

Ovalbumin in breast milk is associated with a decreased risk of IgE-mediated egg allergy in children

To the Editor,

There is an urgent need to delineate early-life factors protecting against food allergy, a rising epidemic affecting up to 10% of children.¹ Prevention strategies now include early introduction of egg and peanut into infant diet to induce oral tolerance.¹ However, recent studies demonstrate a significant proportion of infants already have egg sensitization and clinical reactivity (including anaphylaxis) at 4–6 months of age prior to the first introduction of egg in their solid food diet.¹ This underscores the necessity to identify earlier and safer ways to promote oral tolerance development to food allergens in young infants. In seminal studies, we demonstrated that the presence of hen's egg-derived allergen ovalbumin (OVA) in breast milk induces oral tolerance and prevents allergic reactions in mice.² Here, we addressed whether human data support this observation in a study of 88 breastfed infants at high risk for allergy, who were control participants of a larger double-blind randomized controlled trial (RCT), the Infant Fish Oil Supplementation Study³ (www.anzctr.org.au; ACTRN12606000281594), where all the mothers are allergic.³ Breast milk samples were collected at 3 months (122 samples) and/or at 6 months (102 samples) of lactation, and aliquots were stored at -80° prior to analysis.³ OVA levels were determined by ELISA⁴ (level of detection 50 pg/mL). IgE-mediated egg allergy was defined by a paediatric immunologist on the basis of a questionnaire capturing history of immediate symptoms following ingestion of egg in any form and a positive skin prick test (≥ 3 mm) to egg white.³ Statistical analyses were performed with SAS 9.4 (The SAS Institute) and GraphPad Prism 6 (GraphPad Software).

OVA was detected in 49% and 45% of breast milk samples collected at 3 and 6 months, respectively, with median value (1st quartile; 3rd quartile) of 150 pg/mL (73 pg/mL; 318 pg/mL) and 173 pg/mL (100 pg/mL; 644 pg/mL) (Figure 1A). Strikingly, among the 98 samples for which we had a breast milk sample available at both 3 and 6 months, one third (32/98; 32.6%) had no OVA detected at neither 3 nor 6 months (Figure 1B). Two thirds of breast milk samples had detectable OVA either at both time points of collection (27/98; 27.5%) or only at one time point of collection (39/98; 39.5%) (Figure 1B). To determine whether OVA exposure through breast milk may be associated with decreased egg allergy risk, we compared the prevalence of IgE-mediated egg allergy in children, which had been exposed to breast milk with detectable OVA at 3 and/or 6 months to those exposed to breast milk with undetectable OVA at both 3 and 6 months. Such data were available for 88 children for whom we had a follow-up at 1 year and at 2.5 years. In this high-risk population, 13.6% of infants had IgE-mediated egg allergy at 1 year and 9% at 2.5 years

(Figure 1C). There was no statistically significant difference in population characteristics between egg-allergic and nonallergic children (Table 1). At 1 year of age, relative risks (RR) and confidence intervals (CI) for IgE-mediated egg allergy in children exposed to breast milk with OVA detectable at 3 and/or 6 months vs undetectable at both 3 and 6 months, was 0.55 (0.20; 1.55), *P* Fisher's exact, two-sided = .31. At 2.5 years of age, we observed more than a fourfold reduction in the prevalence of IgE-mediated egg allergy in children exposed to OVA in breast milk as compared to those with no OVA detected at either 3 or 6 months, RR 0.23 (0.06; 0.85), *P* Fisher's exact, two-sided = .03 (Figure 1C). We further addressed whether the time of egg introduction in the child's diet would influence the association between having OVA in breast milk and egg allergy risk. Age of egg introduction was relatively late in this birth cohort of 2011 as compared to current recommendations.¹ The median age of egg introduction was 11 months and was similar between egg-allergic and nonallergic children (Table 1). To adjust for age at egg introduction while reporting exact statistics, we switched to exact logistic regression. This analysis did not provide evidence for confounding since the crude and adjusted odds ratios were very similar (Table S1).

While breastfeeding is the most efficient way for infectious disease prevention, there is inconsistent evidence for its beneficial effect on food allergy risk.^{5,6} Data gathered in mouse model² and in this study suggest that the heterogeneity in breast milk composition, and more specifically its allergen content, contributes to the lack of reproducible evidence for the prevention of allergy by breastfeeding. In this regard, it is noteworthy that maternal egg consumption is only partially predictive of OVA concentration in breast milk.^{7–9} A RCT showed that, in some mothers, a dose-response relationship exists between the amount of egg ingested and OVA concentration in breast milk.⁷ Interestingly, however, this trial also showed that two thirds of lactating mothers allocated to an egg-free diet intervention group have detectable OVA in their milk, which may have resulted from accidental exposure or from environmental exposure due to household egg intake, and that one quarter of mothers allocated to a high-egg diet intervention group (>4 eggs a week) do not have detectable OVA in their milk.⁷ This suggests that maternal dietary egg ingestion is an unreliable predictor of OVA levels in breast milk and strengthens the need to measure egg allergen content in breast milk itself to draw conclusions on possible oral tolerance induction through breast milk allergen exposure. Therefore, a continuous monitoring of OVA levels in breast milk would be ideal but hardly achievable. In this

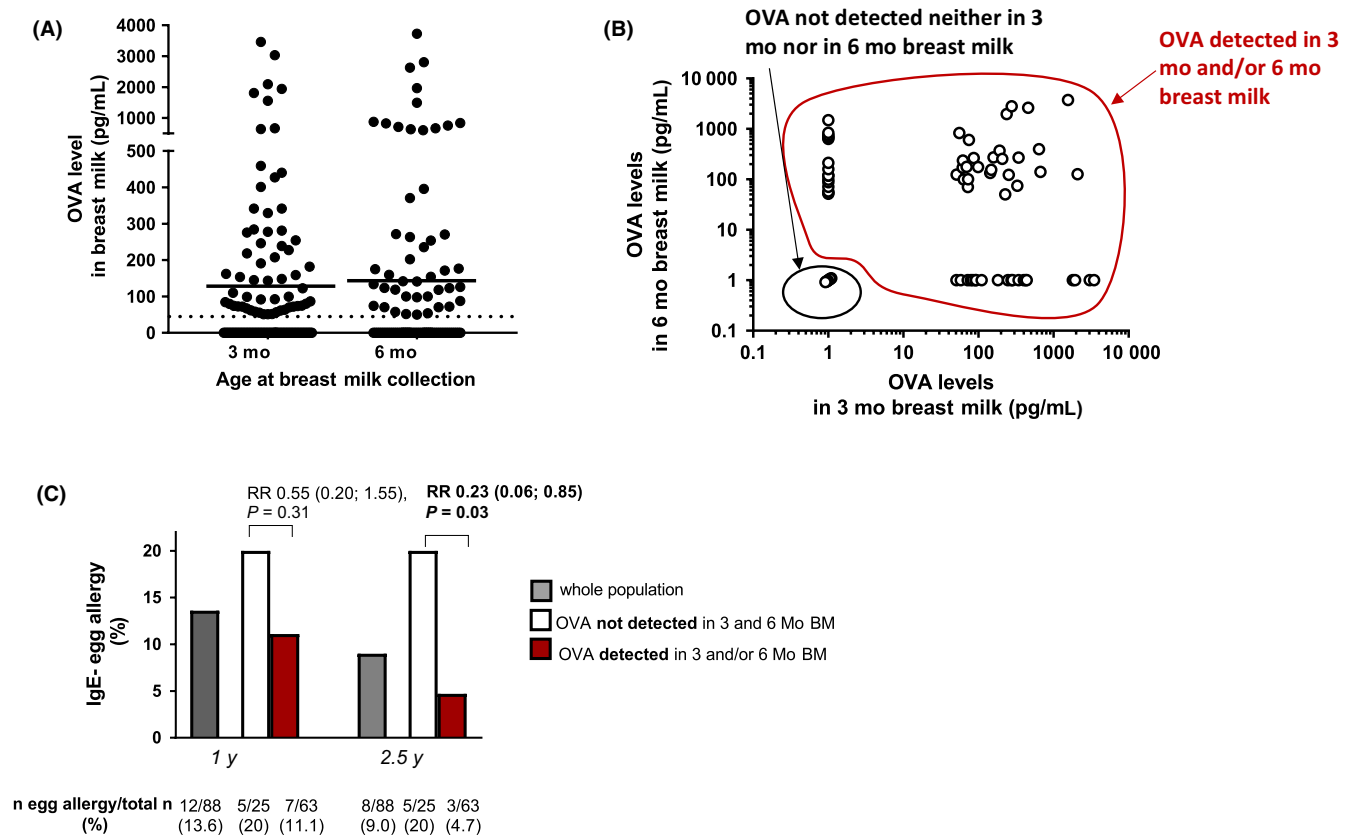


FIGURE 1 OVA levels in breast milk and prevalence of IgE-mediated egg allergy at 1 and 2.5 y according to the presence of OVA in breast milk. A, OVA levels in breast milk samples collected at 3 mo ($n = 122$) and 6 mo of lactation ($n = 102$). Dotted line represents the detection level (50 pg/mL) and horizontal lines median values. B, Correlation of OVA levels found in 3 and 6 mo breast milk samples pairs ($n = 98$, Spearman $r = .2$, $P = .04$). For logarithmic representation of OVA levels, an arbitrary value of 1 was given to samples with undetectable OVA. C, Prevalence of IgE-mediated egg allergy at 1 and 2.5 y according to the presence of OVA in breast milk as defined by not detected in both 3- and 6- mo breast milk (white column) or detected in 3- and/or 6-mo breast milk samples (red column). Analysis is shown for a population of 88 children at high risk of allergy and for whom egg allergy outcomes and breast milk samples were available

	Whole pop (n = 88)	No egg allergy at 1 y (n = 76)	Egg allergy at 1 y (n = 12)	P
Maternal age at delivery (years)	34.7 (32.2; 36.7)	34.8 (32.5; 36.9)	34.0 (28.5; 36.4)	.20
Gestational age at birth (weeks)	39.7 (38.6; 40.3)	39.7 (38.4; 40.3)	39.9 (39.0; 40.0)	.76
Infant birthweight (g)	3598 (3223; 3775)	3594 (3223; 3763)	3630 (3335; 3872)	.45
Caesarean section	21 (24%)	18 (24%)	3 (25%)	.92
Duration of breast-feeding (months)	11.9 (7.5; 12.0)	11.5 (7.3; 12.0)	12.0 (10.5; 12.0)	.26
Age at first egg introduction (months)	11.0 (9.0;12.0)	11.0 (9.0;12.0)	10.0 (9.0;11.5)	.26

TABLE 1 Population characteristics

Note: Characteristics of the study population from the IFOS cohort, which is a cohort where all mothers are allergic. Data show median with 25 and 75 percentile. P values were calculated comparing the last 2 columns using Mann-Whitney U test for continuous and chi-square test for dichotomous variables. For exact age at first egg introduction, data were available for 76, 67 and 9 infants of the whole population, with no egg allergy and with egg allergy at 1 y, respectively.

proof of concept study, we measured levels of OVA in breast milk at 3 and 6 months and showed for the first time that the presence of an egg-derived allergen in breast milk is associated with a reduced risk of egg allergy in children at 2.5 years of age as compared to breast milk with undetectable OVA. A limitation for the interpretation of the data of this study is its small sample size as well as the possibility of reverse causality. Because the majority of mothers avoiding egg consumption still have OVA in breast milk⁷ and because data gathered in a mouse preclinical model showed a causal relationship between OVA in breast milk and tolerance induction in offspring,² we are proposing that reverse causality, although possible, is not the main explanation of the findings we are presenting here. However, to fully confirm that OVA in breast milk is responsible for a decreased egg allergy risk in children, further randomised controlled trials will need to be conducted. The next steps will be to identify maternal interventions leading to consistent presence of OVA in breast milk, in order to move towards a successful prevention of egg allergy by breastfeeding.

KEYWORDS

breastfeeding, egg allergy, Ovalbumin, primary prevention

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

VV proposed the hypothesis, designed the study analysis, performed the statistical analysis with JG, interpreted the data and wrote the manuscript. JM extracted data from the database and contributed to data analysis and their interpretation. AR and MT performed the ELISA and contributed to data analysis. DP contributed to data interpretation. SP set up the IFOS cohort and contributed to data interpretation. All authors contributed to manuscript writing and approved the final version of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Supplementary Table 1. Association between egg allergy risk and OVA detection in breastmilk with or without adjustment for age at egg introduction

Model	Egg Allergy	N	Odds Ratio	Exact 95% CI, two-sided
only OVA	1 yr	88	0.50	(0.12; 2.26)
only OVA, restricted to those with data on introduction and introduction <=12 months	1 yr	68	0.40	(0.07; 2.28)
OVA adjusted for introduction	1 yr	68	0.42	(0.08; 2.39)
only OVA	2.5 yrs	88	0.20	(0.03; 1.16)
Only OVA, restricted to those with data on introduction and introduction <=30 months	2.5 yrs	76	0.22	(0.02; 2.04)
OVA adjusted for introduction	2.5 yrs	76	0.24	(0.02; 2.15)

Data show Odd Ratio for egg allergy at 1 and 2.5 years in children exposed to breastmilk containing OVA at 3 and/or 6 Months versus with no detectable OVA neither at 3 nor at 6 Months. Exact logistic regression analysis was further performed taking into account age at egg introduction in the diet.