

# The Impact of Preterm Infants' Continuous Exposure to Breast Milk Odor on Stress Parameters: A Pilot Study

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## Abstract

**Objective:** This pilot study aimed to assess the effect of continuous exposure to the odor of own mothers' breast milk (BM) on the stress parameters of preterm infants.

**Materials and Methods:** Fifteen healthy preterm infants were included. Mean heart rate and salivary cortisol were measured over three consecutive time periods, each lasting 2 days: (1) preintervention (odor free); (2) intervention, during which a cotton pad soaked with 1.5 mL of BM was placed near the infant's head with the aim of providing continuous exposure to its odor; (3) postintervention period (odor free).

**Results:** Saliva cortisol levels differed significantly between the three exposure periods (pre-, during, and post-BM odor exposure):  $11.38 \pm 5.03$ ,  $9.51 \pm 4.38$ , and  $4.99 \pm 3.42$  nmol/L, respectively. A repeated univariate analysis of the cortisol measure showed a significant difference ( $F=9.34$ ;  $df=2.28$ ,  $p<0.001$ ). There was no difference in mean heart rate over the three study periods.

**Conclusions:** Preterm infants exposed to BM odor from their own mothers demonstrate a persistent decrease in saliva cortisol levels, which continues after termination of the intervention. This finding may suggest that exposure to own mothers' BM odor has a soothing effect on preterm infants. Further randomized controlled studies are needed to evaluate this simple, safe, and inexpensive intervention.

**Keywords:** preterm infants, maternal odor, neonatal stress, breast milk

## Introduction

**I**N CONTRAST TO term newborn infants, preterm infants have a very different postnatal experience, which includes prolonged hospitalization, delayed and limited parental contact, and various unpleasant and painful medical procedures to treat and monitor their medical condition. This experience leads to occasional and prolonged stress that may have an impact on long-term outcomes.<sup>1-3</sup>

Several methods have been investigated to reduce stress among preterm infants, among them are the Neonatal Individualized Developmental Care and Assessment Program (NIDCAP), skin-to-skin contact, and music induction.<sup>4-7</sup>

Several parameters have been used to evaluate the effect of these various procedures on stress relief, including physiologic parameters such as respiratory and heart rates (HRs),

pain scores, and laboratory measurements of stress hormones (mostly saliva cortisol).<sup>4-9</sup>

Several studies examined the effect of olfactory stimulation on newborn infants' stress. The chemosensory system develops anatomically and functionally in the first trimester, and complete differentiation of olfactory cells was demonstrated among human embryos by the 11th week of pregnancy.<sup>10</sup> Therefore, premature infants were able to react positively to maternal odors as a soothing method during painful procedures.<sup>6,8,11,12</sup> Olfactory stimulation during unpleasant and stressful conditions favored a return to a calm state, thereby reducing the expenditure of energy.<sup>6,8</sup> It seems that newborns are particularly sensitive to maternal odors (breast milk [BM], amniotic fluid), presumably due to the link between these odors and feeding and the relationship with intrauterine maternal odor.<sup>11-14</sup>

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To date, the effect of BM odor on relieving stress among preterm infants has only been partially studied.

In the present study, we aimed to assess the effect of the odor of a mother's own BM on her preterm infant's stress parameters. In theory, exposure to mother's BM odor may mimic "continued maternal presence." If effective, this intervention may serve as an innovative, simple, and inexpensive strategy to reduce stress among preterm infants during their stay in the neonatal intensive care units (NICU).

## Materials and Methods

### Setting

The study was conducted in the NICU at the Sheba Medical Center, a large tertiary medical center in Israel, with ~10,000 births per year.

Data taken from the infants' medical files included data on maternal parity and gravida, pregnancy complications such as premature contractions and premature rupture of membranes, and prenatal steroid treatment. Infants' data included gestational age (GA), birth weight, gender, weight for GA (appropriate, small, or large for GA: AGA, SGA- $\leq$  10th percentile, LGA- $\geq$  90th percentile, respectively), Apgar score, and any postnatal complications, such as respiratory distress, need for and duration of mechanical ventilation and oxygen, patent ductus arteriosus, and sepsis.

### Inclusion criteria

Clinically stable preterm infants of at least 1 week of age who were placed in incubators were recruited for the study. We excluded infants with active neonatal diseases, such as respiratory distress with need for respiratory mechanical assistance, sepsis, or cardiovascular instability. Infants with congenital malformations, intraventricular hemorrhage, or neurologic impairment were also excluded. Mothers who expressed BM for their infants were approached to participate in the study. Patients were asked to maintain similar parent-infant interactions throughout the study period, including visiting times, skin-to-skin contact, and hand contact.

### Intervention

The study aimed to measure the effect of continuous exposure to BM odor on stress parameters among hospitalized preterm infants. The study consisted of three consecutive periods of 2 days each: (1) a preintervention, odor-free period, (2) followed by 2 days of intervention, continuous exposure to own mother's BM odor, and (3) ending with two odor-free days. During periods 1 and 3, the nurses were instructed to remove the BM syringes from the incubator as soon as the feeding ended. After giving their informed consent, mothers were instructed to provide eight samples (1.5 mL each) of BM for the third and fourth days of the study, in addition to the regular BM preparation for infants' feedings. The samples were kept in the same refrigerator as the expressed BM prepared for infants' feeding. During the third and fourth days of the study, a cotton pad soaked with 1.5 mL of BM was placed in the incubator about 10 cm from the infant's head to provide continuous exposure to BM odor. This pad was replaced every 3 h. Morning saliva cortisol was sampled at the end of each study day. All studied infants were  $<34$  weeks postmenstrual age during the study and hence fed by nasogastric tube.

Vital signs were continuously monitored so that HR, respiratory rate (RR), and apneic events were recorded in real time. The final scores used for analysis were constructed from all the measurements of each stage of the experiment (the mean of the 2 days of each stage). This yielded three scores for HR and three scores for RR. Apneic events were not included in the analysis, since due to the selection process there were hardly any such events.

### Salivary cortisol

Saliva was collected from infants using cotton-tipped applicators. All samples were taken at the same time (7:00 AM) following the end of each 24-h study period (total of 6 days). All samples were taken 1–2 h after feeding.

After collection, the saliva was frozen immediately and stored at  $-40^{\circ}\text{C}$ . Samples were thawed, centrifuged at 2700 RPM for 10 min, and stored at  $-80^{\circ}\text{C}$  until analysis via an enzyme-linked immunosorbent assay (IBL International GmbH, Germany). Standards, controls, and samples were analyzed in duplicate, and the absorbance was read using the Tecan Sunrise™ automated microplate reader (Tecan, Switzerland). The assay analytical sensitivity was 0.138 nmol/L, and intra-assay and interassay coefficient of variations were  $<7.3\%$  and  $<9.3\%$ , respectively.

The final salivary cortisol scores used for analysis were constructed from all the measurements of each stage of the experiment (the mean of the 2 days of each stage). This yielded three scores for cortisol (preintervention, intervention, and postintervention).

The Sheba Medical Center Ethics Committee approved the study and all participating parents gave their informed consent.

### Statistical analysis

A within-subjects design was chosen to overcome the vast variability found in preterm infants. The analyses used the statistical software for Windows (SPSS, Inc. Chicago, IL). The main analysis was a multivariate repeated-measures model. The repeated variable constituted the three periods: period 1 (days 1 + 2), period 2 (intervention—days 3 + 4), and period 3 (postintervention—days 5 + 6). The multivariate stress measures consisted of two stress measures: HR and saliva cortisol.

TABLE 1. CLINICAL DATA OF THE STUDY POPULATION (N=15)

Variable	
Gestational age at birth, weeks, mean $\pm$ SD (range)	29.3 $\pm$ 2.13 (26–32)
Birth weight, grams, mean $\pm$ SD (range)	1116 $\pm$ 272 (835–1643)
Appropriate for gestational age	13 (87%)
Small for gestational age	2 (13%)
Male/female	7/8
Antenatal steroids N (%)	14 (93.3)
Five-minute Apgar score, mean $\pm$ SD (range)	8.67 $\pm$ 2 (2–10)
Respiratory distress syndrome N (%)	11 (73.3)
Ventilation days, median (range)	0 (0–7)
Oxygen days, median (range)	8 (0–34)
Day of life at study initiation, median (range)	16 (7–54)

TABLE 2. MEANS AND STANDARD DEVIATIONS OF THE STRESS MEASURES OVER THE THREE STAGES: PREINTERVENTION, INTERVENTION, AND POSTINTERVENTION

	<i>Preintervention</i>	<i>Intervention</i>	<i>Postintervention</i>
Respiratory rate			
Mean	48.97	49.30	50.13
SD	6.36	7.39	6.88
Heart rate			
Mean	156.33	155.40	159.33
SD	11.87	12.10	10.78
Cortisol (nmol/L)			
Mean	11.38	9.51	4.99
SD	5.03	4.38	3.42

## Results

We enrolled 17 infants in the study. Multiple apneic episodes were recorded for two infants during the second day of the study (before intervention), leading to sepsis workup and antibiotic treatment. Due to the unstable condition of these infants, we did not initiate intervention and excluded these two infants from the study. The final study group consisted of the remaining 15 infants, who were clinically stable during the study period.

Table 1 shows the characteristics of the study group infants.

It should be noted that the events involving apnea, bradycardia (<100 BPM), or desaturation were rare during the entire study period.

The results of the multivariate repeated measures demonstrate an overall significant difference between the three periods (preintervention, intervention, and postintervention) (Wilks' Lambda  $F=4.71$ ,  $df=6.52$ ,  $p<0.001$ ). However, when we applied a separate univariate repeated analysis to each measure and checked the contrasts between stages, each measure yielded different results (Table 2).

The overall repeated univariate analysis of the HR measure showed significant differences ( $F=6.76$ ;  $df=2.28$ ;  $p<0.004$ ). The contrasts indicated that the minute increase of 2.46% in the postintervention stage compared with the intervention stage was statistically significant. The preintervention stage did not differ significantly from the intervention stage.

The overall repeated univariate analysis of the cortisol measure showed significant differences ( $F=9.34$ ;  $df=2.28$ ,  $p<0.001$ ). The highest score was the preintervention stage. The intervention stage was lower by 16.34%, although the difference was not significant. The postintervention stage was significantly lower—47.53% lower than the intervention stage and 56.15% lower than the preintervention stage.

## Discussion

Hospitalization in a neonatal department requires newborn infants to be separated from their parents and interferes with the natural close maternal–infant interaction during the neonatal period. During hospitalization of a preterm infant, parent–infant contact is intermittent and depends on various parameters, including the infant's medical condition as well as parental, social, and familial factors. Through continuous exposure to BM odor, the study design aimed to mimic ongoing maternal presence even during maternal absence and thus to evaluate the impact of the odor as a soothing tool for lowering infant stress.

Our study group is the first to design and carry out such an intervention. We hypothesized that continuous exposure to BM odor would be associated with a significant decrease in cortisol levels over time. Apnea events could not serve as a measure as they were sparse, most likely due to the good clinical status of the selected cohort. The minimal increase in mean HR at the postintervention stage was not meaningful enough to draw any conclusions.

In response to stress, the autonomic nervous system is activated, as is the hypothalamic–pituitary–adrenal axis (HPA). HPA activation results in excretion and elevation of blood and saliva cortisol. This mechanism appears even among preterm infants. An elevation of saliva cortisol has been observed in response to ACTH administration as early as the first week of life.<sup>15</sup> Nevertheless, the circadian rhythm of the HPA axis is observed only later in gestational maturation, as among term neonates.<sup>16</sup>

Measurement of saliva cortisol is a common method to evaluate stress or stress and pain relief among term and preterm infants.<sup>4,5,7,14,17</sup> Various studies suggest continued activation of the HPA axis following cessation of painful procedures as well as continuation of relaxation following soothing intervention.<sup>5,12</sup> We speculate that in this study, the effect of the soothing odor in maintaining HPA axis reactivity was the reason for the continuous reduction in salivary cortisol levels, especially during the postintervention stage. This speculation is supported by the knowledge that the early anatomic and physiologic maturation of the olfactory system enables preterm infants to sense and react to various odor stimulations. Indeed, a familiar odor was shown to have a soothing effect in painful procedures<sup>6,11,12</sup> and exposure to a pleasant odor was shown to prevent apnea among preterm infants.<sup>8</sup> Furthermore, there is evidence that newborn infants can produce “memories” of odors they were exposed to in the first days of life for at least 2 weeks.<sup>18</sup>

Basal cortisol levels were found to be high among infants of younger GA.<sup>19</sup> Although a few studies revealed low levels of cortisol among preterm infants<sup>1,19</sup> that increased later on during infancy,<sup>1</sup> those studies dealt with sick infants, which our screening process excluded.

In contrast to the changes that were found in the cortisol levels, the odor intervention did not seem to affect physiologic parameters (HR). We believe that this discrepancy is related to the basic differences in these two types of measurements (cortisol level versus mean HR), suggesting the need to use different measures in future studies.

The present study is limited by the small study sample size, yet due to the within-measures design it was possible to show a general pattern. Considering the heterogeneous response of the HPA axis to odor stimulation, individual evaluation of response should be further studied. In addition, the lack of a control group precludes concluding that the decreased cortisol levels derived from the soothing effect of continuous exposure to breast milk odor and not from the infants' non-specific maturation and/or lowered need for care/handling during the study period of 6 days.

## Conclusions

Decreased saliva cortisol was observed during and after continuous exposure to BM odor that mimicked an ongoing maternal presence. This result may indicate the soothing

effect of exposure to BM odor on preterm infants. Further studies using a larger cohort as well as a control group are needed to evaluate this simple, nonharmful, inexpensive, and safe method of soothing for clinical use.

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#### Disclosure Statement

No competing financial interests exist.

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