Transplacental transmission of the COVID-19 vaccine messenger RNA: evidence from placental, maternal, and cord blood analyses postvaccination

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OBJECTIVE: SARS-CoV-2 infection presents substantial challenges to global health, necessitating effective interventions such as COVID-19 vaccination. The initial clinical trials for the COVID-19 messenger RNA (mRNA) vaccines excluded pregnant women, leading to a knowledge gap concerning the potential biodistribution of the vaccine's mRNA to the placenta and/or the fetus after maternal vaccination.

The Pfizer and Moderna Assessment Reports that were provided to the European Medicines Agency[1,2] concluded that in animal models, a fraction of the administered mRNA dose is distributed to distant tissues, mainly the liver, adrenal glands, spleen, and ovaries. Another animal study showed that lipid nanoparticle (LNP) mRNA injections, similar in composition to COVID-19 mRNA vaccines, delivered functional mRNA to the placenta and other fetal organs.[3] Our recently published study demonstrated that the COVID-19 vaccine mRNA administered to lactating mothers can spread systemically from the injection site to breast milk, indicating that it could cross the blood-milk barrier.[4,5] Another study that evaluated the effects of maternal COVID-19 vaccination on the hematopoietic stem progenitor cells in the umbilical cord blood suggested that the LNP mRNA vaccines might reach the fetus following maternal vaccination.[6] This report presents 2 unique cases of pregnant individuals who were vaccinated with the COVID-19 mRNA vaccine shortly before delivery. This study aimed to assess the presence of the COVID-19 vaccine mRNA in the placenta and umbilical cord blood following maternal vaccination during human pregnancy.

STUDY DESIGN: This study involved 2 pregnant individuals. Patient 1, a 34-year-old gravida at 38 weeks and 4 days of gestation had pregnancy-induced hypertension and was vaccinated with 2 Pfizer COVID-19 vaccine doses and 2 booster doses (Pfizer and Moderna). The last dose was a Moderna booster administered 2 days before cesarean delivery of a healthy baby. Samples from the placenta, maternal blood, and umbilical cord blood were collected after delivery. Patient 2, a 33-year-old gravida at 40 weeks of gestation, had an uncomplicated pregnancy and received 2 Pfizer COVID-19 vaccine doses; the last dose was administered 10 days before vaginal delivery of a healthy baby. Only placental samples were collected after birth.

The presence of COVID-19 vaccine mRNA was assayed using Droplet Digital polymerase chain reaction (ddPCR) of the placenta and of umbilical cord and maternal blood. Based on the putative sequences of the mRNA1273 (Moderna) and BNT162b2 (Pfizer) vaccines, 2 PCR assays targeting 2 regions of the vaccine mRNA were designed.[5] Determining vaccine mRNA localization in the placental sections was done by in situ hybridization (ISH) using RNAscope targeting of the BNT162b2 and mRNA1273 vaccine sequences. Placental samples from mothers without COVID-19 (confirmed by PCR) and with no history of vaccination were used as the negative controls. We used placenta explants spiked with diluted BNT162b2 or mRNA1273 as positive controls. Placental expression of spike protein was evaluated using an automated capillary western blot system (WES). The stability of vaccine mRNA can be variable and may degrade during distribution and cellular entry. Because the vaccine's efficacy in activating an immune response is closely associated with the fully intact vaccine amount, we assessed the vaccine mRNA quality and extent of degradation in the samples using a ddPCR linkage duplex assay.[5]

RESULTS: The vaccine mRNA was detected in the 2 placentas evaluated (Table) using quantitative ddPCR and ISH. The localization of the vaccine mRNA was mainly in the villus stroma (Figure 1B and D) with a notably high signal in the decidua of patient 1 (Figure 1A) when compared with that of patient 2 (Figure 1C). Using WES, the spike protein expression was detected in the placenta of patient 2, but not in patient 1 as demonstrated in the Figure 2A. Furthermore, the vaccine mRNA was detected in the umbilical cord and maternal blood of patient 1 using ddPCR (Table). Unfortunately, no umbilical cord or maternal blood samples were available for analysis for patient 2. Finally, the integrity of the vaccine mRNA varied across different samples. In the placentas, 23% and 42% of the original integrity were retained in patients 1 and 2, respectively (Table). The vaccine mRNA in the maternal blood showed a high integrity level of 85%; however, in the umbilical cord blood, it decreased to 13% of the original vaccine mRNA' integrity (Figure 2C and D).

CONCLUSION: Our findings suggest that the vaccine mRNA is not localized to the injection site and can spread systemically to the placenta and umbilical cord blood. The detection of the spike protein in the placental tissue indicates the bioactivity of the vaccine mRNA that reach the placenta. Notably, the vaccine mRNA was largely fragmented in the umbilical cord blood and, to a lesser extent, in the placenta. These 2 cases demonstrate the ability of the COVID-19

TABLE
Summary of vaccination history and vaccine mRNA and spike protein detection

Characteristic	Patient 1	Patient 2
Gestational age	38 wk + 4 d	40 wk + 0 d
Birth type	Cesarean delivery	Vaginal delivery
COVID-19 disease history	1 mo before delivery	No COVID-19 history
Days between the last vaccination and delivery	2	10
COVID-19 vaccine history	Pfizer (3 doses) and 1 Moderna booster	Pfizer (2 initial doses)
Last vaccine type	Moderna booster	Pfizer second dose
Vaccine mRNA detection in the placenta		
By ddPCR	5,033,000[a] (23%)[b]	1,387,000[a] (42%)[b]
By ISH	Detected	Detected
Spike protein detection in the placenta		
By WES	Not detected	Detected
Vaccine mRNA detection in maternal and cord blood		
Maternal blood (by ddPCR)	209,761[c] (85%)[b]	N/A
Cord blood (by ddPCR)	56,653[c] (13%)[b]	N/A

ddPCR, Droplet Digital polymerase chain reaction; *ISH*, in situ hybridization; *mRNA*, messenger RNA; *WES*, automated capillary western blot system.

[a] mRNA copies per gram tissue; [b] Relative linkage; [c] Copies per mL blood.

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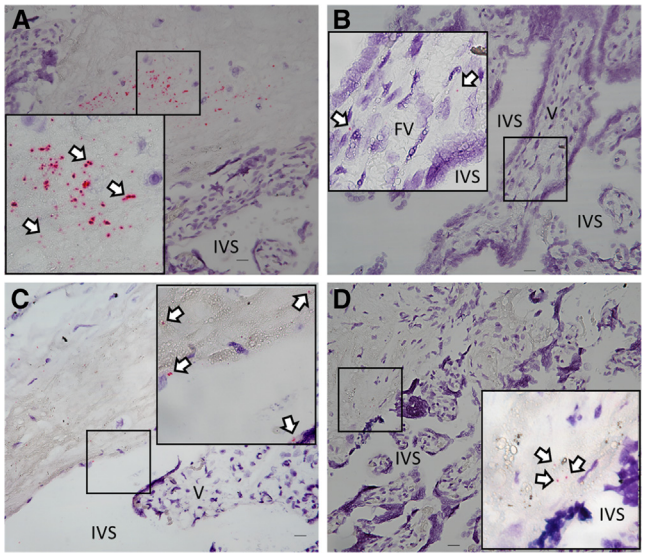
vaccine mRNA to penetrate the fetal-placental barrier and to reach the intrauterine environment.

Two previous human studies by the same research group investigated the presence of COVID-19 vaccine mRNA in the placenta, but with different methodologies and results.[7,8] The first study, in which quantitative reverse transcriptase PCR (qRT-PCR) was used, failed to detect mRNA in maternal blood, umbilical cord blood, or placental tissue, possibly because of the long interval between vaccination and delivery and the use of a single primer set not fully aligned with the mRNA-1273 vaccine.[7] In their subsequent study to improve the sensitivity of the detection, an RNAscope-based ISH assay was used, which also did not detect the vaccine mRNA. However, the probe that was used targeted the SARS-CoV-2 S gene rather than the vaccine mRNA sequence.[8] This can lead to inaccurate results because of the mismatch between the probe and the target sequence. In our study, we adopted a more sensitive and robust approach. We used 2 primer sets that covered ~1.5 kb of the full-length mRNA vaccine to enhance detection sensitivity. Furthermore, we used ddPCR for more precise quantification of the vaccine mRNA, thereby offering superior accuracy and sensitivity over RT-qPCR. Lastly, our RNAscope-based ISH assay used a probe tailored explicitly to the vaccine mRNA, thus ensuring more reliable detection.

In this report, the placental concentration of the vaccine mRNA was higher in patient 1 (delivered 2 days after vaccination) than in patient 2 (delivered 10 days after vaccination). This observation is likely attributable to the short half-life of the vaccine mRNA, leading to rapid degradation by day 10 after vaccination. Conversely, the expression of the spike protein in the placenta of patient 2, but not in patient 1, suggests that more than 2 days are required after vaccination for the mRNA to reach the placenta and to be translated into the spike protein, which is then expressed in the placental tissue. Notably, a significant amount of the vaccine mRNA in the maternal blood of patient 1 was also detected in the umbilical cord blood (approximately one-third) (Table). However, the vaccine mRNA integrity was significantly reduced to 13%. Although the vaccine mRNA in umbilical cord blood seemed fragmented, suggesting limited bioactivity, further investigation is required to determine the minimum amount of mRNA required to elicit an immune response in the fetus. Although our findings are novel, they represent only 2 cases, and validation through subsequent research is needed. Furthermore, the specific mechanisms and contributing factors that facilitate the transplacental transport of vaccine mRNA need further exploration.

The evidence overwhelmingly supports the effectivity of the COVID-19 vaccine in mitigating the morbidity and mortality of COVID-19 in pregnant and nonpregnant individuals. The widespread acceptance and proven safety of mRNA vaccines during the COVID-19 pandemic have opened doors for other mRNA therapies. Although gene therapy, particularly mRNA-based treatments, shows promise, research on its perinatal delivery is still emerging. Prenatal therapy can be advantageous, because it offers early disease intervention and reduced immunogenicity. In experiments

FIGURE 1
COVID-19 vaccine mRNA detection in the placenta by in-situ hybridization



The panel demonstrates COVID-19 vaccine mRNA detected in paraffin embedded placental tissue using in situ hybridization (RNAscope). Panel A and B represent samples from patient 1 and demonstrate positive signals in the decidua (A) and the villi (B) using RNAscope Probe- S-encoding-mRNA-1273-C1. Panel C and D represent samples from patient 2 and demonstrate positive signals in the decidua (C) and the villi (D) using RNAscope Probe- S-encoding-BNT-162b2-C1.

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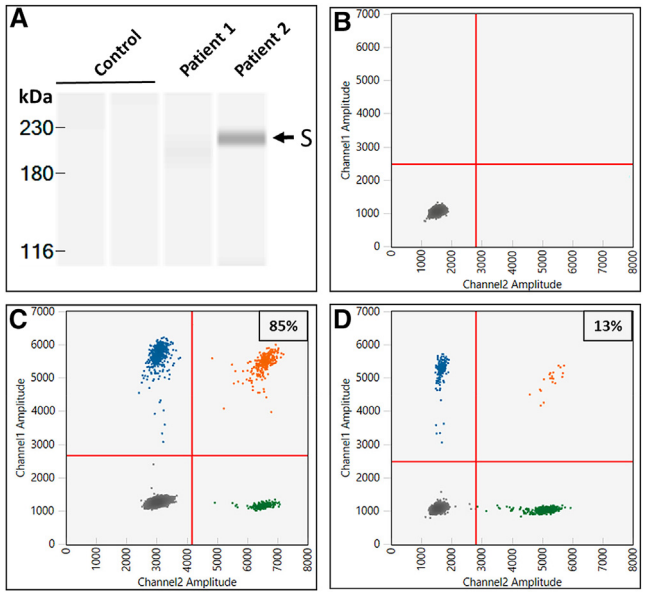
FIGURE 2
The expression of S protein in the placenta and the integrity of vaccine mRNA in cord and maternal blood



A. Expression of S protein in tissue lysate of placental biopsies from patients 1 and 2, analyzed by automated capillary western blot system (WES). The control was a pre-pandemic placenta sample. C and D. Circulating vaccine mRNA integrity was assayed in a duplex ddPCR assay in samples from patient 1 maternal blood (C, relative linkage 85%) and cord blood (D, relative linkage 13%). B. represents a blood sample of an unvaccinated subject showing no positive signal. Droplets emitting 2-dimensional signals were separated into 4 groups, namely gray indicating double negative for mRNA1273-1 and mRNA1273-2; blue indicating positive for mRNA1273-1 but negative for mRNA1273-2; green indicating positive for mRNA1273-2 and negative for mRNA1273-1; and orange indicating double positive for both mRNA1273-1 and mRNA1273-2. The number of droplets in each single or double positive group was calculated using QX Manager Software, and the percentage linkage of each sample was expressed as a percentage of linked molecules in relation to the total molecules detected and normalized to the original vaccine stock solution.

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with pregnant rats, LNPs successfully delivered various mRNAs, including one potentially useful for treating fetal anemia.[3] Although introducing mRNA to the fetus may potentially pose plausible risks, it may also have biologically plausible benefits. The potential of mRNA-based interventions to address maternal and fetal health issues is profound. Such insights could substantially advance the crafting of safer and more effective mRNA-based therapies during pregnancy.

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Xinhua Lin, PhD
Bishoy Botros, BS
Women and Children's Research Laboratory
Department of Foundations of Medicine
New York University—Grossman Long Island School of Medicine
Mineola, NY

Monica Hanna, MD
Division of Neonatology
Department of Pediatrics
New York University Langone Hospital—Long Island
New York University—Grossman Long Island School of Medicine
Mineola, NY

Ellen Gurzenda, BS
Claudia Manzano De Mejia, MD
Women and Children's Research Laboratory
Department of Foundations of Medicine
New York University—Grossman Long Island School of Medicine
Mineola, NY

Martin Chavez, MD
Division of Maternal-Fetal Medicine

Department of Obstetrics and Gynecology
New York University Langone Hospital—Long Island
New York University—Grossman Long Island School of Medicine
Mineola, NY

Nazeeh Hanna, MD
Women and Children's Research Laboratory
Department of Foundations of Medicine
New York University—Grossman Long Island School of Medicine
Mineola, NY
Division of Neonatology
Department of Pediatrics
New York University Langone Hospital—Long Island
New York University—Grossman Long Island School of Medicine
259 First St
Mineola, NY 11501
Nazeeh.Hanna@NYULangone.org

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Raw data for every experiment are available upon request. Upon justifiable request, the sharing of de-identified data should be approved by the board of an investigational ethics committee.

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