

Letters

RESEARCH LETTER

Detection of Messenger RNA COVID-19 Vaccines in Human Breast Milk

Vaccination is a cornerstone in fighting the COVID-19 pandemic. However, the initial messenger RNA (mRNA) vaccine clinical trials excluded several vulnerable groups, including young children and lactating individuals.¹ The US Food and Drug Administration deferred the decision to authorize COVID-19 mRNA vaccines for infants younger than 6 months until more data are available because of the potential priming of the children's immune responses that may alter their immunity.² The Centers for Disease Control and Prevention recommends offering the COVID-19 mRNA vaccines to breastfeeding individuals,³ although the possible passage of vaccine mRNAs in breast milk resulting in infants' exposure at younger than 6 months was not investigated. This study investigated whether the COVID-19 vaccine mRNA can be detected in the expressed breast milk (EBM) of lactating individuals receiving the vaccination within 6 months after delivery.



Supplemental content

Methods | This cohort study included 11 healthy lactating individuals who received either the Moderna mRNA-1273 vaccine (n = 5) or the Pfizer BNT162b2 vaccine (n = 6) within 6 months after delivery (Table 1). Participants were asked to collect and immediately freeze EBM samples at home until transported to the laboratory. Samples of EBM were collected before vaccination (control) and for 5 days postvaccination. A total of 131 EBM samples were collected 1 hour to 5 days after vaccine administration. Extracellular vesicles (EVs) were isolated in EBM using sequential centrifugation, and the EV concentrations were determined by ZetaView (Analytik) (eMethods in the Supplement). The presence of COVID-19 vaccine mRNA in different milk fractions (whole EBM, fat, cells, and supernatant EVs) was assayed using 2-step quantitative reverse transcriptase-polymerase chain reaction. The vaccine detection limit was 1 pg/mL of EBM (eMethods in the Supplement).

Results | Of 11 lactating individuals enrolled, trace amounts of BNT162b2 and mRNA-1273 COVID-19 mRNA vaccines were detected in 7 samples from 5 different participants at various times up to 45 hours postvaccination (Table 2). The mean (SD)

Table 1. Demographic and Clinical Information About Breast Milk Samples Collected From 11 Lactating Individuals After Receiving an mRNA COVID-19 Vaccine

Participant No.	Maternal age, y	Race and ethnicity	Mode of delivery	Gestational age at birth, wk	Vaccine timing after delivery, wk	Vaccine type ^a
1	33	White	Vaginal	26	10	mRNA-1273
2	33	White	Vaginal	39	25	BNT162b2
3	35	White	Vaginal	37	9	BNT162b2
4 ^b	34	Asian	Cesarean	39	18	BNT162b2
5	37	White	Cesarean	39	7	mRNA-1273
6 ^b	37	White	Vaginal	32	6	mRNA-1273
7 ^b	22	White	Vaginal	38	24	BNT162b2
8 ^b	35	White	Cesarean	39	4	BNT162b2
9	38	Black	Vaginal	39	20	BNT162b2
10 ^b	34	White	Cesarean	39	7	mRNA-1273
11	35	White	Cesarean	26	5	mRNA-1273

Abbreviation: mRNA, messenger RNA.

Pfizer-BioNTech.

^a mRNA-1273 was manufactured by Moderna and BNT162b2 by

^b Participants who had detectable vaccine mRNA in their breast milk.

Table 2. Detection of Vaccine RNA in Whole Expressed Breast Milk and Extracellular Vesicles in 5 Patients at Various Time Points Postvaccination

Participant No.	Vaccine type	Time points of vaccine mRNA detection in EBM	Concentration of vaccine mRNA detected in whole milk ^a	Concentration of vaccine mRNA detected in EBM EVs ^a
4	BNT162b2	27-h ^b Sample	Not detected	14.01 pg/mL
6	mRNA-1273	27-h and 42-h ^b Samples	11.7 pg/mL	16.78 pg/mL
7	BNT162b2	37-h ^b Sample	Not detected	4.69 pg/mL
8	BNT162b2	1-h and 3-h ^b Samples	1.3 pg/mL	6.77 pg/mL
10	mRNA-1273	45-h ^b Sample	2.5 pg/mL	2.13 pg/mL

Abbreviation: EBM, expressed breast milk; EVs, extracellular vesicles; mRNA, messenger RNA.

^a Units for concentration are picogram of mRNA per milliliter of whole milk equivalent.

^b Sample used for vaccine mRNA concentration detection.

yield of EVs isolated from EBM was 9.1^{10} (5.0^{10}) particles/mL, and the mean (SD) particle size was 110.0 (3.0) nm. The vaccine mRNA appears in higher concentrations in the EVs than in whole milk (Table 2). No vaccine mRNA was detected in pre-vaccination or postvaccination EBM samples beyond 48 hours of collection. Also, no COVID-19 vaccine mRNA was detected in the EBM fat fraction or the EBM cell pellets.

Discussion | The sporadic presence and trace quantities of COVID-19 vaccine mRNA detected in EBM suggest that breastfeeding after COVID-19 mRNA vaccination is safe, particularly beyond 48 hours after vaccination. These data demonstrate for the first time to our knowledge the biodistribution of COVID-19 vaccine mRNA to mammary cells and the potential ability of tissue EVs to package the vaccine mRNA that can be transported to distant cells. Little has been reported on lipid nanoparticle biodistribution and localization in human tissues after COVID-19 mRNA vaccination. In rats, up to 3 days following intramuscular administration, low vaccine mRNA levels were detected in the heart, lung, testis, and brain tissues, indicating tissue biodistribution.⁴ We speculate that, following the vaccine administration, lipid nanoparticles containing the vaccine mRNA are carried to mammary glands via hematogenous and/or lymphatic routes.^{5,6} Furthermore, we speculate that vaccine mRNA released into mammary cell cytosol can be recruited into developing EVs that are later secreted in EBM.

The limitations of this study include the relatively small sample size and the lack of functional studies demonstrating whether detected vaccine mRNA is translationally active. Also, we did not test the possible cumulative vaccine mRNA exposure after frequent breastfeeding in infants. We believe it is safe to breastfeed after maternal COVID-19 vaccination. However, caution is warranted about breastfeeding children younger than 6 months in the first 48 hours after maternal vaccination until more safety studies are conducted. In addition, the potential interference of COVID-19 vaccine mRNA with the immune response to multiple routine vaccines given to infants during the first 6 months of age needs to be considered. It is critical that lactating individuals be included in future vaccination trials to better evaluate the effect of mRNA vaccines on lactation outcomes.

Nazeeh Hanna, MD

Ari Heffes-Doon, MD

Xinhua Lin, PhD

Claudia Manzano De Mejia, MD

Bishoy Botros, BS

Ellen Gurzenda, BS

Amrita Nayak, MD

Author Affiliations: Division of Neonatology, Department of Pediatrics, NYU Langone Hospital-Long Island, NYU Long Island School of Medicine, Mineola, New York (Hanna, Heffes-Doon, Nayak); Women and Children's Research Laboratory, NYU Long Island School of Medicine, Mineola, New York (Lin, Manzano De Mejia, Botros, Gurzenda).

Accepted for Publication: July 25, 2022.

Published Online: September 26, 2022. doi:10.1001/jamapediatrics.2022.3581

Correction: This article was corrected on September 30, 2022, to fix the year of the study period and other minor typographical errors in the eMethods in the Supplement.

Corresponding Author: Nazeeh Hanna, MD, Division of Neonatology, Department of Pediatrics, NYU Langone Hospital-Long Island, NYU Long Island School of Medicine, 259 First St, Mineola, NY 11501 (nazeehanna@gmail.com).

Author Contributions: Dr Hanna had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Concept and design: All authors.

Acquisition, analysis, or interpretation of data: All authors.

Drafting of the manuscript: Hanna, Heffes-Doon, Lin, Nayak.

Critical revision of the manuscript for important intellectual content: All authors.

Statistical analysis: Hanna, Lin.

Obtained funding: Hanna.

Administrative, technical, or material support: Hanna, Lin, Manzano De Mejia, Botros, Gurzenda, Nayak.

Supervision: Hanna.

Conflict of Interest Disclosures: Dr Hanna reported grants from the National Institute of Child Health and Human Development, National Institute of Environmental Health Sciences, American Lung Association, March of Dimes, New York State Department of Health, and Robert Wood Johnson Foundation. No other disclosures were reported.

Funding/Support: All financial and material support for this work was provided by the Department of Pediatrics, NYU Langone Hospital-Long Island, New York University Long Island School of Medicine.

Role of the Funder/Sponsor: The supporting organization had a role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Additional Contributions: We acknowledge the participants who volunteered for this study. We are thankful to Christie Clauss, PharmD (Department of Pharmacy, NYU Langone Hospital-Long Island), for voluntarily providing unused vaccines for this study and for her critical revision of the manuscript. We are also thankful to the following individuals for their voluntary help in recruitment: Regina Cafferty, RN (Department of Pediatrics, NYU Langone Hospital-Long Island), and Elisabeth Sulger, MD, and Hollisa Rosa, MD (Department of Obstetrics and Gynecology, NYU Langone Hospital-Long Island). None of these individuals received compensation for their contribution.

1. Van Spall HGC. Exclusion of pregnant and lactating women from COVID-19 vaccine trials: a missed opportunity. *Eur Heart J*. 2021;42(28):2724-2726. doi:10.1093/eurheartj/ehab103

2. US Food and Drug Administration. Coronavirus (COVID-19) update: FDA authorizes Moderna and Pfizer-BioNTech COVID-19 vaccines for children down to 6 months of age. Released June 17, 2022. <https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-authorizes-moderna-and-pfizer-biontech-covid-19-vaccines-children>

3. Centers for Disease Control and Prevention. COVID-19 vaccines while pregnant or breastfeeding. Accessed March 8, 2021. <https://www.cdc.gov/coronavirus/2019-ncov/vaccines/recommendations/pregnancy.html>.

4. European Medicines Agency. Assessment report: COVID-19 vaccine Moderna. Published March 11, 2021. http://www.ema.europa.eu/en/documents/assessment-report/spikevax-previously-covid-19-vaccine-moderna-epar-public-assessment-report_en.pdf.

5. Pardi N, Tuyishime S, Muramatsu H, et al. Expression kinetics of nucleoside-modified mRNA delivered in lipid nanoparticles to mice by various routes. *J Control Release*. 2015;217:345-351. doi:10.1016/j.jconrel.2015.08.007

6. Bansal S, Perincheri S, Fleming T, et al. Cutting edge: circulating exosomes with COVID spike protein are induced by BNT162b2 (Pfizer-BioNTech) vaccination prior to development of antibodies: a novel mechanism for immune activation by mRNA vaccines. *J Immunol*. 2021;207(10):2405-2410. doi:10.4049/jimmunol.2100637