The last word on COVID-19 vaccines and breastfeeding?

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More than two years have now elapsed since the COVID-19 vaccination was first authorized and administered worldwide. Over time, evidence on COVID-19 vaccines has been continuously reviewed by regulatory bodies leading to progressive inclusion of even the more susceptible groups (*i.e.*, infants, pregnant and breastfeeding women). The initial lack of consensus, in concert with the absence of any preliminary data about mRNA vaccine transfer and persistence in breast milk (BM), has determined hesitancy and low compliance among breastfeeding mothers.

In this issue of *eBioMedicine*, Hanna and colleagues attempted to put the final word on the COVID-19 vaccine mRNA biodistribution, fragmentation and biological activity in BM.[1] They demonstrated that vaccine mRNA does not persist in the injection site but can be systemically spread, being mainly packaged into BM extracellular vesicles (EVs). In addition, they definitively reported the presence of mRNA vaccine in BM, although displaying large fragmentation and very little translational activity.

Recent scientific literature has mainly focused on determining the presence and functionality of anti-Spike protein antibodies in the milk of vaccinated women,[2,3] but only few studies have investigated the levels of BNT162b2 or mRNA-1273 mRNA in BM.[4] Golan et al., analyzing 13 BM samples collected from 7 breastfeeding women 24 h after vaccination -including multiple time points, up to 48 h, from a single participant-have initially neglected the presence of vaccine-associated mRNA.[5] Low et al. instead reported minimal transfer of BNT162b2 at the sub-picogram level in approximately 10% of samples from BM: the highest concentration detected was 2 ng/mL, which would be consistent with a 0.667% of the original vaccine dose in 100 mL of BM.[6] Yeo et al. also detected transient low intact vaccine mRNA levels in 5/309 (2%) BM samples, but not in serum samples collected from breastfed infants.[7] It is worthwhile to mention that limited sample sizes, low sensitivity techniques and sample storage may have significant impact on the results of these studies.

The ultimate and more convincing evidence for the presence of vaccine mRNA in BM is precisely this study by Hanna et al., in which 154 samples were collected from 13 breastfeeding individuals at different time intervals after the first and/or booster dose.[1] The group detected small amounts of vaccine mRNA in 15 samples up to 48 h post-injection. Interestingly, for the first time, they provided evidence for vaccine mRNA packaging in EVs isolated from milk supernatants, as previously anticipated by the same group,[8] with approximatively 12–90% of vaccine mRNA found in the EV fraction. Similar findings paved the way for the proposal of a new model of vaccine mRNA biodistribution in BM: after intramuscular injection, lipid nanoparticles enclosing vaccine mRNA systemically diffuse to mammary glands *via* hematic or lymphatic pathways; within mammary cells, vaccine mRNA is packaged into developing EVs and released into the BM. Similar results are not surprising for the researchers working on lipid nanoparticles, as off-target distribution has been a major issue of these formulations for the last decade.[9]

Interestingly, the authors moved a step further than other previous publications in this field by exploring the potential translational activity and integrity of vaccine mRNA. Assuming that vaccine mRNA is mainly packaged in BM EVs, which are resistant to gastric and pancreatic digestion and retain the capability to enter human intestinal cells *in vitro*,[10] the decisive breakthrough consists in the demonstration that residual mRNA vaccine is unable to induce Spike protein expression in EV-treated HT-29 cells. This was likely due to poor vaccine mRNA integrity since it was reduced to 12–25% in the BM, as demonstrated by duplex droplet digital PCR (ddPCR) using two probes targeting the flanks of the intact mRNA vaccine. We would like to particularly point out that the study by Hanna and colleagues had the great merit of overcoming some technical limitations of previous research, mainly using ddPCR and linkage duplex assays, since the use of two distinct set of primers allowed a more specific and sensitive targeting of the two respective vaccine mRNAs.

We should keep in mind that transient detection of vaccine mRNA in BM is not synonymous of harm, as suggested by its scarce integrity and biological activity. The potential benefits of maternal vaccination during lactation for the mother-infant dyad outweigh any conceivable hazards, in accordance with recommendations from governmental health agencies. The accumulating evidence, in particular the results emerging from the current study, allows us to reasonably assert that any danger for breastfeeding newborns is, to say the least, remote; however, further safety studies are needed to shed light on the fate of BM-derived EVs in the

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infant's body and to provide the very final word on this still sensitive and controversial issue.

Contributors

Andrea Balduit and Chiara Agostinis: conceptualization and original draft preparation.

Roberta Bulla: review and editing.

Declaration of interests

The authors declare no conflict of interest.

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