LETTER TO THE EDITOR

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BNT162B2 COVID-19 mRNA vaccination did not promote substantial anti-syncytin-1 antibody production nor mRNA transfer to breast milk in an exploratory pilot study

Dear Editor,

Vaccine hesitancy still threatens global efforts to end the COVID-19 pandemic caused by SARS-CoV-2 and its emerging variants. Social media-driven "conspiracy theories" cast doubts on vaccine safety for reproductive health,[1] including concerns that vaccine-induced SARS-CoV-2-neutralising antibodies (NAb) cross-react with human syncytin-1—a protein involved in gamete fertilisation and normal placental development[2]—resulting in infertility or pregnancy loss. Protein sequence similarities between syncytin-1 and SARS-CoV-2 spike protein S2 domain raise this possibility.[3] Concerns over BNT162B2 mRNA persistence may prompt affected women to defer vaccination due to a perceived lack of reproductive toxicity or breastfeeding safety data, though COVID-19 vaccination is not contraindicated pre-conception, in pregnancy, or for breastfeeding.[4] Given the enduring susceptibility of pregnant women to COVID-19 complications, and the risk when facing variants of higher transmission potential, such vaccine hesitancy is worrying.[5]

To address these issues, we performed an observational study of a convenience sample of female at-risk frontline workers receiving BNT162B2 to investigate post-vaccination presence of vaccine mRNA and anti-syncytin-1 antibodies. We analysed 42 plasma and 30 breast milk samples from 15 consented female frontline staff at the National University Hospital, Singapore, collected before and after the first dose of BNT162B2, in this institutional review board-approved study (DSRB2012/00917). The participants included 5 breastfeeding mothers and 2 women inadvertently vaccinated in early pregnancy. The study was approved under National Healthcare Group Domain-Specific Review Board (Domain D) DSRB2012/00917 and the methods were conducted in accordance with the Declaration of Helsinki. All study participants provided written informed consent.

Plasma was collected at Day 0 (pre-vaccination), 1–4 days and 4–7 weeks. Breast milk samples were collected daily for the first week, the timing designed to capture the presence of mRNA in plasma and breast milk (degraded within days), and peak neutralising activity (about 3 weeks post-vaccination) to answer our research questions.[6] Blood and breast milk-derived total RNA was amplified by real-time reverse transcription polymerase chain reaction using TaqMan primers and probe amplifying an 87bp segment of the BNT162B2 mRNA spike-encoding region.[7] Negative controls were water and pre-vaccination plasma RNA; positive controls were day 1 post-vaccination RNA. Illumina Nextera XT DNA Library Preparation Kit was used for library production and barcoding. Paired end-sequencing of 300bp read length was performed on the iSeq100 sequencer and sequenced reads were aligned to human references using HISAT2 with comparison to published Pfizer sequences.

Anti-syncytin-1 antibodies were determined using standard semi-quantitative ELISA methodology, incubating plasma in Maxisorp plates coated with 100ng of human syncytin-1 recombinant protein (MyBioSource). Negative control pre-vaccination plasma gave an optical density reading at absorbance wavelength 492nm (OD_{492}) of ~0.1. Positive controls comprised pre-vaccination plasma spiked 1:250 with rabbit anti-human syncytin-1 antibody (MyBioSource) following optimisation, as it produced consistent OD_{492} of ~0.9. Briefly, all samples and controls were diluted 1:50 with dilution buffer (2% bovine serum albumin [BSA] in phosphate-buffered saline with Tween [PBST]), incubated at room temperature in prepared plates. Positive controls were incubated with goat anti-rabbit secondary antibody (1:1000 dilution) (Thermo Fisher Scientific Inc, Waltham, US), samples incubated with goat anti-rhesus secondary antibody (1:4000 dilution) (SouthernBiotech, Birmingham, US), colorimetric reactions generated with o-phenylenediamine dihydrochloride (Sigma) and OD_{492} analysed with background correction.

SARS-COV-2 NAb were detected using SARS-CoV-2 Surrogate Virus Neutralization Test Kits (GenScript Biotech Corp, Piscataway, US). Samples and controls were pre-incubated with horseradish peroxidase (HRP) Receptor Binding Domain (RBD), allowing binding of circulating NAb to HRP-RBD. The mixtures were added to capture plates (pre-coated with human angiotensin converting enzyme-2 protein), washed to remove circulating NAb HRP-RBD complexes, and colorimetric reactions to the remaining unbound HRP-RBD elicited and read at OD_{450}. Inhibition (inversely

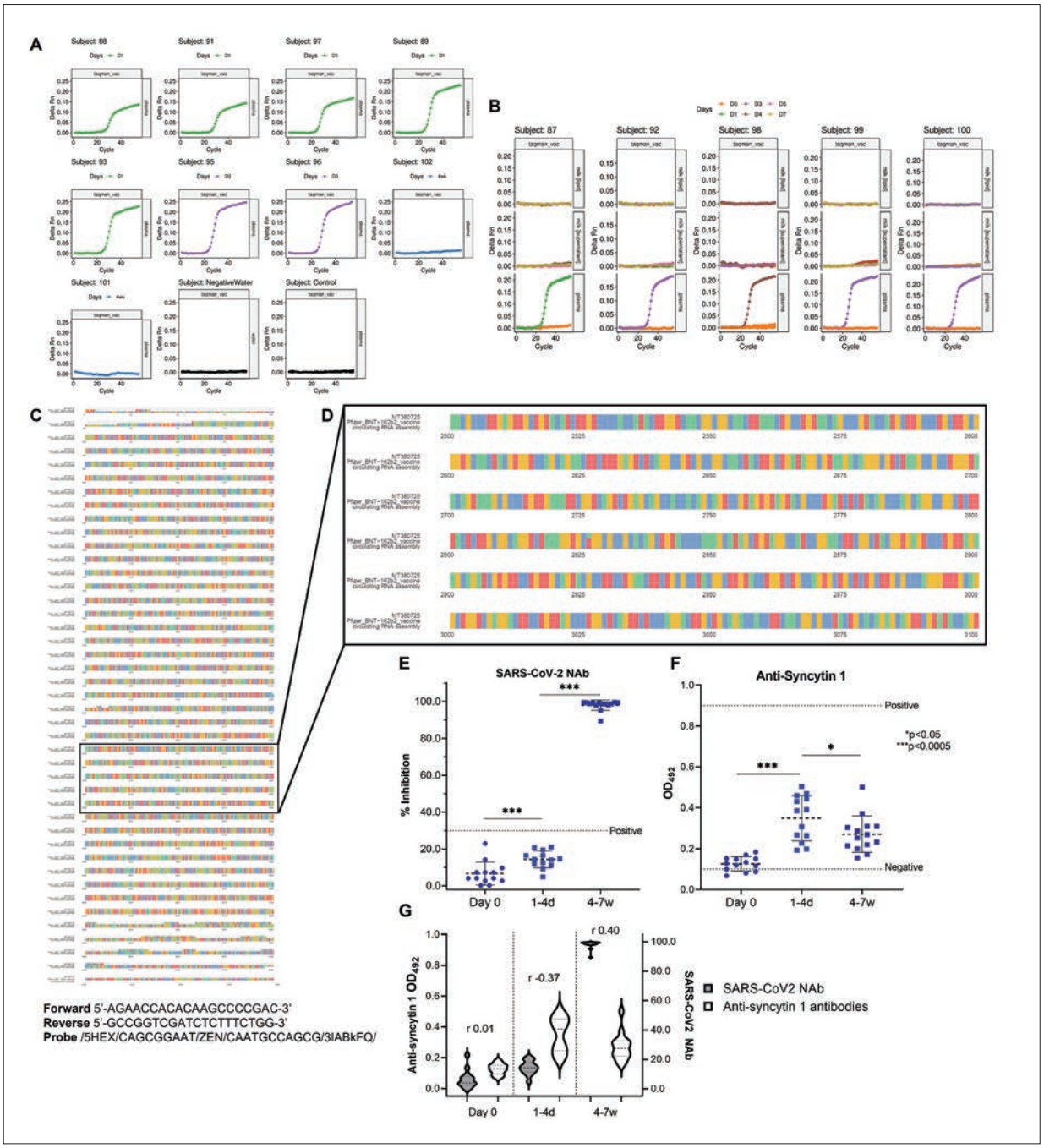

Fig. 1. BNT162B2 mRNA detection, sequence alignment of plasma with vaccine RNA, neutralising activity and anti-syncytin-1 antibodies. (A) Amplification of BNT162B2 mRNA at Ct<30 was observed in all plasma samples between 1 and 4 days post-vaccination, but not in 4-week plasma (subjects 101 and 102), after a single dose. Negative control (pre-vaccination plasma) and water blanks did not amplify, demonstrating TaqMan primers/probe specificity. (B) Five breast milk samples collected on days 0–7 (lipid and supernatant factions analysed separately) did not amplify vaccine mRNA. (C) Spike protein-encoding region of the BNT162B2 mRNA amplified by whole transcriptome sequencing of extracted RNA; primers and probe sequences are listed. (D) Magnified sequence from 2500–3100bp showing almost perfect alignment between sample RNA, BNT162B2 and MT380725 (native SARS-CoV-2 spike protein) sequences. (E) All subjects were negative (<30% inhibition) for SARS-CoV-2 neutralising antibodies (NAb) on days 0–4 and strongly positive by 4–7 weeks (P<0.0005). (F) All samples showed low-level binding antibodies to human syncytin-1 antigen well below the positive limit (0.9 OD492). Small increases were observed at successive time points (P<0.05) but all levels remained below 0.9 OD492. (G) Weak correlation was observed between NAb and anti-syncytin-1 antibodies at all time points (Pearson correlation coefficients r<0.8, not significant).
d: day; w: week
--- dashed line: mean; error bar: standard deviation

proportionate to anti-SARS-CoV-2 NAb titres) was calculated based on OD_{450} absorbance, and ≥30% was interpreted as a positive result (manufacturer's instructions). Results are expressed as mean±SD and analysed by one-way analysis of variance using Tukey correction for multiple comparisons, and Pearson correlation coefficients (GraphPad Prism v9.2.0 for Windows, GraphPad Software Inc, San Diego, US).

Participants were of Malay, Indian and Chinese ethnicities, mean age 40.4±12.2 years, and had all received 2 doses of BNT162B2 according to the prescribed schedule, except the 2 pregnant subjects (101 and 102) who had each received a single dose. Plasma BNT162B2 mRNA was detected within 4 days of vaccination (n=13, Ct<30 in all samples, Fig. 1A), but none was observed in breast milk (n=30 samples, Fig. 1B). Early plasma samples were not obtained from the 2 pregnant participants at the time of vaccination as they were not yet recruited; subsequently their plasma samples did not amplify BNT162B2 mRNA at week 4 after a single vaccine dose. High-quality sequenced reads from whole transcriptome sequencing were assembled to obtain draft genomic scaffolds of 4196bp, comparable in size to BNT162B2 mRNA (Fig. 1C); we observed perfect alignment from 120–4077bp against the published sequence, and multiple sequence alignment to MT380725 (SARS-CoV-2 spike protein, GenBank) demonstrated agreement at the nucleotide level (Fig. 1D). We found weak inhibition <30% at day 0 (mean activity 6.95±6.45%) and day 1–4 (14.68±4.73%), and strongly positive inhibition 4–7 weeks post-vaccination (98.65±1.24%, Fig. 1E), including in the pregnant participants (>89.0%). At the same time points, anti-syncytin-1 binding activity increased from OD_{492} 0.12±0.03 (day 0) to 0.34±0.11 (day 1–4) and 0.28±0.09 (4–7 weeks, both increases $P<0.05$). All values were below the assay's positive threshold and were interpreted as negative (Fig. 1F). Non-significant correlation was found between NAb and anti-syncytin-1 activity (Pearson's r 0.1–0.4, Fig. 1G).

Our finding, that vaccinated women with detectable mRNA and high NAb did not produce a corresponding positive anti-syncytin-1 response, is important because it suggests that cross-reactivity to trophoblast syncytin proteins is unlikely. This is corroborated by a recent study also demonstrating no cross-reactivity between anti-SARS-CoV-2 antibodies and syncytin-1 pre- and post-vaccination.[8] We are unable to comment on the significance of the low-level increase in anti-syncytin-1 levels as clinical thresholds have not been established, but we interpret these as negative results based on the assay limits we established. We encourage a restrained interpretation of our findings, as spontaneous miscarriage is the most common outcome reported after COVID-19 mRNA vaccination.[9] Our pilot study adds a unique angle by demonstrating the absence of anti-syncytin-1 antibodies in parous and non-parous women who were vaccinated in early pregnancy, in the postnatal period and remote from childbirth. Our limited data also complements other breast milk safety studies,[10] and supports recommendations to continue breastfeeding throughout vaccination. While our numbers are small, they support vaccination in this at-risk group, though longitudinal surveillance of a larger cohort of vaccinated women is desirable to parse long-term immunological impact of this mRNA vaccine.

Disclosure

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