Comment on "The Role of Human Breast-Milk Extracellular Vesicles in Child Health and Disease"

Dear Editor:

I enjoyed reading the perspective by O'Reilly et al. on extracellular vesicles (EVs) in human milk (1). A few points warrant clarification or comment by the authors. Although O'Reilly et al. provide an excellent discussion of potential roles of EVs and their cargos in child health and disease, they miss out on the opportunity of commenting on a fundamental question: are milk EVs and their cargos bioavailable? In the discussion of analytical protocols, the authors correctly point out that it is difficult to compare findings from distinct laboratories due to the plethora of different protocols used to purify EVs from milk. In Table 1, O'Reilly et al. list cargos identified in human milk EVs. For microRNA cargos, the table is incomplete because O'Reilly et al. show only those microRNAs that were readily apparent in the papers they cited. O'Reilly et al. should have gone through the exercise of extracting the sometimes hundreds of microRNAs from raw data repositories detected in some of the studies they cited. For protein cargos, it would have been helpful to distinguish between positive and negative markers of EV subsets, particularly markers of exosomes and microvesicles, and contaminants like casein and fat globules. Negative markers are crucial when assessing the purity of preparations. Contrary to a statement by O'Reilly et al., the International Society for Extracellular Vesicles stepped away from prescribing methods for the purification of EVs in its publication *Minimal Information for Studies of Extracellular Vesicles 2018* but is encouraging authors to document how EVs were purified so that others can draw their own conclusions (2). This represents an important change compared with the 2014 guidelines, which had a more prescriptive tone (3). On a more editorial note, when referring to 1 of our publications in Table 1, O'Reilly et al. suggest that the number of samples analyzed was not reported. That is not correct; in our paper we state that 3 milk samples were used in microRNA sequencing analysis and the assessment of EV size, concentration, and storage stability (4).

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Supported by the National Institute of Food and Agriculture (NIFA), USDA, under award numbers 2015-67017-23181 and 2020-67017-30834, NIH grants 1P20GM104320 and R21 OD028749, the University of Nebraska Agricultural Research Division (Hatch Act), and USDA multistate group W4002 (all to JZ).

The granting agencies had no influence on the study design; the collection, analysis, and interpretation of the data; the writing of the manuscript; or the decision to submit the manuscript for publication.

Author disclosures: JZ is a consultant for PureTech, Inc. and serves on the Editorial board of *Advances in Nutrition*; JZ reports no conflicts of interest.

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