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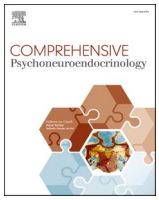

Breast milk oxytocin and s-IgA modulate infant biomarkers and social engagement; The role of maternal anxiety

Orna Zagoory-Sharon [1], Karen Yirmiya [1], Itai Peleg, Ortal Shimon-Raz, Rachel Sanderlin, Ruth Feldman [*]

Center for Developmental Social Neuroscience, Reichman University, Israel

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ABSTRACT

Breastfeeding has long been known to improve infants' health and mental development and to enhance the mother-infant bond, but much less research focused on the biological composition of breast milk and its associations with the infant's biomarkers and social development. In this exploratory study, we measured oxytocin (OT) and secretory immunoglobulin-A (s-IgA), the most abundant antibody in breast milk, and evaluated their associations with the same biomarkers in infant saliva and, consequently, with infant social engagement behavior. Fifty-five mother-infant dyads were home-visit and OT and s-IgA were assessed from breast milk and from infant saliva before and after a free-play interaction. Infant social behavior was coded offline using the Coding Interactive Behavior (CIB) and maternal anxiety self-reported. A path model revealed that mother's breast milk s-IgA impacted child social engagement via its links with child OT. In parallel, maternal breast milk OT was linked with infant social behavior through its association with the infant's immunity. This path was moderated by maternal anxiety; only in cases of high anxiety breast milk OT was positively connected to infant s-IgA. Our study, the first to measure OT and s-IgA in both breast milk and infant saliva in relation to observed social behavior, underscores the need for much further research on the dynamic interplay between breast milk composition, infant biomarkers, maternal mental health, and infant social outcomes. Results may suggest that biological systems in breast milk integrate to prepare infants to function in their social ecology through bio-behavioral feedback loops that signal the degree of stress in the environment.

1. Introduction

The benefits of breast milk nutrition to infants' physical health and psychological well-being have long been documented [1,2]. Studies have shown that breastfed infants are calmer [3], exhibit a more advanced neurobehavioral profile [4,5], and are less prone to illness in the first months of life [6]. The benefits of lactation are not limited to the infant; mothers who breastfeed report lower depression and anxiety, express more maternal behavior, and form a more sensitive relationship with their infant [7,8]. Longitudinal studies spanning infancy to adulthood report better cognitive development and less chronic diseases, such as diabetes, obesity, hypertension, cardiovascular disease, hyperlipidemia, and some types of cancer in adults who were breastfed for at least six months [9], albeit critics argue that breastfeeding was not teased apart from the effects of social class and maternal investment [10]. Such evidence convinced the World Health Organization (WHO) to recommend exclusive breastfeeding for the first 6 months of life [11]. Less research, however, has focused on the biological composition of breast milk and its contribution to infants' physiological and social growth. In the current study, we examined the associations between oxytocin (OT) and immune biomarker in maternal breast milk with the same biomarkers in the child and their potential links with infant social development.

Human breast milk functions as a dynamic feedback loop that adapts to the child's needs at different stages and conditions and its role is to best prepare the child to function in the physical and social ecology [12]. Being a complex substance, breast milk contains the necessary nutrients for the prevention of infections, adequate growth and development, colonization by intestinal microbiota, and proper maturation of the immune system. Understanding the composition of human breast milk and its potential impact on development is important given its connection with numerous developmental processes in newborns and young infants [13]. With this in mind, our study – the first to examine OT and

* Corresponding author.
E-mail address: feldman.ruth@gmail.com (R. Feldman).
[1] These authors share the first authorship of the paper.

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Abbreviations

OT	oxytocin
s-IgA	secretory immunoglobulin-A
CIB	Coding Interactive Behavior

immunity in human breast milk in relation to infant social development – sought to test the dynamic relationships between breast milk OT and immunity, infant biomarkers and social engagement behavior. In addition, we explored how maternal anxiety may moderate the associations between breast milk composition, child biomarkers, and child social behavior.

The postnatal period marks a critical time window for maturation of the infant OT system [14,15]. One source for the development of the infant's OT is maternal breast milk. In rats, neonates whose mothers were administered OT showed stable levels of OT in their gastric contents, suggesting that OT was transferred via milk [16]. In mice, OT detected in neonates' saliva was thought to originate from remnants of maternal milk OT or absorption of OT from mother's milk into the blood through intestinal permeability [17]. OT exerts local effects on the neonatal stomach and intestine [18] and infant OT can influence behavior, either through peripheral mechanisms such as the microbiome [19] or by reaching the brain [20]. We thus speculated that maternal breast milk OT may be dynamically associated with infant OT and social functioning.

Human studies have shown that infant salivary OT contributes to optimal development and links with more synchronous and engaged social behavior [21–23]. Infant social engagement in the first months of life, during the period when children begin to take an active part in social interactions and initiate social bids, serves as a robust index of optimal development. Infant social engagement is individually stable from infancy to adolescence [24] and young adulthood [25] and predicts better cognitive development [26], less externalizing and internalizing symptoms in adolescence [24], and better functionality of the social brain in adulthood [25].

Apart from OT, the rearing environment also impacts the development of the immune system and less favorable rearing conditions increase susceptibility to disease [27,28]. Here, we focused on secretory immunoglobulin-A (s-IgA), the most abundant antibody in mucosal secretions that serves as a first-line mucosal barrier from pathogen penetration [29]. Human breast milk contains large quantities of s-IgA, which constitutes about 80–90% of the total immunoglobulins. These antibody, formed as a consequence of the mother's infectious history, can bind to potential pathogens and prevent their attachment to the infant's cells [30–32]. s-IgA is relatively resistant to proteolysis and can therefore provide protection against pathogens in the gastrointestinal tract [33]. When measured in saliva, elevated levels of s-IgA serve as a biomarker of stress in both adults [34] and children [35,36]. While the beneficial effects of s-IgA concentrations in breast milk on infant health are known, no data, to our knowledge, are available on the relations between early life stress and infants' s-IgA. Similarly, no studies tested the associations between OT and s-IgA in infancy despite the well-known role of OT in modulating inflammation, cytokine production, and wound healing [37].

In light of the above, the goal of the current exploratory study was to examine the relationships between OT and s-IgA in maternal breast milk with OT and s-IgA in infant saliva and whether and how biomarkers in maternal milk and child saliva link with the infant social engagement behavior during the sensitive period for maturation of the immune system. Our secondary goal was to assess whether stress in the rearing environment, as indexed by high maternal anxiety, may alter the associations between the OT and s-IgA systems. It has been shown that while OT links with greater bonding-related behavior and social engagement, in contexts of high stress OT functions in an opposite ways and correlates with lower bonding and sociality [38,39], and numerous explanations have been offered for OT's reversed role in contexts of early life stress [40]. We thus examined how the OT and s-IgA systems differentially tune infants toward their environment, versus inward and toward the preservation of resources in contexts of higher stress.

Guided by the *biobehavioral synchrony* model [41], we examined associations between mother and child's biomarkers and social behavior. As this is the first study on oxytocin in breast milk using enzyme immuno-assay (EIA), our first goal was to provide initial data on oxytocin levels in breast milk. In addition, being that this is the first study to include measures of oxytocin and s-IgA in breast milk, their corresponding biomarkers in infant saliva, and an observed index of infant social behavior, our second goal was to examine the dynamic inter-relationships between these factors. Using path analysis, we examined how variations in oxytocin and s-IgA in breast milk link with these components in infant saliva and chart pathways to infant social behavior both directly and indirectly. Lastly,our final goal was to explore how maternal anxiety may moderate the link between oxytocin levels in breast milk and the immune system of the infant.

2. Method

2.1. Participants

Participants were 55 Israeli breastfeeding mothers and their 3–7 months old infants (M = 4.54 months, SD = 1.16). Mothers were of a middle-class, low-risk background; all above 20 years old, with an average age of 30.19 years (SD = 4.31), and 16.58 (SD = 1.95) years of education (Table 1). Of these, 94.3 % were breastfeeding fully and the rest combined breastfeeding with baby milk formula. All mothers reported breastfeeding 75–100 % of the time, and at least 90 % of the infant's nutrition was based on breast milk. While a small number of infants were introduced to some formula and/or solid foods, none replaced breastfeeding with solid meals. The research was approved by the Institutional Review Board and was conducted according to ethical standards and all participating mothers signed an informed consent.

2.2. Procedure

Mothers were recruited via social media, where they responded to advertisements (a passive recruitment method) by providing their contact details. A research assistant then contacted them to share comprehensive study information, obtain their consent, and schedule a home visit. Mothers and their infants were visited at home in the morning hours (between 8am and 2pm) for approximately 1 h. Prior to the visit, mothers were asked to refrain from eating and not feed their infants for 30 min. Upon arrival, mothers provided informed consent and were given a 10-min rest period. The first saliva sample was then collected from the infants (S1), followed by a 7-min play session with the mother. The experimenter left the room during this interaction in order to not affect the 'free play'. Afterward, a second saliva sample was collected from the infants (S2). Next, right before breastfeeding, mothers provided

Table 1
Sociodemographic characteristic of the participants.

Sample characteristics				
	M	***SD***	***n***	***%***
Infant age (months)	4.54	1.16		
Gender (Female)			31	56.4
Firstborn			20	37.7
Infant weight	6.54	0.99		
Infant height	62.16	6.17		
Mother education (years)	16.58	1.95		
Mother age	33.4	4.31		
No. of feedings per day	7.07	1.71		

an initial breast milk sample (BM1) from the breast they did not plan on feeding their infant. The collection was carried out from the breast that was not intended for breastfeeding, so as not to harm the feeding of the baby. Following the breastfeeding session, a second milk sample was taken (BM2) from the breast they fed their infant. This collection aimed to reflect the actual nutrition the child consumed, to verify that the milk composition is not breast dependent, and, moreover, to verify that the OT concentration in the milk is not influenced by sucking. Mothers were also asked to complete electronic self-reports assessing anxiety and a range of demographic and health variables at the same day of the visit, including weight, height, smoking status, menstrual cycle, and feeding patterns. Mothers received a voucher for 250 NIS (equivalent to 80$) in return for their participation.

2.3. Measures

2.3.1. Child social behavior

Children's social behavior was coded from the mother-infant interaction. Mothers were asked to play with their children as they normally do, therefore no specific position or toys were required. Interactions were coded using the well-validated Coding Interactive Behavior Manual (CIB) [42]. The CIB is a global rating system including multiple scales ranging from 1 to 5 that are integrated into theoretically meaningful constructs. The CIB has been validated in a large number of studies across cultures, ages, and psychopathologies with good psychometric properties (for review; Feldman [43]). The child's social behavior was measured using an engagement score, which included a mean of the following scales: infant's gaze, positive affect, alertness, vocalization, and initiation. Two trained coders, blind to other information, coded the interactions and reliability on 20 % of the interactions exceeded 85 % on all codes. Cronbach's alpha coefficient was computed to evaluate the reliability of the overall measure, yielding a value of 0.88 (95 % 0.82, 0.92), indicating high internal consistency.

2.3.2. Maternal anxiety

The Trait Anxiety Inventory (TAI) is a subscale of the Spielberger State-Trait Anxiety Inventory (STAI), a widely used self-report tool to assess general anxiety [44,45]. The STAI-T comprises 20 descriptive items ranging from 1 (almost never) to 4 (almost always), and the final score is the aggregated sum. The Cronbach's α coefficient reported in the current study was 0.869.

2.3.3. Samples collection and handling

The infant saliva samples were collected using Saliva-Bio (SIS) (Salimetrics USA), which later on placed in sterile spin-down containers for further extraction of the saliva. Breast milk collected via hand expression, or pump into sterile 50 mL cups and then transferred into 15 mL sterile tubes for storage and analysis, at least 5 ml of breast milk were collected. All samples were stored at −20C until underwent vigor centrifugation and the clear supernatant of saliva or milk whey fraction were transferred into a clean tube for further storage. Samples with sufficient volume were further analyzed, for s-IgA and OT assays, at least 50 or 400 micro-liters respectively. Accordingly, of all the 55 dyads' double samples, the following were excluded: infant OT 4 at S1 and 6 at S2; milk OT one at BM1 and 4 at BM2; for infant s-IgA one at S1 and 3 at S2; milk s-IgA one at BM1 and one at BM2.

2.3.4. Oxytocin and S-IgA analysis

Determination of OT concentration was performed by using the OT Enzyme-Linked Immunosorbent Assay (ELISA) kit, by ENZO (USA). On the assay day, all samples were thawed completely, and measured according to the instructions of a commercial kit. The inter-assay coefficients of the controls and samples were less than 10.6 % and 16.8 %, respectively. We verify that the supernatant of milk whey samples are suitable for direct measurement with the OT kit, by comparing the values with extracted samples. Twenty milk samples underwent the following extraction protocol. Two hundreds microliter of milk samples were acidified with TFA 0.1 % centrifuged, and the supernatant was loaded on HBL columns (60 mg, Waters USA), followed by extraction with 3 ml of 80 % acetonitrile, aliquoted into two tubes and dried into powder, and kept in −20C until assayed. On the assay day these samples were reconstituted with assay buffer and measured as saliva. The OT concentration of extracted sample was as received by directed measurement, with CV% less than 10 %.

Determination of s-IgA was performed, using a commercial s-IgA ELISA kit (EUROIMMUN AG; Luebeck, Germany). The kit provides a quantitative in vitro assay for s-IgA in human saliva. On the assay day, all samples were thawed completely, and s-IgA levels were measured according to the kit's instructions. Samples preparation was performed by Freedom-Evo an automatic liquid handler (Tecan Switzerland), and the readings and calculations were conducted by Magellan V.7 software (Tecan Switzerland). The intra-assay coefficient of samples and controls was 2.35 %, and inter-assay coefficients for samples and controls were less than 8.74 %

2.4. Statistical analysis

All statistical analyses were conducted using R 3.3.256 (R Team, 2019). We used the Multiple Imputation by Chained Equations (MICE) technique with predictive mean matching (PMM) to impute the missing data [46]. PMM is an effective method for handling missing data when it is missing at random [47], which is the case in our study. Consistent with prior research, OT and s-IgA in saliva and breast milk were log-transformed to ensure normal distribution [36,48]. Descriptive parameters of study variables were calculated, and paired t-tests were used to examine differences in the two main biomarkers from T1 to T2 for infants' saliva levels and between the two breast sides for breast milk measures. Differences in the main dichotomous sociodemographic variables were examined using $\chi 2$ tests. Next, Pearson correlations examined associations between all study variables and sociodemographic variables, such as age, maternal education, mean breastfeeding times a day. To examine direct and indirect associations between maternal OT and s-IgA and child social behavior as mediated by the child's levels of OT and s-IgA we conducted a structural equation model, with two alternative models to validate the original model. Structural equation modeling (SEM) was performed using the lavaan package for R [49] with child age and gender as covariates, salivary biomarkers' second measurement controlled for the first assessment, and p-values of <0.05 were considered significant. To assess model fit, the following indices were used: χ^2, comparative fit index (CFI), Tucker-Lewis Index (TLI), and the root mean square error of approximation (RMSEA). CFI and TLI ≥ 0.90 and RMSEA ≤ 0.08 values were considered to indicate a good fit [50]. Ideally, the χ^2 statistic is expected to be non-significant in the case of adequate fit, however, this index is no longer used to evaluate fit because of its hypersensitivity to sample size [50]. We performed a Monte Carlo simulation to estimate the indirect effects using the bootstrap method. For each indirect effect, we generated 5000 bootstrap samples. The confidence level was set to 95 % using the MASS package in R [51]. Following SEM, we estimated the conditional effect of OT in breast milk (X axis) on s-IgA in child's saliva (Y axis), with maternal anxiety levels as a moderator (M) using the Emmeans package for R [52]. OT in breast milk and maternal anxiety were mean centered to facilitate the interpretation of the simple and interaction effects [53].

3. Results

This is the first study to measure OT in human breast milk by enzyme immuno-assay (EIA) methodology. We therefore start by providing baseline data on OT in both breast milk samples before and after breastfeeding in addition to s-IgA levels in breastmilk and levels of oxytocin and s-IgA in infant saliva. We then explore differences in these biomarkers related to infant gender and birth order. Following, we

explored correlations between these biomarkers with maternal anxiety and infant behavior.

Oxytocin (OT) concentration in the breast milk samples, in the first sample (BM1), revealed a mean value of MBM1 = 204.17, SEBM1 = 8.6. For the second sample (BM2), the mean value was MBM2 = 199.53 with SEBM2 = 8.677 (Fig. 1). The values for breast milk s-IgA at the two time-points were MBM1 = 148346.5 SEBM1 = 6549.0 and MBM2 = 148889.8 SEBM2 = 6382.3. Infant salivary OT values were MS1 = 134.90 SES1 = 16.41 for the pre free play interaction S1 saliva sample (S1) and MS2 = 141.25 SES2 = 17.58 the post interaction S2 saliva sample. Infant s-IgA values were MS1 = 10471.3 SES1 = 23.2 for S1and MS2 = 1964.8 SES2 = 18.0 for S2.

To test differences in OT concentrations between the two fractions in the two assessment points, a 2 × 2 (Saliva/Milk × Time) repeated measures ANOVA was performed. The analysis revealed a significant Saliva/Milk main effect; $F(1,53) = 16.606$, $p < 0.001$, indicating that OT concentrations in breast milk were significantly higher as compared to their concentrations in saliva samples. There was no significant main effect of Time, $F(1,53) = 0.016$, $p = 0.901$, suggesting no differences between concentrations in the two assessment point in either breast milk or saliva, and no Saliva/Milk × Time interaction was found; $F(1,23) = 0.009$, $p = 0.926$. Repeated measures ANOVA (Saliva/Milk × Time) performed on s-IgA levels, yielded similar results. Breast milk s-IgA concentrations were significantly higher than infant salivary samples; $F(1,54) = 403.561$, $p < 0.001$, no main effect emerged for time; $F(1,54) = 0.018$, $p = 0.894$; and no interaction effect was found; $F(1,54) = 0.065$, $p = 0.8$] (Fig. 1).

Next, we examined associations with demographic factors. No differences in mothers' breast milk and infants' OT and s-IgA levels were found between boys and girls or between firstborns and non-firstborn children (Table 2). Additionally, OT and s-IgA levels in breast milk and infant saliva did not correlate with child height, weight, age, and other lactation-related confounders (all $P_{Pearson} > .05$) (Table 1).

Pearson correlations showed that infant OT and s-IgA were related to biomarkers in mothers' breast milk. Maternal anxiety was negatively correlated with infant OT levels and infant social behavior was negatively related to infant salivary s-IgA (Table 3).

We used structural equation modeling (SEM) to test the mediating role of children's biomarkers on the links between breast milk OT and s-IgA and infant social behavior. Similar considerations were taken by our group previously (Halevi et al., 2017). As breast milk OT and s-IgA levels did not differ between the two time-points (OT: $t(54) = 0.685$, $p > 0.05$; s-IgA: $t(54) = 0.908$, $p > 0.05$), an average was used in these analyses.

The overall model provided a good fit to the data ($\chi^2_{(6)} = 6.99$, $p = 0.332$, CFI = 0.974, TLI = 1.34, RMSEA = 0.055, with lower 90 % CI = 0.000 and higher 90 % CI = 0.19 PCLOSE = .469), and the final path model is presented in Fig. 2. The effect of maternal OT in breast milk on infant social behavior was mediated by the infant 's s-IgA levels. Test of mediation showed that this indirect path was significant (95 % CI = −0.25, −0.022). The effect of maternal s-IgA in breast milk on infant behavior was also mediated by the child's s-IgA levels, which in turn were negatively associated with infant social behavior (95 % CI = −0.24, −0.01). The mediating pathway from s-IgA in breast milk to child social behavior via child OT was not significant (95 % CI = −0.0007, 0.21).

To validate the presented path model, we compared it with two alternative models and these are presented in supplementary Figure 1 and 2. In the first alternative model, infant's behavior mediated the link between breast milk biomarkers and infant's OT and s-IgA levels. In this model, the only variable that charted a significant path was infant salivary s-IgA as predicted from infant social engagement and maternal breast milk s-IgA. In the second alternative model, breast milk biomarkers mediated the association between infant OT and s-IgA levels and infant social behavior. Similar to the original model, maternal s-IgA in breast milk was associated with the infant's s-IgA and OT levels and breast milk OT was linked only to infant s-IgA levels. However, maternal biomarkers did not correlate with the infant's social behavior. Both models did not provide an adequate fit to the data, lending support to the current model.

To further understand the association between OT in breast milk and infant s-IgA we examined the potential moderating role of maternal anxiety on this link using a moderation model. Results indicate that the overall moderation model was significant ($F_{(3, 51)} = 3.494$, $p = 0.022$) and explained 17.05 % of the variance in infant s-IgA. The coefficients of the independent variables showed that maternal anxiety ($\beta = -0.35$, $p = 0.033$) and the interaction between maternal breast milk OT and maternal anxiety ($\beta = 0.149$, $p = 0.035$) were both significant predictors of child s-IgA levels. Further analysis of simple slopes showed that the relationship between breast milk OT and infant s-IgA varied depending on the level of maternal anxiety. The positive association between breast milk OT and infant s-IgA was significant only in cases where mothers reported high anxiety (95 % CI = 0.982, 4.20) but not when mothers reported lower anxiety (95 % CI = -1.200, 2.02; see Fig. 3). We computed a similar moderation model on the association between breast milk s-IgA and infant OT but the model was not significant ($p > 0.05$).

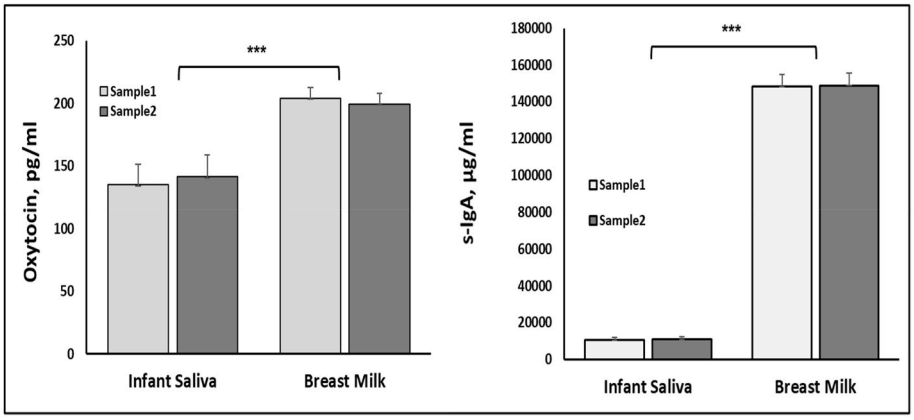

Fig. 1. OT and s-IgA in infant saliva and mother's breast milk
Note: Infants' sliva sample 1and 2 refer to S1 and S1, breast milk sample 1 and 2 refer to BM1and BM2.
2x2 Repeated measures two ways ANOVAs (Saliva/Milk × Time) were conducted separately on OT and s-IgA concentations. Significant main effects for Salive/Breast milk were found in both analyses suggesting that OT and s-IgA concentrations in Breast milk are higher compred to Infant saliva.
Error bars represent standard error of the mean; ***; $P < 0.001$.

Table 2
OT and s-IgA differences between A boys and girls infants B firstborns with non-firstborns.

A	*Group*	*M*	*SE*	*T-test*	*Cohen's d*	*[95 % CI]*
OT S1	Boys	2.045	0.083	t(49) = −1.520, p = 0.134	−0.43	[-0.98, 0.13]
	Girls	2.204	0.065			
OT S2	Boys	2.171	0.084	t(47) = 0.37, p = 0.714	0.11	[-0.46, 0.67]
	Girls	2.128	0.081			
s-IgA S1	Boys	3.941	0.102	t(53) = −0.92, p = 0.361	−0.25	[-0.78, 0.29]
	Girls	4.072	0.097			
s-IgA S2	Boys	3.968	0.092	t(50) = −1.13, p = 0.265	−0.32	[-0.86, 0.24]
	Girls	4.096	0.069			
OT BM1	Boys	2.317	0.021	t(52) = 0.57, p = 0.574	0.15	[-0.38, 0.69]
	Girls	2.303	0.014			
OT BM2	Boys	2.302	0.023	t(49) = −0.08, p = 0.941	−0.02	[-0.57, 0.53]
	Girls	2.304	0.014			
s-IgA BM1	Boys	5.131	0.036	t(52) = −0.4, p = 0.694	−0.11	[-0.64, 0.43]
	Girls	5.15	0.032			
s-IgA BM2	Boys	5.104	0.041	t(51) = −1.57, p = 0.124	−0.43	[-0.98, 0.12]
	Girls	5.177	0.025			
B						
OT S1	Non-firstborn	2.097	0.076	t(47) = −1.00, p = 0.322	−0.29	[-0.87, 0.29]
	Firstborn	2.208	0.070			
OT S2	Non-firstborn	2.188	0.078	t(45) = 0.54, p = 0.593	0.16	[-0.42, 0.74]
	Firstborn	2.123	0.091			
s-IgA S1	Non-firstborn	4.058	0.101	t(51) = 0. 79, p = 0.433	0.22	[-0.33, 0.78]
	Firstborn	3.939	0.100			
s-IgA S2	Non-firstborn	4.064	0.075	t(48) = 0.15, p = 0.878	0.04	[-0.53, 0.62]
	Firstborn	4.046	0.085			
OT BM1	Non-firstborn	2.324	0.014	t(50) = 0. 87, p = 0.388	0.25	[-0.31, 0.81]
	Firstborn	2.303	0.021			
OT BM2	Non-firstborn	2.322	0.018	t(47) = 1.23, p = 0.226	0.36	[-0.22, 0.93]
	Firstborn	2.290	0.017			
s-IgA BM1	Non-firstborn	5.186	0.025	t(50) = 0. 11, p = 0.039	0.6	[0.03, 1.17]
	Firstborn	5.082	0.047			
s-IgA BM2	Non-firstborn	5.190	0.021	**t(49) = 2.20, p = 0.033**	**0.63**	**[0.05, 1.20]**
	Firstborn	5.087	0.049			

Note: OT and s-IgA are log-transformed. Breast milk BM1 and BM2 refer to the first and second milk samples, S1 and S2 refer to infant saliva samples before and after free play interaction.

Table 3
Pearson's correlations between the study's variables.

Variable	*M*	*SEM*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
1. Infant social behavior	3.42	1.02									
2. Maternal anxiety	32.82	7.30	−0.10								
3. OT S1	2.13	0.37	0.01	−.32*							
4. OT BM1	2.31	0.09	0.05	0.18	−0.03						
5. s-IgA S1	4.02	0.52	−0.10	−0.07	.44**	−0.05					
6. s-IgA BM1	5.14	0.18	0.08	0.01	−0.13	0.10	−0.02				
7. OT S2	2.15	0.40	0.16	0.18	0.23	0.01	0.15	0.10			
8. OT BM2	2.30	0.09	−0.07	0.16	−0.09	.66**	−0.07	0.09	0.05		
9. s-IgA S2	4.04	0.41	−.36**	−0.01	0.17	0.20	.44**	0.11	0.26	.36*	
10. s-IgA BM2	5.14	0.17	0.11	−0.08	−0.01	0.20	0.10	.79**	.30*	.35*	.36*

Note. M and *SE* are used to represent mean and standard error, respectively. OT and s-IgA are log-transformed. Breast milk BM1 and BM2 refer to the first and second milk samples. S1 and S2 refer to before and after the free play social interaction. * Indicates $p < 0.05$. ** indicates $p < 0.01$.

4. Discussion

The current study is the first, to our knowledge, to measure OT in human breast milk by EIA and to test the relationships between OT and s-IgA in breast milk with OT and s-IgA in infant saliva and their subsequent connections with the infant's social engagement behavior. Using path model, we identified pathways linking maternal breast milk OT and s-IgA and child social engagement behavior as mediated by the infant's biomarkers. We further explored system-specific mechanisms by which maternal anxiety may modulate the infant's emerging immune reactivity to describe potential early pathways of risk and resilience.

To our knowledge, only one study presents data on OT in human breast milk and showed that human milk OT increases in response to suckling [16]. Since this study used radioimmunoassay, results are not comparable. In contrast, our results showed no significant increase in response to breast feeding. Moreover, the concentration of OT in human breast milk was in the same range as those detected in blood in a large sample of women [54]. It should be noted that our measurement was conducted on the whey fraction of the milk without extraction as this was found to be an unnecessary step, similar to sweat samples [55]. The levels of OT in infant saliva in the present cohort were in the range of infants up to 18 months of age we reported in a previous study [56], and higher than those found in school-aged children aged 6 and 10 years [57], suggesting that salivary OT levels in infancy are higher as compared to lager childhood.

Our descriptive findings revealed that infants' salivary OT and s-IgA levels did not change significantly between the two measurements conducted before and after interactions with their mothers, indicating that, at least in this cohort, these biomarkers were robust and did not show a marked change following a free-play session in the familiar home environment. While other studies found changes in infants' salivary levels from baseline to after the interaction [22,58], these changes

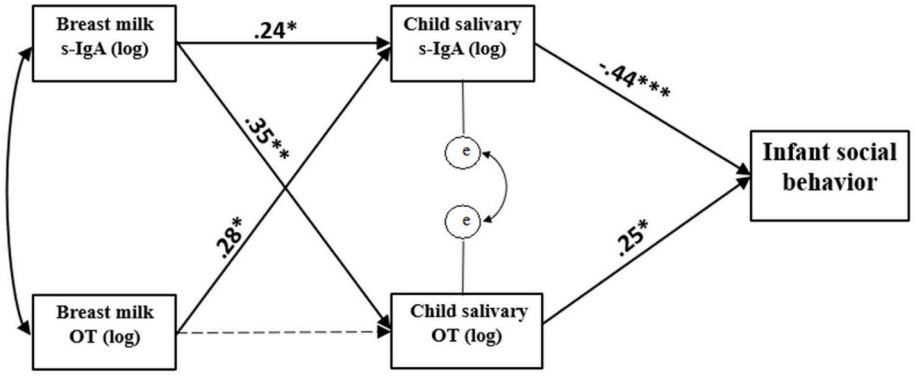

Fig. 2. Path-analysis linking s-IgA and OT in breast milk with child social behavior, with infants' biomarkers as mediators
Note: Path model leading from maternal breast milk to infant social behavior via infant saliva biomarkers. Coefficients represent standardized regression weights. ǂ Controlling for child age and gender. *p < 0.05, **p < 0.01, ***p < 0.001.

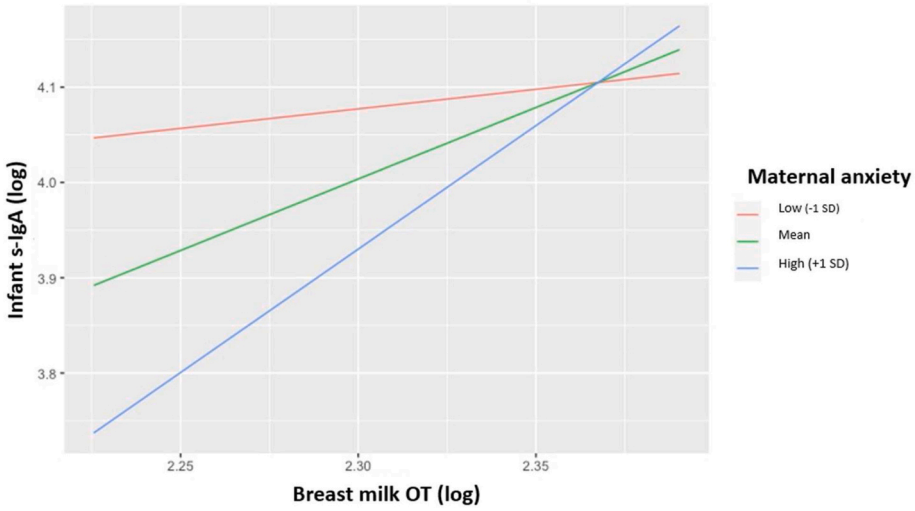

Fig. 3. Simple slopes of the association between maternal OT in breast milk and child s-IgA in saliva as moderated by maternal anxiety
Note: Interaction effects of breast milk OT and maternal Anxiety on infant's s-IgA levels. OT and s-IgA are log-transformed. Simple slope analysis revealed a significant positive correlation between maternal OT and child's s-IgA only in cases of high maternal anxiety.

emerged in a lab setting after interactions that involved specific behaviors or in which parents received specific instructions (for instance, increase their touch) [21], and not in a free naturalistic interaction at the familiar home environment. This may suggest that daily and familiar interactions at home may not be sufficient to induce measurable OT changes.

Results indicate inter-relationships between OT and s-IgA levels in both breast milk and infant saliva and these findings are the first to show such correlations in breast milk and in infant saliva at this age. Previous studies established s-IgA and OT as biomarkers of physical and mental resilience in infants [59–61] and the current study is the first to investigate both biomarkers in the same cohort of breastfed infants. The literature on the nature of these two biomarkers suggests that OT may have a role in the body's adaptive response to immune challenges, since OT stimulates the contraction of mammary gland cells that may lead to increase in the release of IgA into breast milk [37]. In addition, OT has immunomodulatory effects, which may enhance s-IgA production. However, the exact mechanisms that underpin the associations between OT and IgA in breast milk require much further research.

The assessment of OT and s-IgA levels in breast milk in relation to various biological and behavioral factors in the child received very little research. Several studies tested OT from various sources, including saliva, blood, urine [38,62,63], and recently also from sweat [55]. This growing body of research points to the increased attention to the specific roles, distinct correlates, and potential convergence of the various OT measurements. Within this context, the current study presents a unique model that examines the potential transfer of OT and s-IgA from mother to the child via breast milk. This model further enriches our *biobehavioral synchrony* frame [41,64,65] that underscores the dynamic mutual influences of maternal and child biological systems and, consequently, on social behavior. We show here that mother and child's biomarkers chart a biobehavioral feedback loop in which both mother's breast milk and child's biomarkers impact each other and sensitively adapt to environmental stressors, enhancing or attenuating the degree to which infants invest their resources in actively engaging with the environment. The mechanisms by which maternal milk composition responds to signals from the infant and cues from the environment and ultimately shape the infant's behavior mark an uncharted area that requires much further research.

It has been well-documented that mothers' breast milk composition is dynamic and finely-tuned to the infant's requirements [33]. There are daily rhythm variations in levels of proteins, fats, carbohydrates, hormones, immune factors and more [12]. For example, circadian fluctuations in some bioactive components are suggested to transfer chronobiological information from mother to child to assist the development of the biological clock [12].Leukocytes, immune cells, and

immune biomarkers are elevated in human breast milk once the child or the mother are infected. These findings suggest that mother and child interact via breast milk composition [66]. The current study adds to this line of research by pointing to the impact of breast milk composition on child's behavior as mediated by the immune system.

Interestingly, no associations were found between OT in maternal breast milk and infant saliva. Some studies identified links between mothers' and infants' OT levels or fathers' and infants' OT levels [23,38,67]. One possibility is that breastfeeding and sensitive, non-anxious maternal caregiving evoke OT secretion through the oral suckling stimulus, skin-to-skin touch, and a close affiliative relationship [68], but much further research is needed to examine the transfer of OT from mother to child through non-identical fractions, such as breast milk to saliva.

The correlation between maternal anxiety and lower infant OT is consistent with studies on the effect of maternal mental state and sensitive parenting on infant adaptive development [69,70]. Maternal anxiety in infancy links with lower child social engagement, and improvements in maternal anxiety from 3 to 9 months were found to be paralleled by increases in infant social engagement behavior [71]. Maternal anxiety correlates with lower child OT levels across infancy and childhood [35,72]. Maternal anxiety may impede the maturation of children's OT by creating early life stress that interferes with OT production and function [73]. Maternal anxiety not only exerts early life stress that alters the child's OT levels [74,75], but can also reduce maternal sensitivity and increase intrusiveness and negativity [76], further interfering with the quiescence and synchrony needed for the development of infant OT.

Maternal anxiety was not directly related to OT and s-IgA in breast milk. While some studies reported negative correlations between maternal anxiety and breast milk s-IgA [77,78], other studies reported no such correlation [61,79,80]. For instance, perceived stress at day 3 postpartum did not correlate with s-IgA levels in breast milk at 14 days postpartum [81]. One possibility for the lack of direct associations is that our sample was low-risk and anxiety in our mothers did not reach the clinical cutoff, which may have limited the range of maternal anxiety symptoms and their biological correlates.

The pathway linking breast milk OT with infant s-IgA was moderated by maternal anxiety. Under conditions of high anxiety, breast milk OT linked with higher infant s-IgA but not when maternal anxiety was low. One function of maternal OT that transmits through breast milk is to build and prepare the infant's immune system for potential stress, illness, and hazards. When the environment sends signals of stress, the immune system is activated but such activation comes at a cost, as seen by the effects of early life stress on child physical and mental health and social adaptation [82]. We found that when maternal anxiety is high, the link between breast milk OT and infant S-IgA is activated. However, as our model indicates, there is a price for the early activation of immunity. When infants must direct physiological and attentional resources toward managing environmental stress rather than toward active engagement in the social world, they reduce their social engagement as expressed by the negative associations between s-IgA and sociality.

The immune system's activation, indexed by elevated s-IgA, occurs primarily in response to perceived risks. Maternal anxiety is often accompanied by physiological markers such as elevated cortisol levels and increased sympathetic system activity [83] and these may serve to signal to the infant that the environment is unstable. This signal may trigger increase in the child's s-IgA levels but comes at the cost of reallocating resources at the expense of other developmental processes [84,85] and may have long-term consequences for physical and mental health. Specifically, in the case of unstable rearing environments, the child may exhibit decreased attentiveness and allocate fewer resources to the social world, leading to a decrease in social involvement. This effect is particularly pronounced during infancy, a critical period when the infant requires significant resources for learning and navigating the social world.

The negative correlation between infant salivary s-IgA and social engagement is of interest and, to our knowledge, marks the first report at this age in relation to observed social behavior. While high s-IgA levels are usually associated with better infant health outcomes [86], little research addressed the association between s-IgA and infants' adaptive social development. Our study is not consistent with the reported links between breast milk s-IgA and infant responsiveness to visual and auditory stimuli [61]. Still, in contexts of higher risk, s-IgA indexes lower adaptation. For instance, preschoolers with low self-regulation in the context of diminished parent-child synchrony showed higher s-IgA levels [87]. Among children exposed to chronic early trauma s-IgA liked with lower social engagement behavior at age 10 [35] and in adolescence [36]. s-IgA has been associated with greater anxiety, internalizing, and externalizing problems in healthy children [88,89]. These inconsistent findings may highlight the distinct role of s-IgA under different environmental conditions. Overall, our findings contribute to a better understanding on the role of breast milk biomarkers in child development and the interaction between maternal anxiety and infant OT.

Several study limitations should be noted. These include the lack of other immune markers beyond s-IgA, which is one component of a complex system, and future research should examine other innate and adaptive immune parameters. In addition, our sample was low-risk, which limited the range of maternal anxiety and much further research is needed to assess the associations reported in our model in contexts of higher risk. Finally, our study represents one time-point and we did not follow children longitudinally to test the long-term impact of breast milk composition on child development. Furthermore, there is a need to investigate a broader spectrum of infant feeding practices, such as fully breast-fed infants with both skin-to-skin contact, infants primarily fed by expressed milk but without skin-to-skin contact, and formula-fed infants lacking skin-to-skin contact. Such controlled studies may be able to shed further light and begin to chart causal relationships between breastfeeding and child outcomes, which the current study could not define. These additional study groups could help disentangle the various contributing factors, shedding further light on the complex interplay between breast milk composition and infant behavior. However, it is important to note that logistical and ethical considerations may pose challenges in assembling such diverse study groups, and these limitations should be acknowledged.

Despite these limitations, this is the first study that tested the associations between breast milk OT and immunity, similar biomarkers in the child, and social behavior in the context of higher and lower maternal anxiety. Our results are preliminary and call to direct much further effort to understand the biological constituents embedded in maternal breast milk and their impact on children's physical health, mental well-being, and social growth.

CRediT authorship contribution statement

Orna Zagoory-Sharon: Conceptualization, Data curation, Supervision, Writing – review & editing. **Karen Yirmiya:** Formal analysis, Writing – original draft, Writing – review & editing. **Itai Peleg:** Formal analysis. **Ortal Shimon-Raz:** Methodology, Writing – review & editing. **Rachel Sanderlin:** Data curation. **Ruth Feldman:** Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of Competing interest

no conflict.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cpnec.2023.100219.

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