OBSTETRICS

# Enhanced placental antibody transfer efficiency with longer interval between maternal respiratory syncytial virus vaccination and birth

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**BACKGROUND:** Respiratory syncytial virus is associated with significant neonatal and infant morbidity and mortality. Maternal bivalent respiratory syncytial virus prefusion F respiratory syncytial virus vaccination to protect neonates and infants was approved in September 2023 for administration between 32+0 and 36+6 weeks to protect neonates and infants. This approved timeframe is narrower than the 24 to 36 week window evaluated in the clinical trial, due to the possible association between preterm birth and vaccine administration. Currently, data are lacking on how maternal vaccine timing within the approved window affects the transfer of antibodies from mother to fetus, critical information that could influence clinical practice.

**OBJECTIVE:** We sought to examine how gestational age at vaccination and time elapsed from maternal respiratory syncytial virus vaccination to delivery impacted transfer of maternal antibodies measured in the umbilical cord at delivery and in peripheral blood of 2-month infants. We also examined differences in maternal and cord respiratory syncytial virus antibody levels achieved by vaccination vs natural RSV infection.

**STUDY DESIGN:** A prospective cohort study was conducted at 2 academic medical centers between September 20, 2023 and March 21, 2024, enrolling 124 individuals who received the respiratory syncytial virus vaccine during pregnancy. Infant capillary blood was collected at 2 months of age from 29 of the infants. Maternal and cord immunoglobulin G levels achieved by respiratory syncytial virus vaccination were compared to those associated with maternal natural respiratory syncytial virus infection, using banked blood from 20 maternal:cord dyads collected prior to the availability of the maternal respiratory syncytial virus vaccine. Levels of immunoglobulin G against respiratory syncytial virus strain A2 and B fusion (F) and attachment (G) proteins and against pertussis toxin (as a comparator antigen from a vaccine routinely administered earlier in pregnancy) were measured using a Binding Antibody Multiplex Assay. Differences in titers between vaccination and natural infection were examined using Wilcoxon rank-sum test. Differences in cord:maternal transfer ratios and 2-month infant antibody levels by timing of maternal vaccination were evaluated by Kruskal-Wallis testing.

**RESULTS:** Maternal respiratory syncytial virus vaccination resulted in significantly higher maternal and cord antirespiratory syncytial virus F antibody levels than natural infection (5.72 vs 4.82 $\log_{10}$ mean fluorescence intensity, $P<.0001$ maternal; 5.81 vs 5.03 $\log_{10}$ mean fluorescence intensity, $P<.0001$ cord). Maternal vaccination 2 to 3 weeks and 3 to 4 weeks prior to delivery was associated with significantly lower cord:maternal transfer ratios than were observed when vaccination occurred >5 weeks prior to delivery ($P=.03$ for 2–3 weeks, $P=.007$ for 3–4 weeks), and significantly lower transfer ratios than observed for pertussis vaccination administered prior to 30 weeks of gestation ($P=.008$ for 2–3 weeks, $P=.03$ for 3–4 weeks, similar at >4 weeks).

**CONCLUSION:** Vaccine administration earlier in the approved 32 to 36 week window (at least 5 weeks prior to delivery) results in the highest transplacental transfer of maternal antibodies to the neonate. These results should inform the counseling of pregnant individuals on optimal vaccination timing.

**Key words:** Abrysvo, cord blood, maternal blood, neonatal immunity, RSV, transplacental transfer, vaccine

## Introduction

Respiratory syncytial virus (RSV) is a leading cause of infant respiratory disease and hospitalization globally, and the primary cause of infant hospitalization in the United States, affecting 2% to 3% of infants under 6 months of age.[1–4] The highest morbidity occurs in preterm infants, who account for 25% of pediatric RSV hospitalizations.[5] The MATISSE (Maternal Immunization Study for Safety and Efficacy) trial, a large randomized controlled trial of RSV vaccination, demonstrated that vaccination in pregnancy with the bivalent respiratory syncytial virus prefusion F (RSVpreF) recombinant protein (Abrysvo, Pfizer, New York, NY) significantly reduced newborn and infant morbidity and mortality from RSV.[6] In September 2023, the Centers for Disease Control and Prevention recommended administration of this RSV vaccine to pregnant individuals.[4,7] While the vaccine was administered between 24+0 to 36+6 weeks in the MATISSE trial (with a median gestational age at vaccination of 31.3),[6] the approved gestational age for vaccination was limited to 32+0 to 36+6 weeks, due to concerns about an observed association between preterm birth and vaccine receipt.[7–9] As fewer than half the MATISSE trial participants received the RSVpreF vaccine during the ultimately approved dosing window of

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## AJOG at a Glance

### Why was this study conducted?

To determine how timing of maternal respiratory syncytial virus (RSV) vaccination within the approved 32 to 36 week window impacts antibody transfer to the fetus and persistence at 2 months of age.

### Key findings

Vaccination early in the approved window (≥5 weeks prior to delivery) allows for the most efficient placental transfer of maternal antibody.

### What does this add to what is known?

This analysis adds needed data about transplacental antibody transfer efficiency week-by-week after maternal RSV vaccination, suggesting that clinical guidance for vaccination timing within the approved window may need to be refined to optimize neonatal protection.

32 to 36 weeks (1628 of 3568 participants), data are lacking on the impact of timing of maternal vaccination within this window on transplacental transfer of maternal antibody, a correlate of infant protection.

Given that increased transplacental transfer of maternal antibody to the umbilical cord correlates with enhanced infant protection from RSV and other respiratory illnesses,[10–16] detailed quantification of transplacental transfer week-over-week following maternal RSV vaccination could provide information critical to identifying the optimal window for maternal vaccination, and need for administration of RSV monoclonal antibody to neonates and infants. Information about how maternal RSV vaccine timing impacts transplacental transfer is particularly important in light of recent real-world safety data suggesting no clear association between Abrysvo vaccine administration and preterm birth.[17]

Our prior work evaluating prenatal administration of the COVID-19 messenger ribonucleic acid vaccines demonstrated that timing of maternal vaccination was associated with altered maternal responses and transplacental antibody transfer to the fetus,[12,13,18–20] with administration at progressively later gestational ages associated with significantly reduced 6-month infant antibody levels.[12] Thus, we sought to address possible concerns that the narrowed gestational window (32+0–36+6 weeks) for which Abrysvo was approved might similarly impact neonatal and infant immunity. Currently, vaccination at all weeks across the 32+0 and 36+6 period is treated as equivalently protective for the neonate and infant. Here, in a diverse cohort of pregnant individuals and their infants, we address this clinically important question: How does gestational age at vaccine administration within the approved window, or time elapsed from vaccination to delivery, impact transplacental transfer of maternal anti-RSV antibody and durability in infant blood?

## Materials and methods

### Study design and participant population

In this prospective cohort study, pregnant and postpartum individuals at 2 academic medical centers (Massachusetts General Hospital and Mount Sinai School of Medicine) were approached for enrollment of themselves and their infants between September 20, 2023, and March 21, 2024. Eligible participants were: (1) pregnant or 2-months postpartum, (2) at least 18 years old, (3) able to provide an informed consent, and (4) recipients of the Abrysvo RSV vaccine in pregnancy. No additional exclusion criteria were applied. Potential participants were identified via obstetric schedules, referred by practitioners at the participating hospitals, or self-referred. One hundred twenty-four vaccinated pregnant individuals were prospectively recruited for the study, with maternal and/or umbilical cord blood samples from N=122 pregnant individuals and N=29 of their 2-month infants included in the final analyses (Figure 1 and Table). All RSV vaccinated participants were included in the analysis, regardless of the level of RSV F antibodies present.

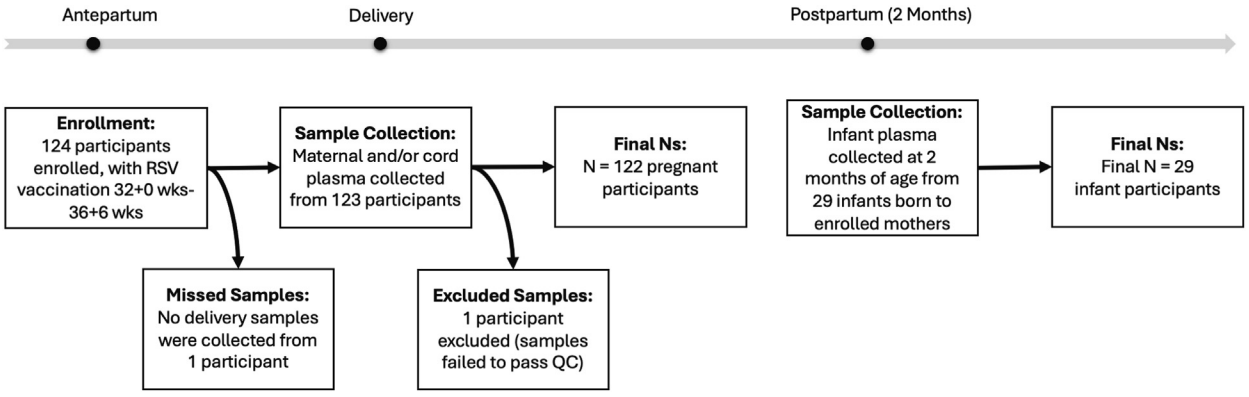
Participants received routine vaccines in accordance with standard clinical care and patient acceptance. Administration of the influenza, COVID-19, and Tetanus, Diphtheria, and Pertussis (Tdap) vaccines varied across the cohort, with N=27 participants receiving vaccines other than RSV during the 32 to 36 week window (Table). In the vaccinated group, transfer of antipertussis antibodies was assessed in participants who received the Tdap vaccine at <30 weeks of gestational age. This comparison was made to benchmark placental transfer efficiency of RSV vaccine week-over-week against that of a vaccine demonstrated in prior studies to transfer most efficiently when administered in the 27 to 30 week window.[21–23]

Banked samples from 20 maternal-cord dyads collected prior to the availability of the Abrysvo RSV vaccine (enrolled between March 15, 2021 and November 11, 2021) were also included in the final analysis. These samples were used as comparator group to evaluate differences between the top quartile of RSV antibody responses elicited by natural infection[24] vs those elicited by vaccination. All pregnant participants signed informed consent for sample collection (MGB IRB #s 2023P001874, 2020P003538, and MSSM IRB 23-01383), and birthing parents signed consent for their infants (MGB IRB #2020P000955).

### Sample collection, processing, and antibody quantification

Maternal and umbilical vein blood samples were collected by venipuncture into EDTA tubes (BD Vacutainer K3 EDTA, Becton Dickinson, Franklin Lakes, NJ) at delivery admission and birth; respectively. Samples were collected between October 25, 2023 and April 4, 2024. Blood was centrifuged at

FIGURE 1
**Flow diagram of participant enrollment, sample collection, and exclusion**




Of the 124 vaccinated pregnant individuals included in the study, there were 119 corresponding cord bloods available for analysis, including N=114 vaccinated maternal:cord dyads; 5 individuals for whom only maternal delivery blood but no cord blood was collected; and 5 individuals for whom only cord blood but no maternal delivery blood was collected.

*RSV*, respiratory syncytial virus.

1000 g for 10 minutes at room temperature. Plasma was aliquoted into cryogenic vials and stored at −80°C until antibody quantification. Infant capillary plasma was collected via microneedle device (TAP II blood collection device, YourBio Health, Medford, MA) from January 19, 2024 to April 9, 2024.

IgG antibodies against RSV strain A2 and B fusion (F) and attachment (G) proteins and against pertussis toxin (a comparator antigen from a vaccine routinely administered earlier in pregnancy) were quantified using a Binding Antibody Multiplex Assay (a highly specific Luminex based assay capable of measuring multiple analytes/antibodies simultaneously) as previously described,[25,26] and detailed in Supplemental Methods. All samples were run in duplicate, and coefficient of variation was evaluated with acceptance below 30%, consistent with recommended thresholds for this assay.[27–29]

## Statistical analysis

Statistical analyses were performed in R (v4.3.2). Luminex data were $\log_{10}$ transformed prior to analysis. For unpaired analysis comparing maternal, cord, and infant samples, or samples stratified based on the timing of the maternal RSV relative to delivery, a Kruskal-Wallis test was performed, followed by Dunn's post hoc test. *P* values were further corrected for multiple comparisons using the Benjamini-Hochberg procedure. Unpaired analyses of 2 groups were performed using Wilcoxon rank-sum test. Paired analyses between the maternal and cord dyads were performed using Wilcoxon signed-rank test. Transfer ratios were calculated by dividing the cord plasma median fluorescence intensity (MFI) value by the respective maternal plasma MFI value. The relative contributions of time from vaccination to delivery and gestational age at vaccination to transplacental transfer of maternal IgG were examined in a linear mixed effects regression model.

# Results

## Prior RSV infection vs vaccine-induced immunity

Vaccinated participants had significantly higher median antibody levels against the RSV fusion protein (anti-RSV F) than the upper quartile of unvaccinated participants (individuals who were previously infected with RSV) in both maternal blood (5.72 vs 4.82 $\log_{10}$MFI, $P<.0001$) and cord blood (5.81 vs 5.03 $\log_{10}$MFI, $P<.0001$, Figure 2, A). However, anti-G antibody levels (antibodies against the RSV attachment protein, G) were significantly higher in unvaccinated than vaccinated participants in both the maternal and cord blood (3.8 vs 4.2 $\log_{10}$MFI, $P<.001$ and 3.9 vs 4.3 $\log_{10}$MFI, $P<.0001$; respectively, Figure 2, B), an expected result since the unvaccinated group represents the upper quartile of individuals with RSV antibodies from prior infection. Similar results were identified for RSV B (Figure 2, bottom row). These results suggest that vaccination with the bivalent preF vaccine does not boost anti-G antibody levels in either maternal or cord blood, and confirm that the magnitude of F antibody levels are significantly greater after vaccination than those present in the top quartile of unvaccinated individuals.

## Vaccine-induced immunity across the maternal, cord, and infant triad

Anti-RSV F and anti-RSV G A/B antibody levels were quantified in triads of maternal, cord, and 2-month infant plasma (Figure 3). As expected in efficient transplacental transfer (cord:maternal ratios ≥1),[30,31] levels of anti-F A2 antibody were significantly higher in cord blood than in maternal blood (5.81 vs 5.72 $\log_{10}$MFI, $P<.05$). Anti-F A2 antibodies waned by 2 months of age, with significantly lower levels of anti-F A2 antibodies in 2-month infant plasma compared to both cord (5.35 vs 5.81 $\log_{10}$MFI, $P<.0001$) and maternal

TABLE
**Participant demographics and clinical characteristics**

| Weeks between RSV vaccination and delivery | | | | | | |
|---|---|---|---|---|---|---|
| Characteristic | All *N*=142 | RSV unvaccinated *N*=20 | <2 wk *N*=4 | 2–4 wk *N*=21 | 4–6 wk *N*=49 | >6 wk *N*=48 |
| Maternal age (y), mean (SD) | 34.7 (5.0) | 34.3 (4.4) | 39.0 (4.1) | 34.2 (5.9) | 35.6 (4.8) | 33.8 (4.9) |
| Race, N (%) | | | | | | |
| White | 99 (70) | 14 (70) | 1 (25) | 16 (76) | 35 (71) | 33 (69) |
| Black | 10 (7) | 0 (0) | 1 (25) | 1 (5) | 5 (10) | 3 (6) |
| Asian | 19 (13) | 5 (25) | 1 (25) | 2 (10) | 4 (8) | 7 (15) |
| Multiracial | 7 (5) | 0 (0) | 0 (0) | 1 (5) | 3 (6) | 3 (6) |
| Unknown | 7 (5) | 1 (5) | 1 (25) | 1 (5) | 2 (4) | 2 (4) |
| Ethnicity, N (%) | | | | | | |
| Hispanic or Latino | 16 (11) | 0 (0) | 0 (0) | 2 (10) | 7 (14) | 7 (15) |
| Not Hispanic or Latino | 122 (86) | 19 (95) | 4 (100) | 17 (81) | 41 (84) | 41 (85) |
| Unknown | 4 (3) | 1 (5) | 0 (0) | 2 (10) | 1 (2) | 0 (0) |
| Public insurance | 25 (18) | 2 (10) | 0 (0) | 5 (24) | 7 (14) | 11 (23) |
| Body mass index | 26.1 (5.8) | 24.5 (5.5) | 28.7 (3.7) | 27.6 (7.2) | 27.3 (6.8) | 24.7 (3.5) |
| Gestational age at RSV vaccination, mean (SD) | 33.7 (1.4) | N/A | 34.9 (1.6) | 35.4 (1.2) | 33.8 (1.3) | 32.6 (0.6) |
| Nulliparous | 64 (45) | 10 (50) | 2 (50) | 12 (57) | 16 (33) | 24 (50) |
| Gestational age at delivery, mean (SD) | 39.1 (1.3) | 39.2 (1.1) | 35.9 (1.5) | 38.5 (1.3) | 38.8 (1.2) | 39.7 (0.8) |
| Preterm birth (<37 wk) | 8 (6) | 1 (5) | 3 (75) | 2 (10) | 2 (4) | 0 (0) |
| Infant female sex | 64 (45) | 8 (40) | 3 (75) | 10 (48) | 18 (37) | 25 (52) |
| Infant birthweight (grams), mean (SD) | 3352.5 (495.3) | 3416.3 (405.7) | 2555.0 (567.5) | 3202.4 (610.6) | 3406.3 (462.2) | 3403.0 (447.9) |
| Received Tdap | 140 (99) | 20 (100) | 4 (100) | 20 (95) | 48 (98) | 48 (100) |
| Gestational at Tdap[a], mean (SD) | 28.7 (1.7) | 28.0 (2.2) | 30.6 (3.1) | 28.8 (1.4) | 29.0 (1.8) | 28.6 (1.3) |
| **Included infants** | | | | | | |
| Characteristic | N=29[b] | N=0 | N=1 | N=5 | N=14 | N=9 |
| Race, N (%) | | | | | | |
| White | 23 (79) | 0 (0) | 1 (100) | 5 (100) | 10 (71) | 7 (78) |
| Black | 1 (3) | 0 (0) | 0 (0) | 0 (0) | 1 (7) | 0 (0) |
| Asian | 3 (10) | 0 (0) | 0 (0) | 0 (0) | 1 (7) | 2 (22) |
| Unknown | 2 (7) | 0 (0) | 0 (0) | 0 (0) | 2 (14) | 0 (0) |
| Ethnicity | | | | | | |
| Hispanic or Latino | 2 (7) | 0 (0) | 0 (0) | 1 (20) | 1 (7) | 0 (0) |
| Not Hispanic or Latino | 25 (86) | 0 (0) | 1 (100) | 4 (80) | 11 (79) | 9 (100) |
| Unknown | 2 (7) | 0 (0) | 0 (0) | 0 (0) | 2 (14) | 0 (0) |
| Female sex | 16 (55) | 0 (0) | 1 (100) | 3 (60) | 8 (57) | 4 (44) |

*(continued)*

TABLE
**Participant demographics and clinical characteristics** *(continued)*

**Included infants**

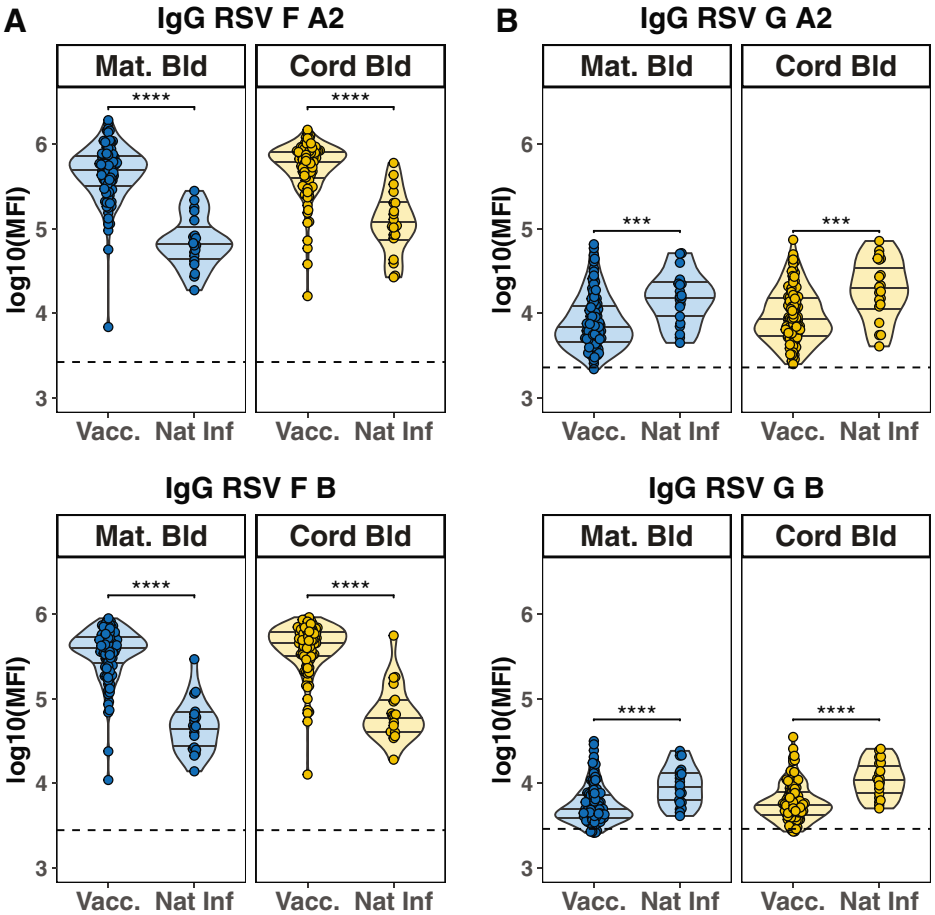
| Characteristic | N=29[b] | N=0 | N=1 | N=5 | N=14 | N=9 |
|---|---|---|---|---|---|---|
| Birthweight (g), mean (SD) | 3376.4 (505.4) | 0.0 (0.0) | 2625.0 (0.0) | 3548.0 (753.9) | 3353.9 (467.0) | 3399.4 (405.9) |
| Received nirsevimab | 1 (3) | 0 (0) | 1 (100) | 0 (0) | 0 (0) | 0 (0) |

Continuous variables are recorded as mean (standard deviation [SD]); categorical variables are recorded as N (%).

*RSV*, respiratory syncytial virus; *Tdap*, Tetanus, Diphtheria, and Pertussis.

[a] Twenty-seven participants received other vaccines during the 32—26 week window, including 13 who received COVID-19, 10 who received Tdap, and 10 who received influenza; [b] Twenty-five of the 29 included infants born to vaccinated mothers were breastfed through 2 months of age. Breast milk-acquired anti-RSV antibodies would not contribute to the plasma anti-RSV IgG levels reported here.

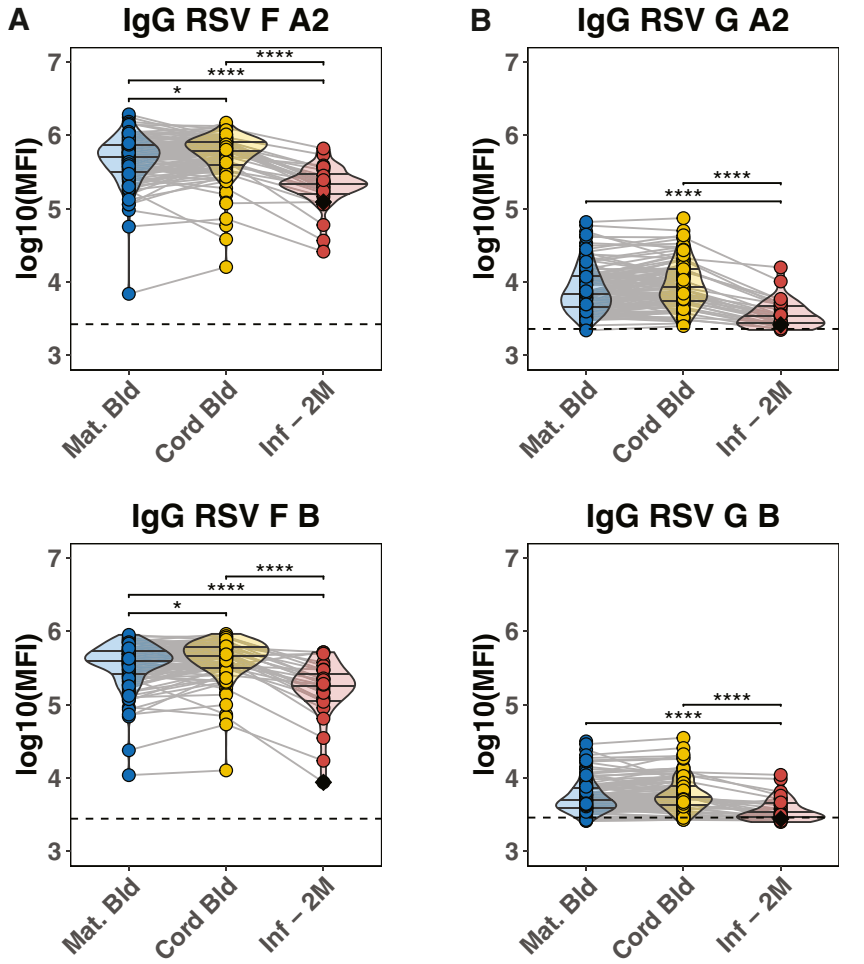
FIGURE 2
**Higher maternal:cord antibody levels after RSV vaccination versus prior RSV infection**




IgG antibody levels against RSV A2 and B fusion (F) and attachment (G) proteins in vaccinated vs naturally infected maternal:cord dyads. The violin plots show the $\log_{10}$ transformed levels of IgG antibodies targeted against **A,** RSV A2 F (*top*) and RSV B F (*bottom*) in addition to **B,** RSV A2 G (*top*) and RSV B G (*bottom*) in N=119 vaccinated maternal and cord samples, and N=20 naturally infected/unvaccinated maternal and cord samples. The *dashed line* indicates the threshold of detectability, defined as the median + 3 standard deviations above phosphate buffered saline background levels. Differences between antibody levels in the vaccinated and naturally infected/unvaccinated maternal and cord samples were assessed by Wilcoxon rank-sum test (***$P<.001$, ****$P<.0001$).

*Cord Bld*, cord; *Mat Bld*, maternal; *MFI*, median fluorescence intensity; *RSV*, respiratory syncytial virus; *Vacc.*, vaccinated; *Nat Inf.*, naturally infected/unvaccinated.

FIGURE 3
**RSV IgG antibody levels in maternal blood, cord blood, and 2-month infants**




Maternal vaccine-generated antibodies demonstrated expected transplacental transfer and waning in 2-month infants. Antibodies from natural infection (anti-G antibodies, not boosted by vaccination) demonstrate similar transplacental passage and waning, but levels are likely overall lower than those of vaccine-generated anti-F antibodies. Violin plots show $\log_{10}$ transformed levels of IgG antibodies against **A,** RSV F A2 (*top*) and RSV F B (*bottom*) and **B,** RSV G A2 antigen (*top*) and RSV G B antigen (*bottom*) in N=119 vaccinated maternal cord samples, and N=29 2-month infants. The *black diamond* represents a sample from an infant who received nirsevimab at 2 weeks old. The *dashed line* indicates the threshold of detectability, defined as the median + 3 standard deviations above phosphate buffered saline background levels. Differences between groups were assessed by Kruskal-Wallis test, followed by Dunn's post hoc test when relevant. *P* values were further corrected using the Benjamini-Hochberg procedure (*$P<.05$, ****$P<.0001$).

*Cord Bld*, cord; *Inf -2M*, 2-month infants; *Mat Bld*, maternal; *MFI*, median fluorescence intensity; *RSV*, respiratory syncytial virus.

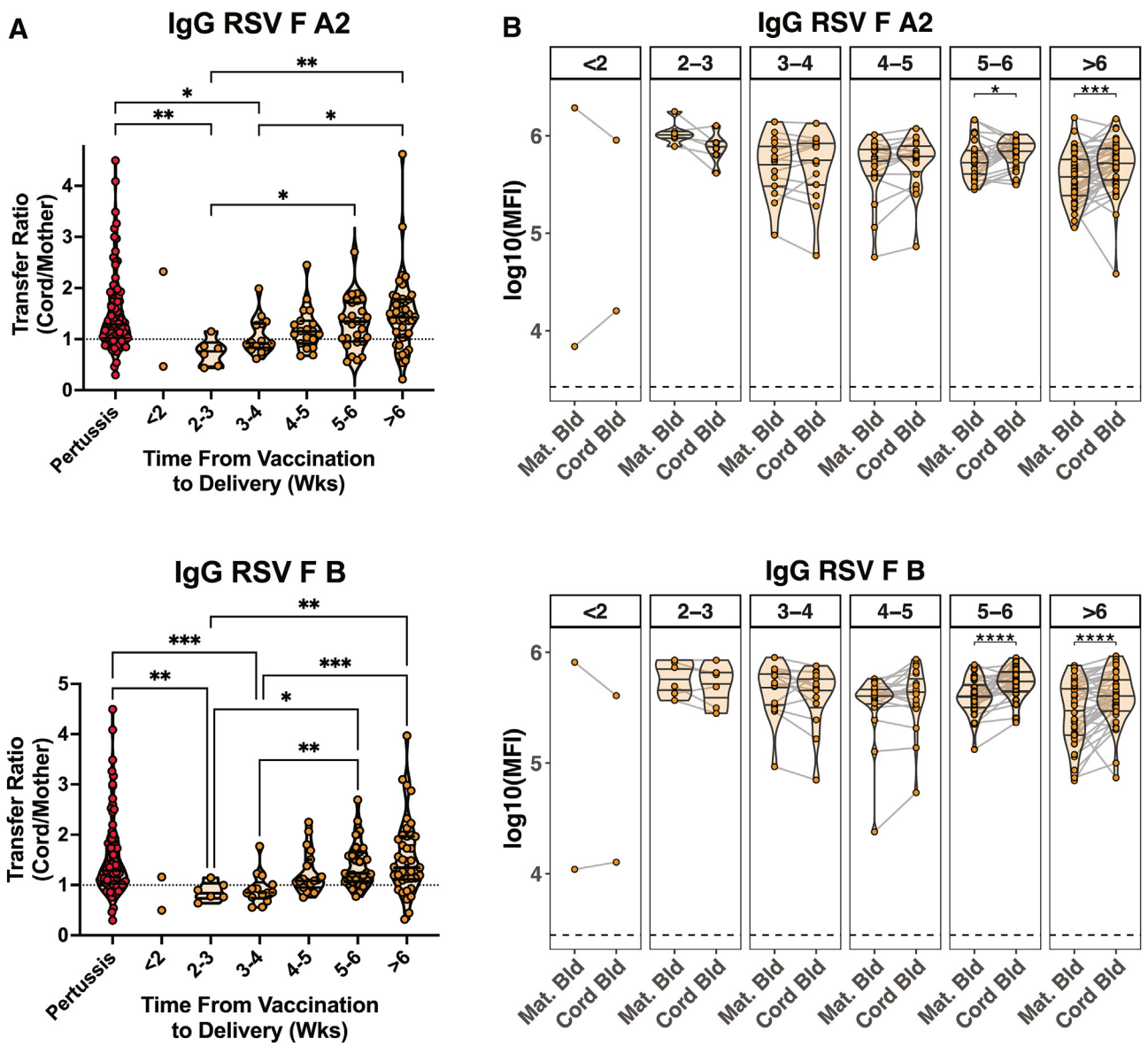
plasma (5.35 vs 5.72 $\log_{10}$MFI, $P<.0001$, Figure 3, A/Supplemental Figure 1, A). Despite the expected waning observed, antibodies detected at 2 months of age are still expected to contribute to the demonstrated protection against medically attended lower respiratory tract illness with RSV at 90 days and even (to a lesser extent) at 180 days, based on the results of the MATISSE trial.[6] Anti-RSV G antibodies (a marker for RSV infection) also waned from birth to 2 months of age (3.92 $\log_{10}$MFI in cord blood vs 3.52 2-month infant, $P<.0001$, Figure 3, B/Supplemental Figure 1, A). Similar patterns were noted for RSV B strain (Figure 3 bottom row/Supplemental Figure 1, C). An analysis of antibody persistence in 2-month-old infants using the area under the curve (AUC) of antibody levels from birth (umbilical cord) to 2 months of age demonstrated that anti-RSV F antibodies (boosted by vaccination) had significantly greater persistence than did anti-RSV G antibodies (presumably from prior infection, AUC value 0.7 vs 0.3, $P<.0001$) - Supplemental Figure 1, B and D.

## Antibody transfer efficiency by weeks from vaccination to delivery

The transplacental transfer of maternal anti-RSV F IgG by weeks elapsed from vaccination to delivery was then evaluated using cord:maternal transfer ratios (a measure of transfer efficiency Figure 4, A). Vaccination 2 to 3 weeks prior to delivery was associated with significantly lower cord:maternal transfer ratios than were observed when vaccination occurred 5 to 6 weeks prior to delivery (0.76 vs 1.35, $P=.03$), or >6 weeks prior to delivery (0.76 vs 1.43, $P=.008$). Similarly, vaccination 3 to 4 weeks before delivery also resulted in lower cord:maternal transfer ratios than vaccination >6 weeks prior to delivery (0.92 vs 1.43, $P=.03$) indicating that vaccination earlier in the approved 32 to 36 week window, at least 5 weeks prior to delivery, results in significantly more efficient antibody transfer to the fetus. Further, the cord:maternal transfer ratios of RSV vaccination 2 to 3 and 3 to 4 weeks prior to delivery were significantly lower ($P=.008$ and $P=.03$, respectively) than the transfer ratios for antipertussis antibodies generated from routine Tdap vaccination, which typically occurs prior to 30 weeks of gestation (Figure 4, A). In contrast, RSV vaccination 4 to 5 and >5 weeks prior to delivery had similar transfer ratios to those generated from pertussis vaccination.

Plotting individual mother-cord dyads by weeks elapsed between vaccination and delivery further demonstrated that the expected significant increase in cord relative to maternal levels of anti-RSV F antibodies only occurred when the vaccine was administered ≥5 weeks prior to delivery (Figure 4, B). Maternal, cord, and

FIGURE 4
**Transplacental transfer of maternal anti-RSV IgG by weeks elapsed from RSV vaccination to delivery**




The most efficient transfer of anti-RSV F antibodies (indicated by significantly higher cord:maternal transfer ratios) occurs when delivery is ≥5 weeks postvaccination. The violin dot plots show the **A,** transfer ratio (cord:maternal IgG) for antibodies against RSV F A2 (*top*) and RSV F B (*bottom*) stratified by weeks elapsed from vaccination to delivery. These transfer ratios were compared to those for antipertussis antibodies in dyads where the mother received the Tetanus, Diphtheria, Pertussis vaccine at <30 weeks of gestational age. The *dashed line* indicates a transfer ratio of 1 (definition of efficient transfer, or equivalent antibody levels in the cord blood and maternal blood). Differences between the groups were assessed by Kruskal-Wallis test, followed by Dunn's post hoc test. *P* values were further corrected using the Benjamini-Hochberg procedure (**P*<.05, *****P*<.0001). **B,** The violin dot plots represent levels of IgG against RSV F A2 (*top*) and RSV F B (*bottom*) in maternal and cord samples, stratified by weeks elapsed from RSV vaccination to delivery (weeks elapsed=numbers in box header). Lines represent maternal-cord dyads. *Dashed line* indicates the threshold of detectability, defined as the median + 3 standard deviations above phosphate buffered saline background levels. Significance between groups was assessed by Wilcoxon signed-rank test (**P*<.05, ***P*<.01, ****P*<.001, *****P*<.0001).

*Cord Bld*, cord; *Mat Bld*, maternal; *RSV*, respiratory syncytial virus.

2-month old infant anti-RSV F antibody levels by weeks elapsed from vaccination to delivery are depicted in Supplemental Figure 2, A–C and demonstrate a significant impact of timing of RSV vaccination on maternal antibody levels, which are highest 2 to 3 weeks after vaccination, and require time to transfer transplacentally to the umbilical cord, as reflected in the higher cord:maternal transfer ratios observed >5 weeks postvaccination.

We applied a series of nested linear mixed effects models of transplacental transfer of maternal RSV-specific IgG, to examine whether gestational age at vaccination was an independent factor in the efficient transplacental transfer of maternal anti-RSV IgG, and whether addition of specific clinical characteristics improved model fit. For anti-RSV F A2, weeks elapsed from vaccination to delivery significantly associated with transplacental transfer efficiency, as measured by improved fit in the model of cord:maternal IgG ratios, while gestational age at vaccination did not improve model fit (Supplemental Table 1) either alone or in combination with weeks elapsed. For anti-RSV F against B strain, however, both weeks elapsed from vaccination to delivery and gestational age at vaccination were significantly associated with transplacental transfer efficiency of vaccine-elicited maternal IgG (Supplemental Table 2). There was no statistically significant interaction between these 2 variables for either strain.

## Comment

### Principal findings

We have demonstrated, in a cohort of 142 individuals (122 RSV-vaccinated and 20 unvaccinated) and their newborns, that maternal RSV vaccination with bivalent preFusion protein (Abrysvo, Pfizer) at least 5 weeks prior to delivery is associated with optimal transplacental transfer of maternal anti-RSV IgG. As RSV-specific antibody levels at birth have been demonstrated to be correlates of protection against RSV infection in infants too young to yet to receive their own vaccines,[14–16] maternal RSV vaccination earlier in the approved 32 to 36 week window (to maximize the likelihood that at least 5 weeks elapse between vaccination and delivery) may confer the best possible protection for the neonate and infant. This finding has important implications for counseling pregnant patients about vaccine timing; currently, maternal vaccination at any point within the approved 32 to 36 week window is presumed to be equally effective for newborn protection.

We also found that RSV vaccination from 32 to 36 weeks of gestation generated significantly higher anti-F antibody levels than did natural RSV infection and that the Abrysvo vaccine does not boost anti-RSV G antibody levels, raising the possibility of using anti-RSV G antibodies as an internal marker of prior natural infection, just as antibodies against SARS-CoV-2 Nucleocapsid (N) were used to distinguish natural infection from vaccine-induced antibodies. Finally, we demonstrated that anti-F antibodies derived from vaccination have significantly greater persistence in 2-month-old infants than do anti-G antibodies, which presumably reflect prior natural infection. The greater durability of anti-F antibodies compared to naturally elicited anti-RSV G antibodies highlights the importance of maternal vaccination in neonatal and infant protection.

### Results in the context of what is known

Concerns about possible vaccine-associated preterm birth drove the U.S. Food and Drug Administration and Advisory Committee on Immunization Practices's approval of the Abrysvo RSV vaccine in pregnancy for administration only between 32+0 and 36+6 weeks of gestation. This approval was based on limited data from 45% of the phase 3 randomized controlled trial, and 38% of the phase 2b randomized controlled trial, who received vaccine or placebo during the 32 to 36 week dosing interval.[6,32] Prior work by our group examining infant protection from the COVID-19 messenger ribonucleic acid vaccines found that vaccination later in the 32 to 36 week time period was associated with lower 6-month infant IgG levels compared to vaccination earlier in the 32 to 36 week time period.[12] Our results, consistent with this prior work,[12] point to the need to examine transplacental antibody transfer week-by-week within this approved vaccine window.

If delivery occurs within 14 days of maternal vaccination, both the American College of Obstetricians and Gynecologists (ACOG) and the CDC's ACIP agree that protection of the neonate is likely insufficient. Thus, if mothers are unvaccinated, or vaccinated less than 14 days prior to delivery, the monoclonal antibody nirsevimab (which provides passive immunity by inhibiting the RSV F protein, preventing viral entry into the cells) is recommended to ensure adequate protection of newborns against RSV infection.[4,33,34] However, both ACOG and the ACIP acknowledge that this is an understudied area, with "*at least* 14 days…likely needed after maternal vaccination for development and transplacental transfer of maternal antibodies to protect the infant."[4,23,33] The lack of available data about transplacental transfer efficiency of anti-RSV antibodies week-by-week after maternal vaccination hampers evidence-based guidance not only about maternal vaccine timing, but also regarding indications for neonatal monoclonal antibody administration.

Our finding that maternal RSV vaccination at least 5 weeks prior to delivery was associated with optimal transplacental transfer of maternal anti-RSV IgG is consistent with studies in Tdap vaccines demonstrating higher antibody levels in the cord blood of neonates after maternal immunization at 27 to 30+6 weeks, compared to maternal immunization at >31 weeks of gestation.[21,22,35] These findings suggest that efficient transplacental transfer of maternal antibody following any vaccination requires at least 30 days from vaccination to delivery.[36] In other studies of maternal Tdap vaccination, efficient transplacental transfer was noted at a median of 7.5 weeks prior to delivery,[37] with enhanced durability of antipertussis antibodies in infants noted with vaccination at 27 to <32 weeks of gestational age.[38] We identified that transplacental transfer efficiency for anti-RSV F IgG was noninferior (not significantly lower) than that resulting from pertussis vaccination prior to 30 weeks even at 4 weeks post-vaccination, although vaccination ≥5 weeks prior to delivery was associated with the best transplacental transfer.

It is important to note that while the concept of antibody titer as a correlate of protection has been well-established,[10–16,21–23,39] there is not an

established minimum amount of antibody transfer and/or infant plasma antibody levels needed to confer adequate infant protection against RSV. While available data from RSV vaccine trials suggest that efficient transplacental transfer is associated with infant protection from moderate to severe RSV-associated respiratory tract infection,[6,40] none of the vaccine trials identified a minimum adequate antibody titer to confer protection. Prior studies in other pathogens, such as evaluations of the persistence of anti-Spike antibodies in infants after maternal COVID-19 vaccination, have demonstrated that increased transplacental transfer of maternal antibody is associated with higher levels of detectable antibody (and improved protection against severe disease) in infants through 6 months of age.[11–13,41,42]

Our group has previously demonstrated in a geographically diverse 300 person cohort that antibodies to RSV-F and -G antigens from naturally occurring infection were nearly ubiquitous in pregnant individuals and their neonates,[24] but the significantly enhanced infant protection conferred by maternal vaccination[6,9] suggests key differences in antibody titer and/or quality generated by vaccination. Here we found that vaccine-induced antibody titers are higher than those induced by natural infection and persist longer in infants. These findings are consistent with our findings for anti-Spike antibodies induced by COVID-19 vaccination vs natural infection in mother-infant dyads and 6-month infants.[13,20] Multilevel linear regression models demonstrated a gestational-age-specific impact of vaccination on transplacental transfer for B strain only, suggesting additional evaluation is needed in larger cohorts to determine if gestational age within the 32 to 36 week window exerts a further effect on transplacental antibody transfer.

## Clinical implications

As cord blood and infant antibody levels after maternal vaccination in pregnancy have been demonstrated to be important correlates of infant protection from respiratory illnesses,[10–13] our results provide rationale for changing how we counsel pregnant patients about receiving the RSV vaccine within the approved window, and reconsidering the current timing recommendations as real-world vaccine safety and efficacy data continue to emerge.[17] The finding of optimal transplacental transfer of maternal antibody at ≥5 weeks post-vaccination may inform future evaluations of vaccine timing, as a recent study demonstrated no association between nonadjuvanted bivalent RSV preF vaccination and preterm birth in a cohort study of 2973 patients who delivered during the 2023 to 2024 RSV vaccine season at 2 New York City hospitals.[17] In addition, given that preterm infants account for 25% of RSV related hospitalizations,[5] vaccinating earlier in the 32 to 36 week window or expanding the window may have greatest benefit for infants born to mothers with spontaneous preterm birth, often an unpredictable event. Finally, our work raises the question of whether indications for the monoclonal antibody nirsevimab postnatally should be expanded to infants born beyond the 2-week post-vaccination window, which would require much larger studies to determine more precisely what antibody levels are required for optimal protection.

## Research implications

This work highlights the power of detailed antibody analyses to provide clinically useful information. Given insights into transplacental transfer and immune cell activation in the infant provided by analyses of canonical (neonatal Fc receptor) and noncanonical (FcγR1-3) Fc-receptor binding properties of antibodies,[12,18,19,30] future work should evaluate FcR binding, antibody functions, and the glycan profile of vaccine-generated antibodies in maternal, cord, and infant blood through 6 months of age. Understanding the potential additive protection for infants provided by breast milk from RSV-vaccinated mothers and characterization of the contribution of maternal vaccination to infant mucosal immunity against RSV is an important area for future research. Finally, evaluating the impact of neonatal and infant sex on antibody properties and transplacental transfer is an important future direction, given the known impact of fetal sex on maternal and infant immunity.[43–45] This work also has the potential to provide insights into maternal vaccination relevant far beyond RSV vaccination itself, informing development of rational vaccines for maternal and infant protection and definitions of optimal vaccination strategy.

## Strengths and limitations

A strength of this study is the relatively large and diverse cohort enrolled across 2 academic medical centers in New York and Boston. Timing of Tdap vaccination was also known precisely, facilitating biological comparisons between transfer of antibody from a vaccine administered 27 to 30 weeks vs 32 to 36 weeks. This group also had no possible prior exposure to RSV vaccine, which will not be the case for future cohorts. A further

### GLOSSARY OF TERMS

Anti-RSV F antibody to the RSV fusion (F) protein
Anti-RSV G antibody to the RSV attachment (G) protein
MATISSE The phase 3 clinical trial evaluating the safety and efficacy of the RSVpreF vaccine (Maternal Immunization Study for Safety and Efficacy)
RSV Respiratory Syncytial Virus
RSVpreF A recombinant protein vaccine targeting the pre-fusion form of the F protein of Respiratory Syncytial Virus
RSV A2 The Respiratory Syncytial Virus is classified into two main subgroups: RSV A and RSV B. The A2 strain is a subgroup of RSV A
RSV B The B strain of the Respiratory Syncytial Virus
Tdap Tetanus Diphtheria and Pertussis vaccine

strength is the use of 2 biological comparator groups (1) maternal-infant dyads with prior natural RSV infection and (2) maternal-infant dyads with Tdap-generated antibodies.

Despite inclusion of a relatively large cohort, one limitation is the relatively small numbers of participants for week-over-week analysis, precluding analysis of the impact of fetal and infant sex. A larger future cohort might also permit discrimination between the relative effects of gestational age at vaccination vs time elapsed from vaccination to delivery. Breast milk antibodies also remain a critical and understudied area with respect to RSV vaccination, especially the potentially additive or synergistic protection these antibodies could confer. Finally, while antibodies are correlates of protection, determining whether the more efficient transfer of antibodies will lead to enhanced protection for infants through 2 and 6 months of age will require larger studies. Importantly, this study was designed to evaluate transplacental antibody transfer and other antibody features, and not to evaluate clinical endpoints. Thus, this study is not able to demonstrate the degree of infant protection against severe RSV disease that is conferred by vaccination week-over-week across the approved interval, nor can it establish the extent of infant protection with vaccination <5 weeks prior to delivery vs >5 weeks prior to delivery. The results do not demonstrate that vaccination >2 but <5 weeks prior to delivery provides inadequate infant protection against RSV, but rather show that transplacental transfer of antibody to the fetus is less efficient during this interval.

## Conclusions

Our results suggest that maternal RSV vaccination at least 5 weeks prior to delivery is associated with optimal RSV-specific IgG transplacental transfer. Additional larger studies are needed to further refine these findings and their clinical implications, particularly whether the timing indication for monoclonal antibody should be expanded beyond infants who delivered within 2 weeks of maternal RSV vaccination. ■

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## Supplemental methods

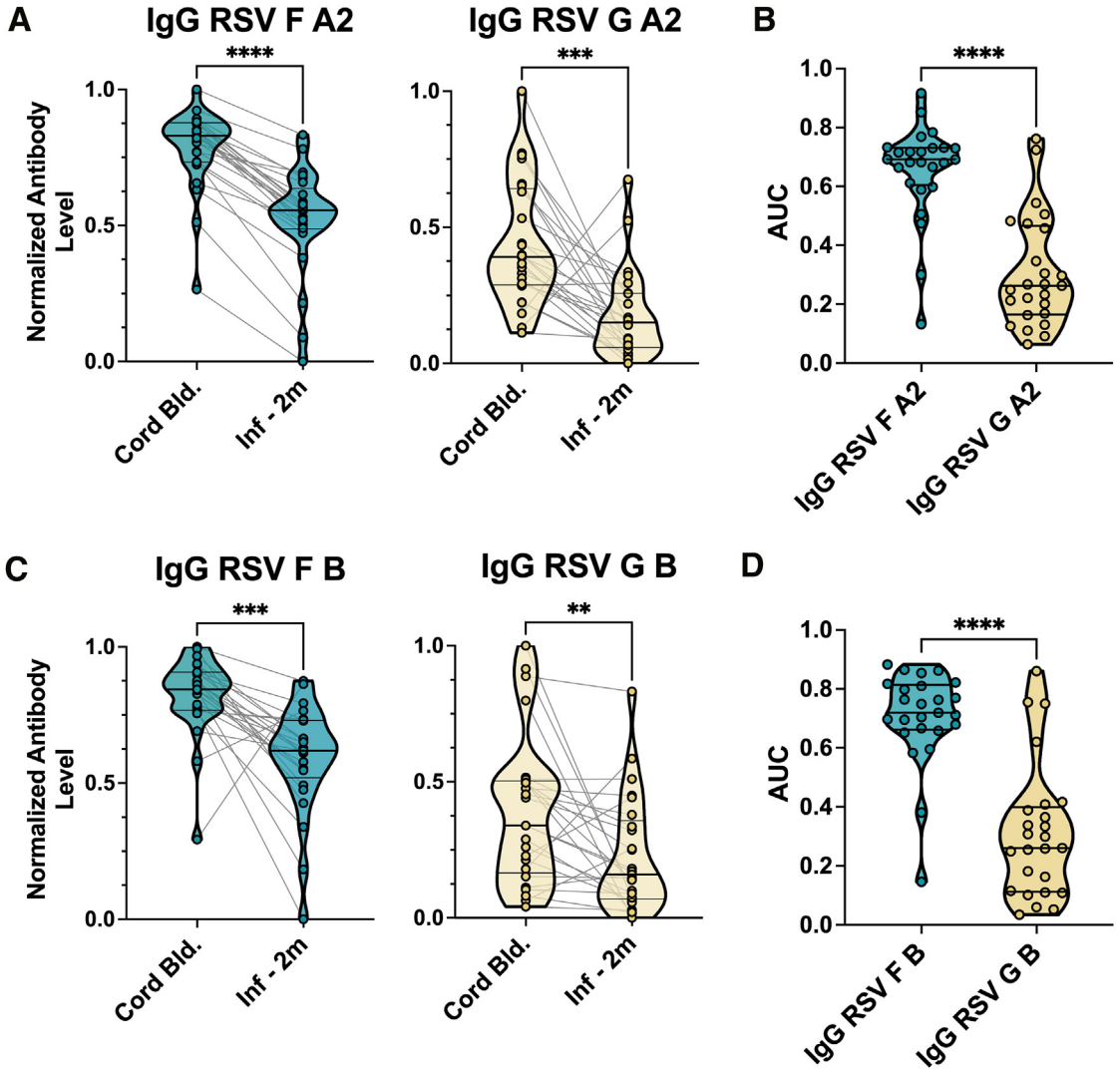
### Antibody quantification: Binding Antibody Multiplex Assay

RSV and Pertussis toxin antigens were conjugated to MagPlex microspheres (Luminex) as previously described.[20,21] The antigens conjugated to the MagPlex microspheres are as follows: Pertussis Toxin (Sigma-Aldrich, Cat# P7208), RSV-F Strain A2, RSV-G Strain A2, RSV-F Strain 18537(B), RSV-G Strain 18537(B).

Conjugated microspheres were incubated on 384-well black Aurora plate (Millipore Sigma, Cat# ABB2-00160A) for approximately 30 minutes before standards, controls and plasma samples were added. The standards, which included Pertussis Antiserum (NIBSC, WHO 06/140), Synagis/Palivizumab (Arexis AB, Sobi, Waltham, MA) and Human Reference Immune Globulin to RSV (BEI, Cat#NR-21973), were diluted in assay diluent (1% dry milk + 5% goat serum + 0.05% tween-20 in 1X phosphate buffer saline, pH 7.4.) at starting concentrations of 10 IU/mL, 10 ug/mL, 250 ug/mL respectively and serially diluted 2- or 3-fold (Pertussis Antiserum, Synagis, Human Reference Immune Globulin to RSV, respectively). Additional controls, which included: (1) Human Antiserum to RSV, High Control (BEI Resources, Cat#NR-4021), (2) Human Antiserum to RSV, Medium Control (BEI Resources, Cat#NR-4022), (3) Human Antiserum to RSV, Low Control (BEI Resources, Cat#NR-4023), (4) Human Reference Antiserum to RSV (BEI Resources, Cat#NR-4020), and (5) Human IgG-Depleted Serum (BEI Resources, Cat#NR-49447), were added undiluted. The plasma samples were diluted in assay diluent at a 1:400-point dilution. Beads and diluted samples were incubated for 30 minutes, then IgG binding was detected using a PE-conjugated mouse antihuman IgG.

(Southern Biotech, Cat#9040-09) at 2 $\mu$g/mL. The beads were washed and acquired on an xMap Intelliflex instrument (Luminex), and IgG binding was expressed as median fluorescence intensity (MFI). To assess assay background, the MFI of binding to wells that did not contain beads or sample (blank wells) and nonspecific binding of the samples to unconjugated blank beads were evaluated during assay analysis. An antigen-specific antibody response was considered positive if above the lower limit of detection, defined as the mean + 3SD of phosphate buffered saline blank well MFIs for each antigen. To check for consistency between assays, the EC50 and maximum MFI values of the standards was tracked by Levy-Jennings charts.

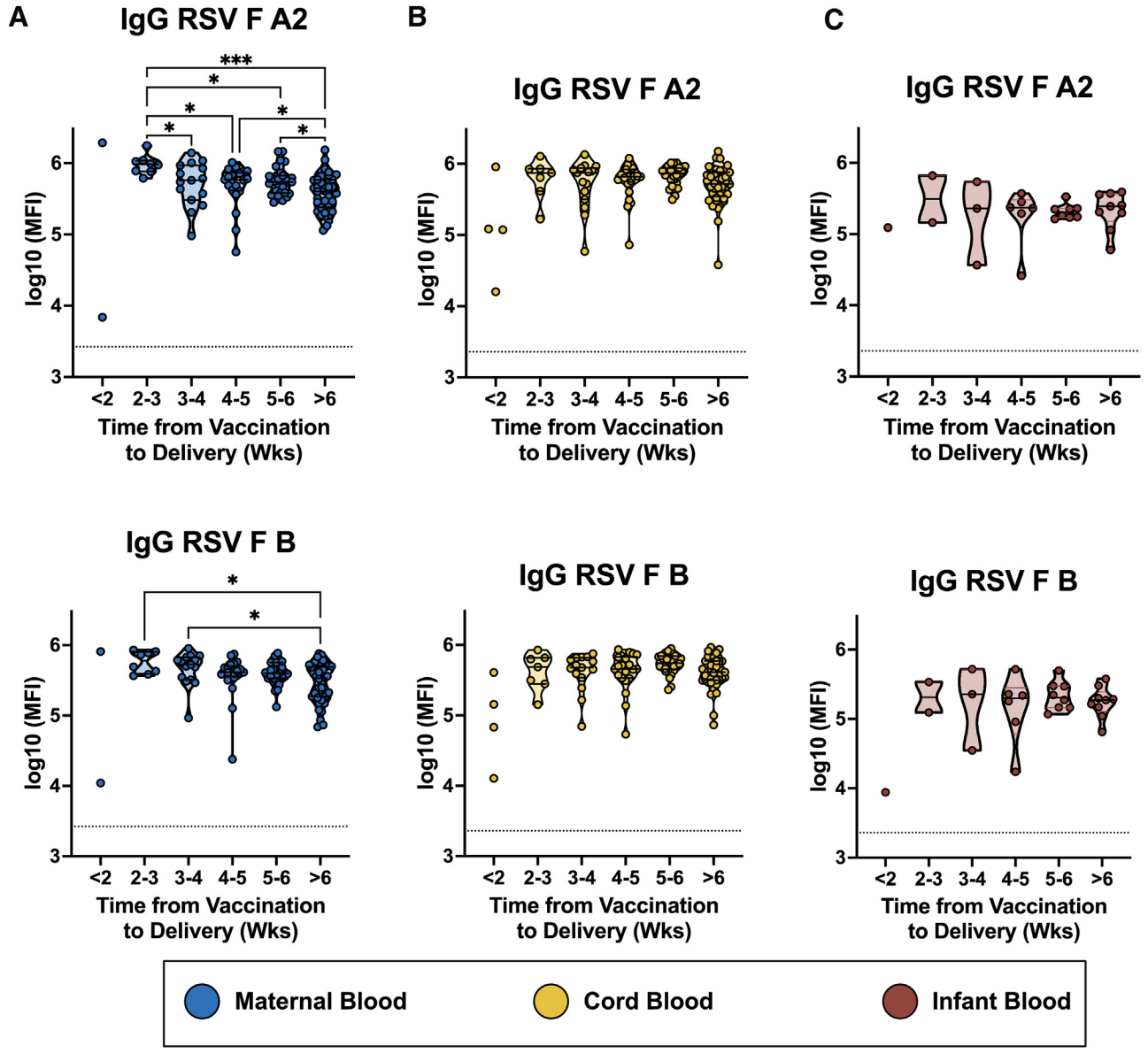
SUPPLEMENTAL FIGURE 1
**Persistence of RSV IgG from birth to 2 months of age**




**A,** Violin plots represent normalized IgG antibody levels targeted against RSV Fusion (F) A2 (*left*) and G A2 (*right*) in N=25 cord and 2-month infant (Inf -2M) pairs, with lines connecting birth and postpartum samples from the same child. Significance between groups was assessed by Wilcoxon signed-rank test (***$P<.001$, ****$P<.0001$). **B,** Violin plots represent IgG antibody persistence for anti-F A2 antibodies (from vaccination) vs anti-G A2 antibodies (from natural infection), defined by area under the curve (AUC) from birth to 2 months. AUC was calculated by taking the area beneath the matched cord-infant dyad line. Differences in AUC were assessed by Wilcoxon rank-sum test (****$P<.0001$). **C,** Violin plots represent normalized IgG antibody levels targeted against RSV F B (*left*) and RSV G B (*right*) in N=29 cord (**C**) and Inf -2M, with lines connecting birth and postpartum samples from the same child. Significance between groups was assessed by Wilcoxon signed-rank test (***$P<.001$, **$P<.01$). **D,** Violin plots represent IgG antibody persistence for anti-F B antibodies (from vaccination) vs anti-G B antibodies (from natural infection), defined by AUC from birth to 2 months. Differences in AUC were assessed by Wilcoxon rank-sum test (****$P<.0001$).

*Cord Bld*, cord; *Inf -2m*, 2-month infants; *RSV*, respiratory syncytial virus.

SUPPLEMENTAL FIGURE 2
**Maternal, cord, and 2-month infant RSV IgG levels by weeks elapsed from vaccination to delivery**




Violin plots show the levels of IgG against RSV Fusion A2 (*top*) and B (*bottom*), stratified by the number of weeks elapsed from vaccination in **A,** maternal, **B,** cord blood, and **C,** 2-month old infant samples. The *dashed line* indicates the threshold of detectability, defined as the median + 3 standard deviations above phosphate buffered saline background level. Differences between the groups were assessed by Kruskal-Wallis test, followed by Dunn's post hoc analysis if relevant. *P* values were further corrected for multiple comparisons using Benjamini-Hochberg procedure (*$P<.05$, ***$P<.001$).

*MFI*, median fluorescence intensity; *RSV*, respiratory syncytial virus.

## SUPPLEMENTAL TABLE 1
### Testing the influence of vaccination timing on placental transfer efficiency of anti-RSV-F A2 IgG

| Model | −2 Log likelihood | Likelihood ratio test (LRT) Statistic (*G*) | Degrees of freedom | *P* value |
|---|---|---|---|---|
| Intercept Only | −97.15721 | | | |
| Weeks Between Vax and Delivery[a] | −90.81157 | 12.69128[a] | 6 | .0129 (*) |
| GA of RSV[b] | −93.60024 | 7.113928[b] | 6 | .1299 (ns) |
| Weeks Between Vax and Delivery + GA of RSV[c] | −89.53744 | 2.548261[c] | 10 | .6360 (ns) |
| Weeks Between Vax and Delivery + GA of RSV + (Weeks Between Vax and Delivery) * (GA of RSV)[d] | −86.06822 | 6.938445[d] | 20 | .7312 (ns) |

Weeks between vaccination and delivery has a significant impact in the placental transfer efficiency (measured by cord: maternal transfer ratio) of anti-RSV-F A2 IgG (*P*=.0129). Gestational age at RSV vaccination did not have a significant impact (*P*=.1299).

GA of RSV = Gestational age at RSV vaccination (weeks); Weeks Between Vax and Delivery= number of Weeks Between Vaccination and Delivery.

*GA*, gestational age; *RSV*, respiratory syncytial virus.

*$P<.05$.

[a] Likelihood ratio test (LRT) comparing Weeks Between Vaccination and Delivery model with Intercept Only model; [b] LRT comparing GA of RSV model with Intercept Only model; [c] LRT comparing Weeks Between Vaccination and Delivery + GA of RSV model with Weeks Between Vaccination and Delivery Only model; [d] LRT comparing Weeks Between Vaccination and Delivery + GA of RSV + (Weeks Between Vaccination and Delivery * GA of RSV) model with Weeks Between Vaccination and Delivery + GA of RSV model.

## SUPPLEMENTAL TABLE 2
### Testing the influence of vaccination timing on placental transfer efficiency of anti-RSV-F B IgG

| Model -2 | Log likelihood | Likelihood ratio test (LRT) Statistic (*G*) | Degrees of freedom | *P* value |
|---|---|---|---|---|
| Intercept Only | −96.51907 | | | |
| Weeks Between Vax and Delivery[a] | −87.64828 | 17.74156[a] | 6 | .0014 (**) |
| GA of RSV[b] | −89.57928 | 13.87958[b] | 6 | .0077 (**) |
| Weeks Between Vax and Delivery + GA of RSV[c] | −86.79797 | 1.700634[c] | 10 | .7906 (ns) |
| Weeks Between Vax and Delivery + GA of RSV + (Weeks Between Vax to Del.) * (GA of RSV)[d] | −81.85371 | 9.888517[d] | 20 | .4503 (ns) |

Weeks between vaccination and delivery has a significant impact in the transfer ratio of anti-RSV-F B IgG of maternal-cord dyads (*P*=.0014). Gestational age at RSV vaccination also has significant impact (*P*=.0077).

GA of RSV = Gestational age at RSV vaccination (weeks); Weeks Between Vax and Del. = number of Weeks Between Vaccination and Delivery.

**$P<.01$.

[a] Likelihood ratio test (LRT) comparing Weeks Between Vaccination and Delivery model with Intercept Only model; [b] LRT comparing GA of RSV model with Intercept Only model; [c] LRT comparing Weeks Between Vaccination and Delivery + GA of RSV model with Weeks Between Vaccination and Delivery Only model; [d] LRT comparing Weeks Between Vaccination and Delivery + GA of RSV + (Weeks Between Vaccination and Delivery * GA of RSV) model with Weeks Between Vaccination and Delivery + GA of RSV model.