller Health Foundation for TB-related research. They also received an honorarium for an invited review article on neonatal vaccination. FN, GS, MGC, RB, RC, FM, GT, and ST have no conflicts to disclose.

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Table 1. Characteristics of maternal-infant cohorts.

	Unvaccinated mothers who tested positive for SARS-CoV-2 during late pregnancy (INF, N = 28)	SARS-CoV-2 uninfected mothers who received 2 doses of BNT162b2 mRNA vaccine during pregnancy (VAX-P, N = 11)	SARS-CoV2 uninfected mothers who received 2 doses of BNT162b2 mRNA vaccine after delivery (VAX-L, N = 12)
Maternal age, median (IQR), years	32 (6.5)	35 (4.5)	34 (2.5)
SARS-CoV2 infection severity		na	na
Asymptomatic, No. (%)	6 (21)	na	na
Symptomatic, No. (%)	22 (79)	na	na
Hospitalized and/or received medication for COVID-19, No. (%)	9 (32)	na	na
Delivery indicated for worsening maternal COVID-19 illness, No (%)	0	na	na
Twin pregnancies, No.	2	1	0
Enrolled newborns, No.	30	12	12
Female newborns, No. (%)	12 (40)	3 (25)	6 (50)
Birthweight, grams, median (IQR)	3175 (745)	2960 (510)	3545 (583)
Gestational age at delivery, median (IQR), completed weeks	39 (2.75)	38 (2)	40 (2)
Weeks of gestation at SARS-CoV-2 vaccination (1st dose), median (IQR)	na	26 (4.75)	na
Weeks of gestation at SARS-CoV-2 vaccination	na	29 (4.75)	na

(2nd dose), median (IQR)				
Days post-delivery at SARS-CoV-2 vaccination (1st dose), median (IQR)	na		na	233.5 (134)
Days post-delivery at SARS-CoV-2 vaccination (2nd dose), median (IQR)	na		na	254.5 (134)
	INF_2d N=28	INF_2mo N=26		
Days from infection[a] or 2nd vaccine dose to cord blood/neonatal blood sampling, median (min-max)	5 (2-19)	67 (44-104)	56 (21, 98)	na
Days from infection[a] or 2nd vaccine dose to maternal blood sampling, median (min-max)	5 (2-19)	67 (44-104)	58 (23, 100)	9 (2-17)
Days from infection[a] or 2nd vaccine dose to breastmilk sampling, median (min-max)	5 (2-19)	65 (62-79)	60 (25, 102)	9 (2-17)

[a] Days from infection were calculated from the date of positive SARS-CoV-2 testing if asymptomatic, or from the date of symptom onset in symptomatic cases.

Table 2. Relative spike-specific (anti-S) antibody concentrations in maternal serum, cord blood/neonatal serum and breastmilk from infected or vaccinated maternal cohorts.

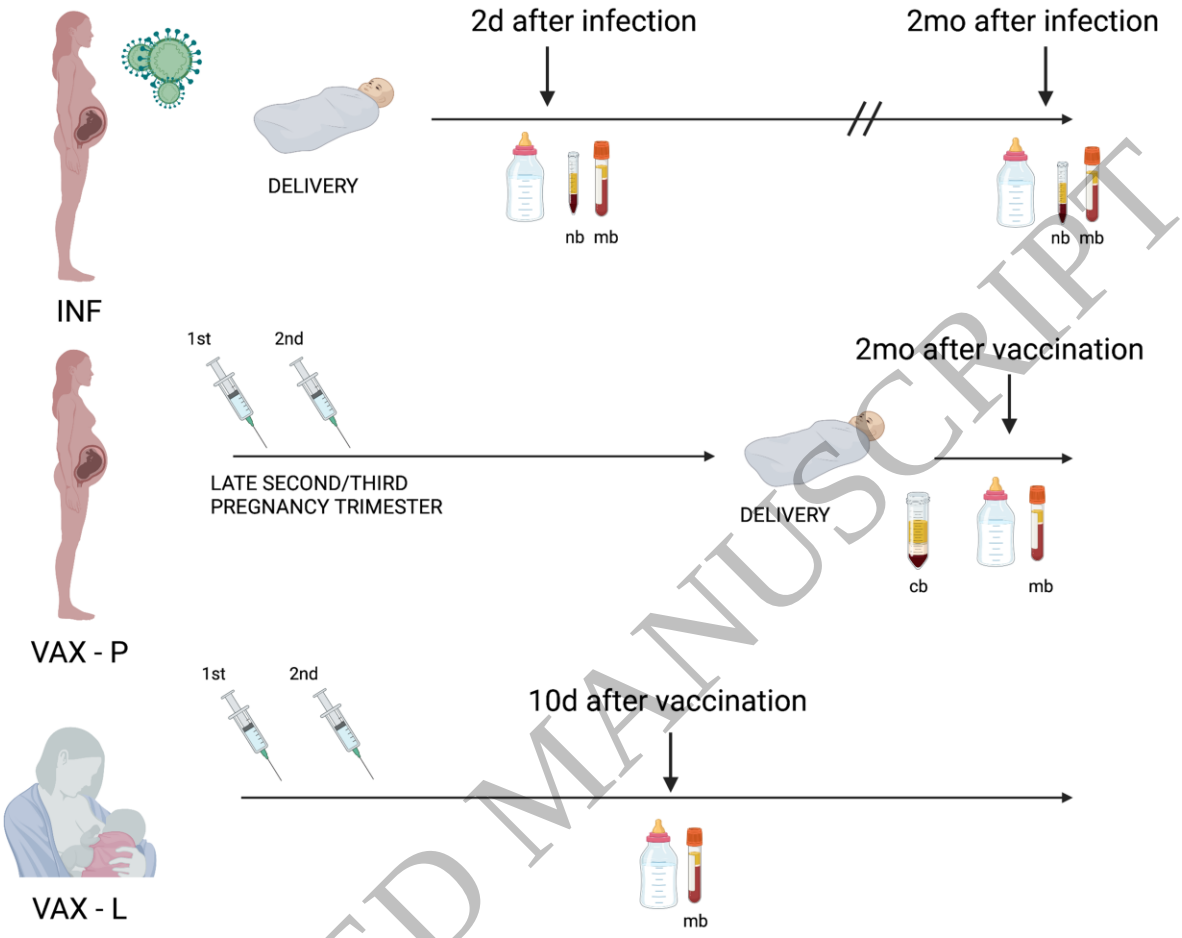
	Unvaccinated mothers who tested positive for SARS-CoV-2 during late pregnancy (2d)		**Unvaccinated mothers who tested positive for SARS-CoV-2 during late pregnancy (2mo)**		**Vaccinated mothers (2 doses of BNT162b2 mRNA vaccine) during pregnancy**		**Vaccinated mothers (2 doses of BNT162b2 mRNA vaccine) after delivery**		**P value**
Anti-SARS-CoV-2 antibody (OD ratio)	N	Median (IQR)	N	Median (IQR)	N	Median (IQR)	N	Median (IQR)	
Maternal serum anti-S IgG	28	1.4 (2.2)	26	5.8 (5.6)	11	31.8 (28.7)	12	64.1 (26.9)	****<0.0001
Maternal serum anti-S IgA		1.8 (4.2)		2.6 (2.9)		1.2 (19.5)		47.8 (22.7)	****<0.0001
Cord blood/ Neonatal serum anti-S IgG	30	0.06 (0.1)	26	0.18 (0.3)	12	31.8 (24.8)	na	na	****<0.0001
Cord blood/ Neonatal serum anti-S IgA		0.01 (0.02)		0.09 (0.2)		0.15 (0.11)		na	****<0.0001
Breastmilk anti-S IgG	6	0.1 (0.22)	10	0.1 (0.06)	10	0.2 (0.38)	12	0.7 (0.8)	***0.0001
Breastmilk anti-S IgA		1.7 (2.6)		0.7 (0.75)		1.1 (1.7)		1.6 (2)	*0.046

Figure legends

Figure 1. Study design and sample collection. The three convenience cohorts used for immunological analysis are depicted. INF were mothers infected in late pregnancy, VAX-P were mothers vaccinated against SARS-CoV-2 in the late second or third trimester of pregnancy and VAX-L were mothers vaccinated against SARS-CoV-2 during lactation. nb= neonatal peripheral blood; mb= maternal peripheral blood; cb=cord blood. Figure was created in BioRender.com.

Figure 2. **Anti-S IgG and IgA measurements in maternal and neonatal serum.** Dot plots show maternal serum anti-S IgG **(A)** and IgA **(B)** across study groups (INF_2d *n= 28*; INF_2mo *n= 26*; VAX-P *n= 11*; VAX-L *n= 12*). Serum anti-S IgG **(C)** and IgA **(D)** from neonates born to mothers infected or vaccinated during pregnancy (INF_2d *n= 30*; INF_2mo *n= 27*; VAX-P *n= 11*). There is a significant correlation between VAX-P maternal and neonatal serum anti-S IgG **(E**), but not anti-S IgA **(F)**. There was no correlation between INF_2mo maternal and neonatal serum anti-S Ab. In graphs A-D, results are reported as optical density (OD) ratios and the dotted line represents the assay detection threshold (OD ratio=1.1). Median values are plotted, and statistical significance was determined using unpaired Mann-Whitney tests (compare ranks). For correlation graphs, *p* value and Pearson *r* are reported. *p < 0.05; **p<0.01; ***p< 0.001; ****p<0.0001.

Figure 3. Anti-S IgG and IgA levels in maternal breastmilk (BM). A) BM anti-S IgG shows a similar pattern to serum but lower magnitude of the Ab response. **B)** BM anti-S IgA are significantly higher in VAX-L compared to VAX-P or INF mothers. BM-to-maternal serum ratio for each cohort is shown for anti-S IgG **C)** and IgA **D)**. There is a significant correlation between BM and maternal serum anti-S IgG concentrations in VAX-P (**E**) and VAX-L (**G**), but not anti-S IgA concentrations in the same cohorts (**F** and **H,** respectively). Dotted lines indicate the mean value of anti-S IgA (OD ratio=0.2) and IgG (OD ratio=0.1) from control BM samples provided by uninfected unvaccinated mothers (*n = 7*). Median values are plotted, and statistical significance was determined using unpaired Mann-Whitney tests (compare ranks). For correlation graphs, *p* value and Pearson *r* are reported. *p < 0.05; **p<0.01; ***p< 0.001; ****p<0.0001.



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2 **165x140 mm (.50 x DPI)**

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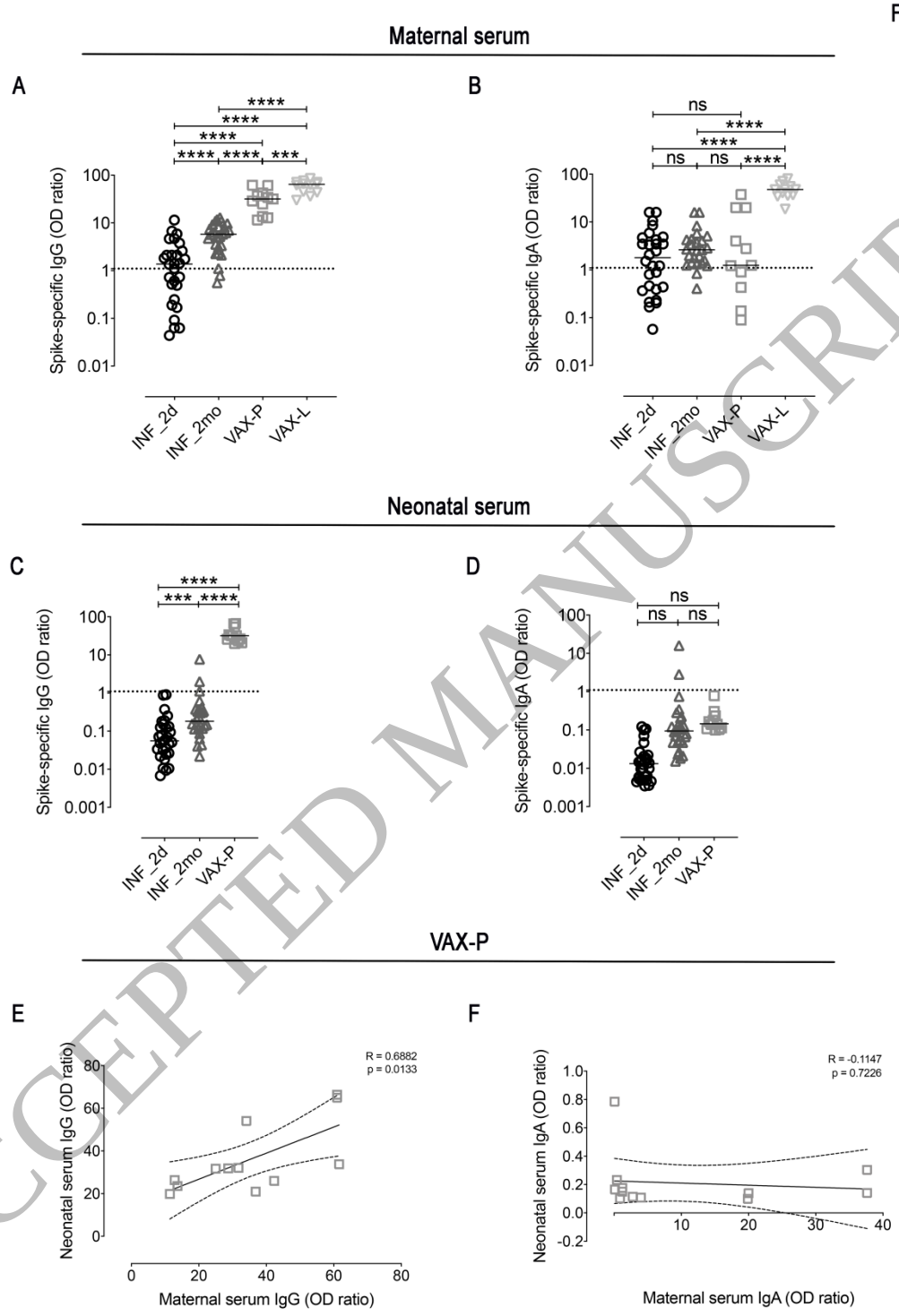
Figure 2



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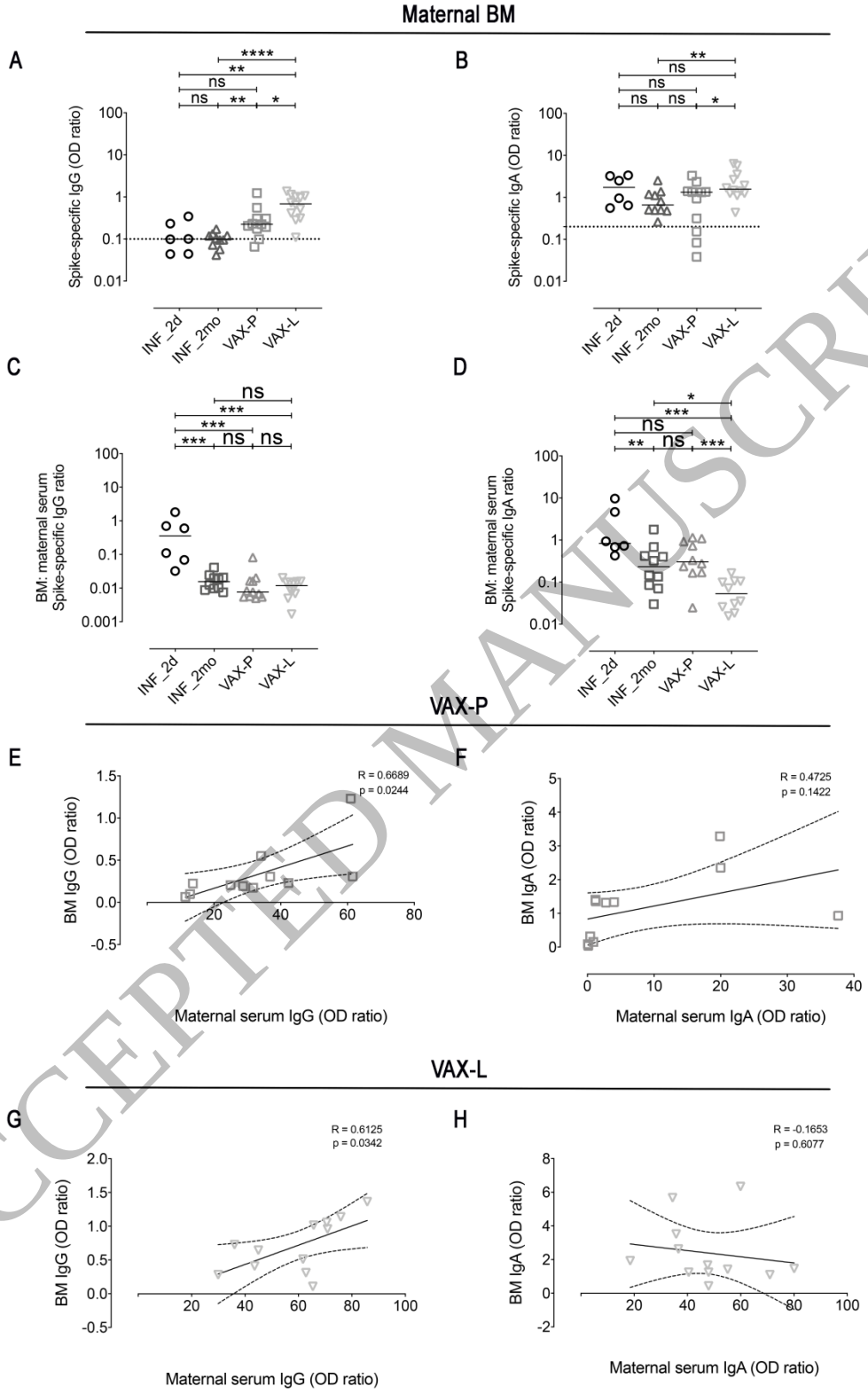
2 **162x229 mm (.50 x DPI)**

Figure 3



162x229 mm (.50 x DPI)