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Case Report

Detection of Pfizer BioNTech Messenger RNA COVID-19 Vaccine in Human Blood, Placenta and Semen

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Abstract

This study examines the persistence of synthetic mRNA from the COVID-19 vaccine Comirnaty in the blood, placenta, sperm, and seminal fluid of both vaccinated and unvaccinated individuals. Samples were collected from 34 participants, including 22 pregnant women, four male patients from a fertility clinic (providing eight samples), and eight additional individuals. RNA was extracted and analyzed using nested PCR, and the resulting amplicons were confirmed by Sanger sequencing. Vaccine mRNA was detected in most samples from vaccinated individuals including their blood, placenta tissue, sperm and seminal fluid samples. Notably, vaccine mRNA remained detectable in approximately half of the samples collected more than 200 days after vaccination, indicating prolonged persistence in the body. These findings expand the limited data available on the biodistribution of the Comirnaty vaccine and its potential implications for pregnancy and fertility.

Keywords: Biodistribution; BNT162b2; Comirnaty; COVID-19; mRNA vaccine; pharmacokinetics; placenta; semen

Introduction

Pandemics have plagued humankind throughout history, from the Plague of Justinian in 543 BC, through the bubonic plagues, cholera, flu and AIDS to the recent COVID-19 pandemic. Since the start of the 21st century, the world has been plagued by three deadly pandemics associated with novel coronaviruses, namely SARS, MERS and COVID-19. The spread of COVID-19 was extraordinarily rapid, with countries across the world being affected in a matter of weeks, leading many national healthcare systems to the verge of collapse [1]. As of April, 2023, globally confirmed cases of COVID-19 numbered over 764 million, with about 7 million deaths being reported to the WHO. By the beginning of 2023, more than 13 billion vaccine doses have been administered [2]. Comirnaty (BNT162b2), developed by Pfizer and BioNTech, accounted for about 4.5 billion of these COVID-19 vaccine doses [3].

Comirnaty successfully reduced the occurrence of severe disease, hospitalization and death. The vaccine is composed of lipid nanoparticles (LNPs) [4] engulfing 30 μg ($1.3*10^{13}$ molecules) of stabilized synthetic N1-methyl pseudouridine-modified mRNA encoding the SARS-COV-2 spike glycoprotein. The full sequence of Comirnaty is publicly available [5] and contains a cap, a 5′-untranslated region (UTR) from the human α-globin gene, sequence encoding the full-length SARS-COV-2 protein

containing two introduced proline-encoding mutations, a 3′-UTR containing part of the human mitochondrial 12S rRNA and human TLE5 gene segments with two C→U mutations and a poly (A) tail. The urgent need to curb the COVID-19 pandemic necessitated that only short-term biodistribution and pharmacokinetics studies of the vaccine were performed, with more detailed analyses being performed later [6,7]. The persistence of Comirnaty vaccine mRNA in lymph nodes after vaccination was assessed by in situ hybridization of needle biopsies collected 7–60 days after the second dose of vaccination. The results show vaccine mRNA in samples from vaccinated individuals up to 60 days after this second dose (no later time points were measured), raising the possibility of vaccine mRNA remaining in the body for several months [8].

Several studies have shown placental transfer of anti-SARS-COV-2 antibodies following Comirnaty immunization [9-12] but none have shown transfer of the vaccine mRNA itself. In addition, testing for the presence of SARS-COV-2 RNA in the sperm of males in fected with COVID-19 by real-time RT-PCR and nested PCR [13] showed no evidence of SARS-COV-2 RNA in any of the 36 patients enrolled, although other studies did detect SARS-COV-2 RNA in semen [14]. In the present study, we used nested PCR to test for the presence of synthetic mRNA of the Comirnaty COVID-19 vaccine in blood, placenta, sperm and seminal fluid collected from individuals vaccinated with Comirnaty. Our results indicate the persistence of the vaccine mRNA in these fluids and tissues for an extended period (200 days +) after vaccination.

Materials and Methods

Blood and placenta samples were collected from 22 pregnant women. Blood was also collected from eight additional participants. Sperm and seminal fluid samples were collected from four participants attending the Bartoov Medical Center Fertility Clinic in Petah Tikva, Israel, yielding a total of eight samples. All samples were collected after writing and informed consent, in accordance with the rules of the Declaration of Helsinki of 1975 and following approval from the relevant ethics committees.

Whole blood (1 to 3 ml) was collected in EDTA-coated tubes and centrifuged for plasma separation. Pieces of placenta were dissected and frozen at -80°C until RNA extraction.

Total RNA was extracted from blood cell pellets using the Monarch total RNA kit (New England Biolabs) according to the manufacturer's standard protocol. Total RNA was extracted from placenta, sperm and seminal fluid using trizol (Bio-Tri reagent, Biolab) according to the manufacturer's standard protocol. The placenta samples were homogenized with trizol, using a homogenizer in the first step of the extraction protocol. RNA amounts and quality were determined using Qubit and Bioanalyzer. Equal amounts of RNA was used for cDNA synthesis. cDNA was prepared using a Thermo-Fisher High capacity cDNA reverse transcription kit.

For nested PCR, several sets of primers were tested (Table 1). The set that gave the best results in terms of both sensitivity and specificity were selected. These primers amplified the sequence coding for viral spike protein S1, corresponding to bases 1254 to 1791 of the vaccine sequence. Primers amplifying the region spanning bases 16 to 178 in the 5'-UTR of the vaccine sequence, and primers amplifying the 3'-UTR at the junction of the two 3' sequences used, namely, bases 3962 to 4092, gave non-specific amplification and were not used. RNA extracted from a donor blood a couple of years before the COVID-19 pandemic was used as a negative control throughout the experiments. PCR using primers specific to the housekeeping gene GAPDH were used to verify the samples integrity and amount.

Location on vaccine mRNA sequence	Orientation	Sequence	Outside (O) or nested (N)
16	F	TTCTTCTGGTCCCCACAGAC	O
52	F	ACCATGTTCGTGTTCCTGGT	N
125	R	AGCTGTGTTCTGGTGGTCAG	N
178	R	CCTTGTCGGGGTAGTACACG	O
1254	F	CGTGATCCGGGGAGATGAAG	O
1337	F	ACTTCACCGGCTGTGTGATT	N
1745	R	TGCTGGAATGGCAGGAACTT	N
1791	R	GGGATCTCTAACGGCGTCTG	O
3962	F	CAGGTATGCTCCCACCTCCA	O
3989	F	CACTCACCACCTCTGCTAGT	N
4045	R	TGTGGCTAGGCTAAGCGTTT	N
4092	R	GGTTAATCACTGCTGTTTCCCG	O

Table 1: PCR primers tested. Details of thePCR primers used in this study. F- Forward primer, R- Reverse primer, O- Outside (first PCR round) primers, N- nested (second PCR round) primers.

PCR amplification involved 20 amplification cycles using the outer primers, followed by PCR product purification using NucleoSpin Gel and PCRClean-up kits, followed by 30 amplification cycles using the inner primers. Products were separated by electrophoresis on 2% agarose gels and shown to be of the expected size. The bands were excised, purified and subjected to Sanger sequencing, which verified the expected vaccine sequence in the PCR product.

Results

Cohort

We studied 16 pregnant women who received Comirnaty COVID-19 (Pfizer-BioNTech) vaccine during pregnancy between December, 2020 and September, 2021. One woman received the first two doses before pregnancy, while all others received their doses when pregnant. All participants gave birth between June and October, 2021. The time between the first mRNA vaccine dose and delivery ranged from 30 to 277 days, and the time between the last dose received and delivery ranged from 27 to 250 days. Four of the women received three vaccine doses, eleven received two doses and one received a single dose. Six additional non-immunized women were included in the study.

Trans-placental transfer of vaccine mRNA

To test for trans-placental transfer of vaccine mRNA, we examined placenta tissue and coding for the viral spike protein. As expected, RNA presence in both blood and placenta diminished with time from the last vaccination received (Table 2a). Vaccine sequences were detected in blood and placenta in 88% of subjects who received their last vaccination less than 100 days prior to sample collection. In samples collected between 100 and 200 days from the last injection, vaccine RNA was detected in 60% of the subjects, while in about half of the women examined, vaccine RNA was still detectable after more than 200 days from the last vaccination. Results from blood and placenta from all vaccinated women were in agreement, namely, that the vaccine was either detected in both blood and placenta or in neither sample. Interestingly, vaccine sequences were detected in both the blood and the placenta of two of the six non-vaccinated women. One non vaccinated woman presented vaccine sequences in blood alone. These findings were validated using two independent concentration assays.

Participant ID	Detection in placenta	Detection in blood	# Vaccine doses	Days from last vaccine dose
14	+	+	3	27
22	+	+	1	30
12	+	+	3	32
20	-	-	3	40
10	+	+	3	44
2	+	+	2	83
1	+	+	2	85
16	-	-	2	104
3	-	-	2	110
18	+	+	2	133
6	+	+	2	156
9	+	+	2	162
21	-	-	2	203
17	-	-	2	220
15	+	+	2	230
13	+	+	2	251
4	+	+	0	

a: Mothers - unvaccinated				
5	-	-	0	
7	-	+	0	
8	-	-	0	
11	+	+	0	
19	-	-	0	
b: General population				
291 (m)		+	3	55
292 (m)		+	2	212
293 (m)		-	2	238
294 (m)		-	0	
296		-	0	
295		-	0	
340 (m)		-	0	
337*		-	2 (Sputnik)	299

Table 2: Characteristics of enrolled participants and PCR results. **a**: Mothers. Characteristics of enrolled participants and PCR results. (m) designates males. *Participant #337 was vaccinated with the Sputnik vaccine. + designates positive results by nested PCR in at least three of four technical repeats.

Vaccine mRNA persistance in the blood

In addition to the samples described above, eight more blood samples were analyzed, three from participants vaccinated with Comirnaty, one from an individual vaccinated with Gam-COVID-Vac (Sputnik V) and four from non-vaccinated subjects. No RNA was detected in the non-vaccinated and the Sputnik V-vaccinated samples. In blood samples from vaccinated participants, two were positive (55- and 212-days post-vaccination), and one was negative (238 days post-vaccination) (Table 2b). These results corroborate the results from pregnant women, demonstrating that vaccine mRNA molecules can be de- 148 tected in the blood of about 50 percent of the population after more than 200 days following immunization.

Vaccine mRNA persistance in sperm and seminal fluid

We also analyzed for the presence of Comirnaty RNA in the sperm and seminal fluid of four vaccinated donors frequenting a fertility clinic (Table 3). Two of the donors, vaccinated 14 and 27 days prior, had RNA in both their sperm and seminal fluid. One of these individuals, vaccinated 168 days prior, only presented RNA in his sperm. Another participant who produced only seminal fluid and who was vaccinated only 5 days before, had no vaccine RNA in the fluid.

Participant ID	Sperm cells	Seminal plasma	# vaccine doses	Days from the last vaccine dose
76893*	+	-	1	5
76915	+	+	3	14
76894	+	+	3	27
61917	+	-	2	168

Table 3: Sperm Donors. Characteristics of enrolled men and samples analyzed. + designates positive results by nested PCR in at least 3 of 4 technical repeats. *Participant #76893 produced no sperm.

Discussion

The biodistribution and pharmacokinetics of the Comirnaty vaccine were analyzed in rodents for 14 days by Pfizer BioNTech [4]. Human pharmacokinetics studies showed that vaccine mRNA persisted in circulation for at least 2 weeks [6], and in breast milk for 45 hours [7] post-vaccination. Prahl et al. [12] evaluated the trans-placental transfer of Comirnaty RNA, vaccine products and functional anti-SARS-CoV-2 antibodies during pregnancy, and failed to detect vaccine RNA. They estimated the sensitivity of their method (qPCR) to be 1.5 pg/µl, corresponding to about $3*10^5$ vaccine mRNA molecules. The use of nested PCR in the present study enabled us to achieve much higher sensitivity, and reliably detect the vaccine mRNA in placenta, albeit non-quantitively. Still, the presence of vaccine mRNA in the placenta itself does not necessarily reflect trans-placental transfer.

We also detected vaccine mRNA in the sperm of all three vaccinated donors who produced sperm. Interestingly, this contrasts with the presence of the SARS-COV-2 itself.

To date, only a fraction of publications dealing with the presence of SARS-COV-2 in semen found viral-specific sequences [11]. Most of the participants who had viral sequences detected in the semen in that study were in the acute phase of the disease [12]. These studies however, used RT-qPCR, a technique that we have shown to be much less sensitive than the nested PCR employed in this work.

We detected vaccine mRNA in the blood, placenta and semen of some subjects over 200 days post-immunization. The use of nested PCR with 20 and 35 cycles in the first and second PCRs, respectively, combined with relatively large amount of RNA used for analysis, enabled detection of even very few vaccine molecules [15].

We found no indication whether the full-length vaccine mRNA is still present after more than 200 days in the body. However, in a previous study [16], we detected 17,000 reads mapped across 98% of the vaccine spike protein mRNA sequence in the blood seven days after vaccination. Castruita et al. [17] also detected 12,000 mapped reads covering 86% of the vaccine mRNA in the blood 15 days after vaccination, suggesting that in somecases, full-length vaccine mRNA can remain in the blood for extended periods of time. The encapsulation of the mRNA within nanoparticles, together with the modifications to the RNA nucleotides, appear to bestow enhanced stability on the vaccine mRNA.

In two of the six non-vaccinated mothers considered here, trace amounts of vaccine RNA were detected in both the placenta and blood, although the source of this RNA has yet to be investigated. Further studies are also needed to ascertain whether the vaccine retains its ability to induce S-protein expression. Finally, our analysis has several limitations. First, our study cohort was small. Second, we did not perform quantitative analysis, since we had no vaccine to use as a standard, and the large number of PCR cycles required made quantitative estimation unreliable.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of the Hillel Yaffe Medical Center (Approval # 0084-21-HYMC).

Informed Consent Statement: All samples were collected after written and informed consent, in accordance with the rules of the Declaration of Helsinki of 1975 and following approval from the relevant ethics committees.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

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