Effect of Continuous Olfactory Stimulation Using Breast Milk on Physiological Indices and Behavioral Responses of Preterm Neonates

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Abstract

Background: Breast milk odor is considered one of the updated developmental care used for stabilizing vital signs and decreasing distressed behaviors of preterm newborns. **The present study aimed** to evaluate the effect of continuous olfactory stimulation using breast milk on physiological indices and behavioral responses of preterm neonates. **Research design:** quasi experimental design was utilized. **Setting**: It was conducted at Neonatal Intensive Care Unit of EL Menshawy Hospital. **Subjects**: purposive sample of 60 premature neonates who were randomly divided into two groups; study group as breast milk olfactory stimulation and control group. **The study tools**: Assessment of Preterm Neonates Structured Interview Schedule, Preterm Neonate's Physiological Indices and Neonate's Behavioral State Assessment Scales were used for data collection. **Results**: there were statistically significant difference among control and study groups regarding physiological indices and behavioral responses of premature neonates pre and post intervention. **Conclusion**: stimulation with breast milk smell improves physiological parameters and behaviors of preterm hospitalized newborns. **Recommendations:** breast milk olfactory stimulation should be applied as a routine supportive care for all premature neonates at Neonatal Intensive Care Units.

Key words: Behavioral Responses, Breast Milk, Olfactory Stimulation, Physiological Indices, Preterm Neonates

Introduction

Prematurity is the primary cause of morbidity and mortality in neonates as it is associated with multiple health problems that require specialized medical attention and assistance **(Pavlyshyn., Sarapuk., Tscherning., & Slyva, 2023)**. Premature neonates are born before 37 gestation weeks. An estimated 15 million neonates were born preterm in 2022; more than 1 in every 10 livebirths **(World Health Organization, 2022)**. Increased mothers' age during pregnancy triggered the rise in the rates of premature labor

and high-risk neonates. Most neonates delivered prematurely had to be admitted to Neonatal critical Care Units for management of multi systems problems **(Elewa., Amin., & Ayed, 2021)**

Premature birth lead to immature body systems of the newborn, resulting in increasing the vulnerability for respiratory difficulty and underdeveloped regulatory center that causes irregular breathing, hypoventilation and repeated apneic episodes thus, they may have physiological problems such as a low respiratory rate and oxygen saturation, apnea and increased gastric residual **(Punthmatharith & Mora, 2018)**.

In addition, Neonatal Intensive Care Unit environment is upsetting for preterm newborns. They are subjected to a range of environmental stimuli all the time, including excessive noises, bright lights, repeated manipulations, painful invasive procedures, and separation from their mothers, resulting in decrease in the sensory stimulation needed for the neonatal development. These multiple stimuli can also have a negative impact on the premature infant's health, changing heart rate and oxygen saturation levels, increasing blood pressure fluctuations, restlessness and sleep disruption. All these factors affect the whole health outcomes of premature neonates **(Vahdati., Mohammadizadeh., & Talakoub, 2017; Correia & Lourenço, 2020)**.

Olfactory preferences of newborns have their origins in the prenatal period. The last trimester of pregnancy is when nasal chemoreception starts to work. During this time, the amniotic fluid is filled with odorous substances that are controlled by mother's genetic, immunological and physiological structure, moreover her dietary, cosmetic and addictive preferences. These substances are also influenced by stress level and general health of mothers **(Schaal ., Saxton., Loos., Soussignan., & Durand, 2020)**. A newborn's body odor can become strong, owing to the easy passage of odorous metabolites into amniotic fluid or through transplacental penetration.Newborns are able to recognize and recall the odors of their mothers' breast milk and amniotic fluid from the moment they are born. In addition, they are able to discriminate and show distinguished reactions in response to different scents (**Park & Im, 2020**; **Maya-Enero et al., 2022)**

Several studies have examined the relaxing effects on newborns after olfactory breast milk stimuli and revealed that, comforting and familiar smell like mothers' milk decreased behavioral distress and crying throughout painful interventions **(De Clifford-Faugere et al., 2020**; **Tasci & Ayyildiz, 2020)**. Furthermore, human milk odor stimulation maintains stable heart rate, respiration and blood oxygen saturation variations. Olfactory stimulation is safe and useful supportive care that minimizes the stress facing the newborns, enhances their health, growth and development **(Lee & Ra, 2021)**.

Nurses inside neonatal intensive care units play a crucial role in the care of premature infants. It is their responsibility to implement strategies that improve physiological signs and behavioral response of preterm neonates **(Hendy., Alsharkawy., & El-Nagger, 2021)**. They can encourage and teach mothers to maintain continuous flow of breast milk and provide developmental based care for their preterm neonates to promote recovery and

quality of life. Nurses can aid mothers by offering guidance, advice, support, and counseling regarding behavior cues and parenting skills to take care of their premature infants after discharge **(Correia & Lourenço, 2020).**

Significance of the Study

Preterm birth complications are the leading cause of death among neonates, approximately 1 million of newborns who born prematurely die each year. Furthermore, survivors of preterm infants are at a higher risk of adverse developmental disabilities **(World Health Organization, 2022)**. In Egypt, 38% of neonatal deaths caused by the prematurity associated problems. Three-quarters of these deaths could be prevented with current, cost-effective interventions **(Muhe etal., 2019)**.

Continuous Olfactory stimulation of breast milk is a new developmental based approach that can effectively control the neonates' respiratory responses **(Ataei Nakhaei et al., 2019)**.Various studies have shown that stimulation of the olfactory system expands the chest volume, produces higher peak expiratory flow, enhances lung function and significant changes included peripheral oxygen saturation and respiratory retention time **(Rustaee etal., 2023; Duchamp-Viret ., Nguyen., & Maucort-Boulch, 2021)**. Behavioral studies of the olfactory system development of neonates indicating the calming effects of proper olfactory stimuli which enhances behavioral states and sleep of preterm infant (**Sheikh Shoaei., Begjani., & Hoseini, 2023)**. Thus, the study's objective was to evaluate the impact of breast milk smell stimulation on physiological and behavioral parameters of preterm newborns.

Aim of the study was to:

Evaluate the effect of continuous olfactory stimulation using breast milk on physiological indices and behavioral responses of preterm neonates.

Research hypotheses

-Implementing of continuous olfactory stimulation using breast milk is expected to improve physiological indices of preterm neonates than those in the control group.

-Behavioral responses of preterm neonates is expected to be improved after implementation of continuous breast milk olfactory stimulation than those in the control group.

Subjects and Method

Research design

A quasi experimental design was used to fulfill the aim of the present study.

Settings:

The study was carried out at Neonatal Intensive Care Unit at Elmenshawy Hospital in El-Gharbia Governorate which is affiliated to Ministry of Health and populations, Egypt. This unit locates in the fourth floor. It consists of three holes A, B and C contain five incubators in each with two servo devices.

Subjects of the study:

A purposive sampling of sixty (60) preterm neonates who were equally divided into study group as olfactory stimulation group (30) neonates subjected to continuous breast milk odor and control group (30) neonates subjected to routine care applied in the previously stated setting.

The inclusion criteria of neonates:

1. Ppreterm neonates (gestational age: 28 < 38 weeks)
2. Ppreterm is not on mechanical ventilator or receiving continuous positive airway pressure or nasal cannula.
3. RReceiving oxygen therapy via oxygen hood.

Data collection tools

Three tools were used to collect data and included the following:

Tool I: Assessment of Preterm neonates structured Interview Schedule

It was generated by the researchers to obtain the neonates' baseline data. It consisted of two main parts:

Part (1): Personal characteristics of preterm neonates such as: gestational age, postnatal age, sex, birth weight and current weight.

Part (2): Medical history of the preterm neonates such as delivery type, feeding, experience of breast milk, and diagnosis.

Tool II: Preterm Neonate's Physiological Indices

This tool was used to determine physiological indices of preterm neonate such as heart rate, respiratory rate and saturation of oxygen. It was measured for the studied subjects every 3 hours for 5 days according to hospital policy. This was performed one day before using continuous olfactory stimulation with breast milk and throughout 3 days of this intervention as well as one day after termination of this intervention for experimental group and for five days to control group receiving routine care. Data for heart rate and oxygen saturation was obtained by inpatient monitor attached to preterm neonate and respiratory rate was calculated by researchers and nursing staff during their continuous physiological assessment for every neonate inside the incubator. The mean score for each item of physiological indices was calculated every day during the period of the study.

Tool III: Neonate's Behavioral State Assessment Scales

It was created by researchers following a review of related literatures **(Anderson, 1996 & Lawrence etal., 1993)**. This tool was used to assess behavioral response of premature infant. The scale incorporates six categories such as **eye openness, calmness /agitation, respiratory regularity, crying, physical movement and facial tension**. The scoring of each category is ranged from 0 - 2 so the total score for all items are 12. Lower score indicated more stable state while higher score was indicated a wake fullness state with more activity. The categories of the scale were as following: quiet sleep state ranged from (1-5), quiet awake state ranged from (6-8) and restless state ranged from (9-12). The data was collected three times at 8 hour interval for 24 hours and the median was calculated .This measurements was taken for five days for control and experimental groups. It was assessed one day before application of continuous breast milk olfactory stimulation and three days throughout its application and one day after termination of this intervention.

Method

The study was conducted via performing the subsequent steps:

1-Administrative process; The Dean of the Faculty of Nursing at Tanta University and the administrators of El Menshawy Hospital granted official permission to secure their approval and cooperation for the study.

2-Ethical and Legal Considerations

a. The study didn't result in any discomfort or suffering for all of the sample group.
b. Data collection process ensuring privacy and confidentiality for all neonates.
c. After describing the study's purpose to parents, the written informed consents were signed by the parents. The consent form showed that participation was completely voluntary, and they could withdraw from the study at any time
d. Ethical committee approval was obtained from Faculty of Nursing, **code Number of approval** : **154-12-2022**

3- **Development of the study tools:** The researchers collected data using three tools; Tool I: Tool II, Tool III.

4-Content Validity:

The tools of the study were examined for content validity by the experts in the field of pediatric nursing and experts in the field of Neonatal Intensive Care Unit. Modifications were carried out accordingly. Content validity index was 98%.

5-Tools reliability:

The developed tools was tested for reliability through Crombach's alpha coefficient which was 0.91 for tool I., 0.90 for tool II and 0.89 for tool III.

6-A pilot study

To determine the tool' s viability, clarity and comprehensiveness, a pilot study was done.This phase applied on ten percent of the total sample size (six neonates) and subsequently dropped from the main sample. Additionally, adjustments were performed.

The study was done through four phases

Phase of Assessment

- The baseline data for all the study participants collected by the researchers which was consisted of neonates' personal characteristics and medical history, physiological indices (heart rate, respiratory rate, and oxygen saturation) and behavioral state utilizing tool I, II, and III.

-The assessment was done one day before application of continuous mothers' milk olfactory simulation for every preterm neonate in the experimental group.

-Subsequent assessments was done for three days during continuous olfactory simulation with breast milk for experimental group to evaluate preterm neonate progress.

- Control group assessment was done at o, 24,48,72,96 hours of first assessment.

Phase of Planning: Preparation of the procedures and equipments was done by the researchers following exhaustive review of pertinent literatures. The researchers met all nursing staff caring for neonates at NICU to seek their help during data collection.

Phase of Implementation

- Mothers were asked to express their breast milk and bring it at NICUs
- An amount of 5 cc of the mothers' breast milk was submerged in 2cm × 2 cm piece of sterile gauze
- The gauze was placed 2 cm away from the nose near head and was putted on addressing of 7cm×7cm to avoid wetting of mattress.
- The gauze soaked with breast milk was provided about 30 minutes before feeding as preterm become alert during this period.
- New gauze soaked in breast milk at the starting of each intervention period to avoid decomposition of breast milk and infection inspired by breast milk in the warm and humid environments of incubators
- Sterilized wet breast milk gauze was provided before each feed and the feeding was every 3 hours so, the soaked gauze with breast milk was provided eight times per day for 3 consecutive days to ensure continuous olfactory stimulation.
- To reduce the impacts of additional olfactory stimulation other than maternal breast milk, doctors and nursing staff in the NICU who directly contacted the preterm neonates in our study were told not to use any perfume nor fragrant products during the intervention period.
- Notices were allocated at incubator of each participant and a notice was published in a place easy to be observed for being sure that the NICU staff implemented these requirements.
- Each nurse told the instructions to the following nurse who received and continued the neonatal care during the shifts change.
- No intervention was provided to the control group except for routine care from medical and nursing staff in NICU unit.

Phase of Evaluation

-Each preterm neonate in the study was reevaluated after continuous olfactory stimulation by breast milk at 24 ,48,72 hours of its application and one day after termination of intervention using tool II and III.

-The control group reevaluated at 24, 48, 72 and 96 hours after first assessment using tool II and III. Comparison between the findings before and 24, 48, 72 hour after continuous olfactory stimulation with the use of breast milk, and one day after termination of intervention and between the results of the control group subjected to routine care were carried out using appropriate statistical analysis in order to determine effect of continuous olfactory stimulation using breast milk on physiological indices and behavioral state among studied preterm neonate.

-Collection of data began at the starting of July 2023 and ended at December 2023.

Statistical Analysis:

- SPSS software (Statistical Package for the Social Sciences, version 26, SPSS Inc. Chicago, IL, USA) used to clean,tabulate and statistically analyze the collected data.The range, mean and standard deviation for the quantitative data were calculated. For qualitative data that calarifies a categorical set of data by frequency, percentage or proportion of each category, comparison between two groups and more was done using Chi-square test ($\chi 2$). For comparison between means of two different groups of parametric data of Independent Samples t-test was used. For comparison in between more than two means of parametric data, F value of ANOVA test was estimated . Correlation between variables was assessed using Pearson's correlation coefficient (r). A significance was adopted at $P<0.05$ for interpretation of results of tests of significance (*). Also, a highly significance was adopted at $P< 0.01$ for interpretation of results of tests of significance **(White, 2019)**.

Results

Table (1) presents the percentage distribution of the studied neonates according to their personal characteristics and medical history. Regarding their gestational age, it was noticed that 50% and 30% of them were between 33 to less than 35 weeks in control and olfactory group respectively. While 46.7% and 43.3% of them their present age were between 7 to less than 14 days in control and olfactory group respectively. It was observed that 66.7% and 56.7 of the preterm neonates were low birth weight in control and olfactory group respectively. While 53.3% and 40 % of them, their present weight ranged from 2000 to less than 2500 gram in control and olfactory group respectively. Regarding their type of feeding, it was noticed that 50% and 53.3% of them took bottle feeding in control and breast milk group respectively. It was found that 86.7% didn't experience breast milk in control group while 60% of them experienced it in olfactory group. As regards their diagnosis, it was found that, 96.7% and 86.7% of the preterm neonates had respiratory distress in the control and olfactory groups respectively.

Table (2) clarifies the mean scores of the studied neonates' physiological indices regarding heart rate. It was observed that, there were highly statistically significant difference and statistically significant difference (P=0.006 and P=0.022) among control and olfactory group regarding the mean of heart rate in the 3rd day of intervention and one day after intervention respectively. While one day before intervention, 1st and 2nd day of intervention there were no significant differences (P=0.079, P=0.647 and P=0.234) between control and olfactory group regarding the heart rate mean respectively.

Table (3) shows mean of the studied preterm neonates' physiological indices regarding oxygen saturation. It was found that, there were statistically significant difference and highly statistically significant difference (P=0.015 and P=0.0001) among control and olfactory group in the 1st and 3rd day of intervention respectively. Whereas, one day before intervention, 2nd day of intervention and one day after intervention, there were no statistically differences (P=0.952, P=0.952 & P=0.937) among control and olfactory group regarding oxygen saturation mean respectively.

Table (4) illustrates a highly statistically significant differences (P=0.0001) inbetween control and breast milk group regarding the mean of respiratory rate in the 3rd day of intervention and one day later. However, it was observed non significant differences (P=0.113, P=0.146 & P= 0.331) in one day before intervention, 1st and 2nd day of intervention among control and olfactory group regarding the mean of respiratory rate respectively.

Table (5) shows percentage distribution and mean scores of the neonates regarding behavioral responses. It was found a highly statistically significant differences (P=0.0001)

between control and breast milk olfactory group regarding the mean of behavioral responses in the 2nd, 3rd day of intervention and one day later. But, there were no significant difference (P=0.455) one day before intervention among control and olfactory group respectively.

Table (6) clarifies a highly significant differences (P=0.001, P= 0.007 , P= 0.007 & P=0.008) inbetween control and olfactory groups regarding the mean of feeding amount in one day before intervention, 1st and 2nd and 3rd day of intervention. In addition, one day after intervention there was statistically significant difference (P=0.042) among control and olfactory groups.

Figure (1) illustrates distribution of the studied neonates regarding their ability for continuing breast feeding intake after discharge. It was observed that 40% and 90% of the control and olfactory group had the ability for continuing breast feeding intake after discharge respectively. Whereas, 60% and 10% of them had no ability for continuing breast feeding intake after discharge among the control and olfactory groups respectively.

Table (7) demonstrates correlation between physiological indices and behavioral responses of neonates. It was observed that there were positive correlation between heart rate, oxygen saturation, respiratory rate and behavioral responses in the second, third and one day after the intervention (r=0.641, p=0.0001, r=0.053, p=0.781, r=0.478, p=0.008, r=0.466, p=0.009, r=0.127, p=0.505, r=0.045, p=0.815, r=0.266, p= 0.156, r=0.188, p= 0.319, r=0.125, p= 0.509).

Table (1): Percentage Distribution of the Studied Neonates according to their Personal Characteristics and Medical History

Personal Characteristics and Medical History	(n=60)				
	Control group (n=30)		Olfactory group (n=30)		χ^2 P
	No.	%	No.	%	
Gestational age (weeks)					
28: < 30	1	3.3	7	23.3	7.023 0.071
30:< 33	5	16.7	7	23.3	
33 : < 35	15	50.0	9	30.0	
35 : < 38	9	30.0	7	23.3	
Post-natal or present age (days)					
1 -	1	3.3	5	16.7	3.304 0.347
7 -	14	46.7	13	43.3	
14 -	9	30.0	7	23.3	
21 – 28	6	20.0	6	16.7	
Sex					
Male	10	33.3	16	53.3	FE 0.192
Female	20	66.7	14	46.7	
Birth weight					
Low birth weight	20	66.7	17	56.7	FE 0.596
Very low birth weight	10	33.3	13	43.3	
Present weight (grams)					
1000 -	4	13.4	10	33.3	3.365 0.186
1500 -	10	33.3	8	26.7	
2000 -<2500	16	53.3	12	40.0	
Delivery type					
Cesarean section	26	86.7	27	90.0	FE 0.500
Vaginal delivery	4	13.3	3	10.0	
Type of feeding					
Bottle feeding	15	50.0	16	53.3	1.072 0.585
Gavage feeding	12	40.0	13	43.3	
Bottle and gavage feeding	3	10.0	1	3.4	
Experience of breast milk					
Yes	4	13.3	18	60.0	**FE**
No	26	86.7	12	40.0	**0.0001****
Diagnosis					
Respiratory distress	29	96.7	26	86.7	FE
Non-respiratory distress	1	3.3	4	13.3	0.353

**** Highly Statistically significant difference at (P<0.01) FE: Fisher's Exact test**

Table (2): Mean Scores of the Studied preterm neonates' physiological indices scores regarding heart rate

Heart rate	Control group (n=30)	Olfactory group (n=30)	t-test	P
	Range Mean±SD	Range Mean±SD		
One day before intervention	122 – 167 146.9 ±10.66	131 – 169 152.13 ±11.65	1.791	0.079
1st day of intervention	124 – 165 148.5 ± 10.04	125 – 167 149.76±11.26	0.460	0.647
2nd day of intervention	125 – 163 149.93 ± 10.06	118 – 165 146.66 ±10.96	1.202	0.234
3rd day of intervention	126 – 167 151.30 ± 10.18	119 – 160 143.70 ±10.29	**2.874**	**0.006****
One day after intervention	130 – 168 152.83 ± 11.07	116 – 165 146.46±9.92	**2.347**	**0.022***
χ2 value **P**	**47.412** **0.0001****	**79.937** **0.0001****		
Change of heart rate after intervention **P**	(-14) – (20) 5.866 ± 7.181	(-18) – (5.6) -5.673 ± 5.634		
#χ2 value **P**	**31.927** **0.0001****			

***Statistically significant difference at (P<0.05) ** Highly Statistically significant difference at (P<0.001) χ^2 value of Freedman test #χ^2 value of Kruskal Wallis test**

Table (3): Mean of the Studied Preterm Neonates' Physiological Indices regarding Oxygen Saturation

Oxygen saturation	Control group (n=30)	Olfactory group (n=30)	t-test	P
	Range Mean±SD	Range Mean±SD		
One day before intervention	89 – 99 94.90 ±2.42	89 – 99 94.91 ±2.12	0.060	0.952
1st day of intervention	91 – 98 94.50 ±1.96	91 – 98 95.05 ±1.77	**2.498**	**0.015***
2nd day of intervention	93 – 99 95.60 ±1.69	93 – 99 95.80 ±1.64	0.941	0.952
3rd day of intervention	92 – 99 95.03 ±1.49	92 – 99 95.91 ±1.53	**5.440**	**0.0001****
One day after intervention	92 – 99 95.46 ±1.47	92 – 99 95.45 ±1.53	0.079	0.937
χ2 value **P**	**14.682** **0.005****	**46.849** **0.0001****		
Change of oxygen saturation after intervention **P**	(-5) – (6) 0.566±2.144	(-1) – (4) 0.500±1.358		
#χ2 value **P**	0.002 0.963			

***Statistically significant difference at (P<0.05) ** Highly Statistically significant difference at (P<0.01)**
#χ^2 value of Kruskal Wallis test χ^2 value of Freedman test

Table (4): Mean of the Studied Preterm Neonate's Physiological Indices regarding Respiraory Rate

Respiratory rate	Control group(n=30)	Olfactory group (n=30)	t-test	P
	Range Mean±SD	Range Mean±SD		
One day before intervention	35 – 68 49.16 ±8.40	39 – 70 52.96 ±9.84	1.608	0.113
1st day of intervention	40 – 70 53.43 ±7.93	38 – 60 50.57 ±7.05	1.475	0.146
2nd day of intervention	40 – 67 51.10 ±7.78	39 – 56 49.43 ±5.11	0.980	0.331
3rd day of intervention	41 – 70 53.73 ±7.59	35 – 54 46.50 ±5.51	**4.221**	**0.0001****
One day after intervention	34 – 67 53.73 ±7.86	40 – 53 46.60 ±4.23	**4.373**	**0.0001****
χ2 value **P**	**13.618** **0.009****	**35.865** **0.0001****		
Change of respiratory rate after intervention **P**	(-11) – (21) 4.566±7.67	(-26) – (11) -6.366±10.226		
#χ2 value **P**	**17.401** **0.0001****			

**** Highly Statistically significant difference at (P<0.01)**

χ^2 value of Freedman test **#χ^2 value of Kruskal Wallis test**

Table (5): Percentage Distribution and Mean Scores of Preterm Neonates regarding Behavioral Responses

Total scores of behavioral responses	Control group(n=30)		Olfactory group (n=30)		χ^2 P
	No.	%	No.	%	
One day before intervention					
Sleep (1-5)	2	6.7	5	16.7	1.487 0.475
Awake (6-8)	15	50.0	14	46.7	
Restless (9-12)	13	43.3	11	36.6	
Range	4 – 12		3 – 11		**t-test**=0.753
Mean±SD	8.200 ± 2.04		7.766± 2.40		**P=** 0.455
1st day of intervention					
Sleep (1-5)	2	6.7	8	26.6	**7.281 0.026***
Awake (6-8)	15	50.0	17	56.7	
Restless (9-12)	13	43.3	5	16.7	
Range	4 – 11		2 – 10		**t-test= 2.382**
Mean±SD	8.066 ± 1.74		6.766± 2.43		**P= 0.021***
2nd day of intervention					
Sleep (1-5)	0	0.0	18	60.0	**25.942 0.0001****
Awake (6-8)	20	66.7	9	30.0	
Restless (9-12)	10	33.3	3	10.0	
Range	6 – 10		2 – 9		**t-test= 6.801**
Mean±SD	8.000 ± 1.20		5.066± 2.03		**P= 0.0001****
3rdday of intervention					
Sleep (1-5)	1	3.3	27	90.0	**45.643 0.0001****
Awake (6-8)	21	70.0	3	10.0	
Restless (9-12)	8	26.7	0	0.0	
Range	5 – 10		1 – 7		**t-test= 11.373**
Mean±SD	7.833± 1.08		4.033±1.47		**P= 0.0001****
One day after intervention					
Sleep (1-5)	1	3.3	24	80.0	**38.705 0.0001****
Awake (6-8)	16	53.3	6	20.0	
Restless (9-12)	13	43.3	0	0.0	
Range	4 – 11		2 – 8		**t-test= 8.757**
Mean±SD	8.03±1.44		4.566±1.61		**P= 0.0001****

***Statistically significant difference at (P<0.05) ** Highly Statistically significant difference at (P<0.01)**

Table (6): Mean scores of Preterm Neonates regarding Amount of Feeding

Amount of feeding	Control group (n=30)	Olfactory group (n=30)	t-test	P
	Range Mean±SD	Range Mean±SD		
One day before intervention	20 – 50 32.06 ±8.05	10 – 45 23.13±11.57	3.470	0.001**
1st day of intervention	15 – 55 33.00 ±9.91	13 – 45 25.70±10.13	2.820	0.007**
2nd day of intervention	25 – 55 35.00±8.20	15 – 50 28.56±9.68	2.776	0.007**
3rd day of intervention	30 – 55 36.83±7.36	17 – 50 31.13±8.66	2.774	0.008**
One day after intervention	30 – 55 38.36 ±6.27	17 – 60 33.70±10.60	2.074	0.042*
χ2 value P	71.271 0.0001**	102.735 0.0001**		
Change of amount of feeding after intervention P	(0) – (20) 6.300±5.187	(0) – (20) 10.566±5.739		
#χ2 value P	11.590 0.0001**			

*Statistically significant difference at (P<0.05) ** Highly Statistically significant difference at (P<0.01)
#χ^2 value of Kruskal Wallis test χ^2 value of Freedman test

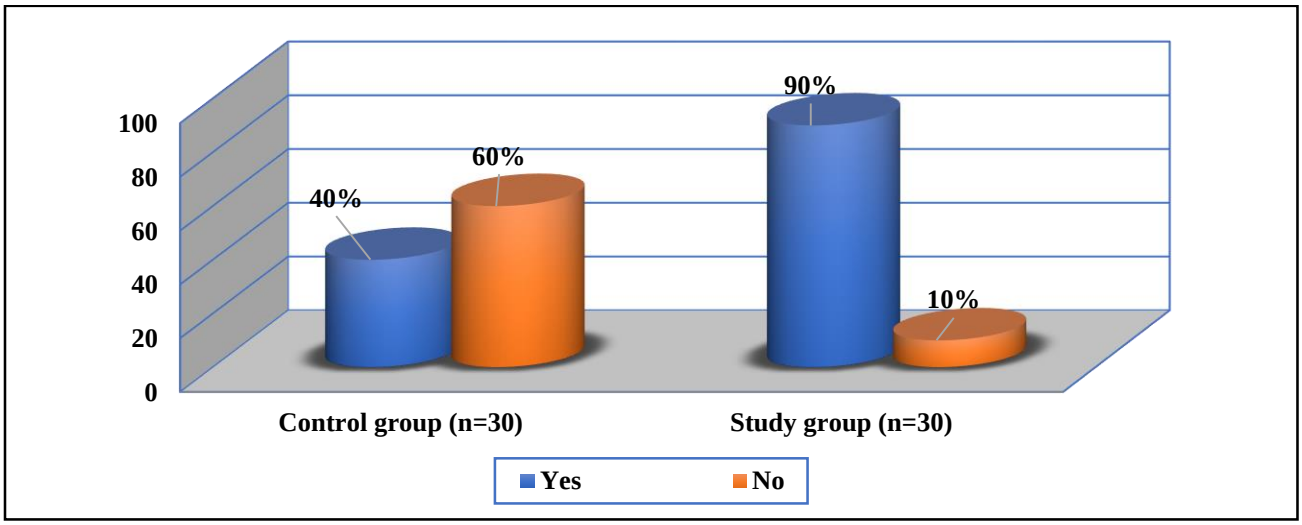

Figure (1): Distribution of the Studied preterm Neonates regarding their Ability for Continuing Breast Feeding Intake After Discharge

Table (7) Correlation between Physiological indices and behavioral responses of neonates

Variables	Physiological Indices (n= 60)					
	Control Group (N=30)			olfactory group (n=30)		
	HR	O_2	RR	HR	O_2	RR
	r P	r P	r P	R P	r P	r P
Behavioral Responses						
One day before intervention	**0.505** **0.004****	.0380 0.844	-0.183 0.333	**0.534** **0.0001****	0.343 0.063	**0.517** **0.003****
1st day of intervention	**0.538** **0.002****	0.212 0.260	0.065 0.732	**0.527** **0.003****	**-0.452** **0.012***	**0.732** **0.0001****
2nd day of intervention	0.157 0.408	0.203 0.282	-0.088 0.642	**0.641** **0.0001****	0.053 0.781	**0.478** **0.008****
3rd day of intervention	0.161 0.396	-0.060 0.752	0.304 0.103	**0.466** **0.009****	0.127 0.505	0.045 0.815
One day after intervention	0.265 0.158	0.064 0.737	0.119 0.530	0.266 0.156	0.188 0.319	0.125 0.509

***Statistically significant difference at (P<0.05) ** Highly Statistically significant difference at (P<0.01)**

Discussion

Premature infants are exposed to excessive painful invasive procedures leading to increased susceptibility to physiological,neurological, behavioral and psychological changes in response to pain. These changes include abnormal vital signs, excessive crying and abnormal facial movements **(Louyeh et al., 2020)**.

Stimulation of premature infants with the smell of the mothers' milk is an effective way which acts as an entrance for improving physiological responses such as increasing oxygen delivery, improving heart rate, developing the infant's sucking reflex and lowering pain especially following procedures inducing pain **(Alemdar & Snal, 2020; Zhang., Su., Li., & Chen., 2018).**

Regarding neonatal heart rate, the study demonstrated a highly statistically difference between control and olfactory groups in terms of the mean scores of the heart rate in the 3rd day of intervention and one day after intervention. This could be explained in the light of the soothing effect of breast milk odor activate the parasympathetic nervous system, which enhances relaxation and reduces distress resulting in stable vital signs **(El-hadary., Abozed., & Alaswad., 2022)**. Furthermore, pleasant odors may distract preterm infants from environmental stress such as excessive light and pain associated with frequent treatment procedures in NICU that increase heart rate variance. This result was in harmony with **Park & Im (2020)** who stated that experimental group presented more stable heart rate with significantly reduced heart rate variations a at all-time points during the study. Similarly, **Bagheri**., **Heidari**., **& Manzari. (2023)** who reported that pleasant olfactory stimuli with mothers' milk is easy and inexpensive technique that stabilize physiological indicators as heart rate and blood oxygen saturation.

On the opposite side, the finding of the current study was inconsistent with **lee**

(2019) & Sheikh Shoaei et al. (2023) who found that there was no significant difference between control and experimental group regarding heart rate scores.

As regards oxygen saturation, it was showed that the olfactory group had a highly significant improvement regarding scores of oxygen saturation in the 1st and 3rd day of intervention. This could be attributed to the importance of continuous breast milk smell in regulating respiration and improving level of oxygen saturation. This result was in agreement with **Rustaee etal. (2023)** & **Sheikh Shoaei et al. (2023)** who stated that preterm neonates exposed to aromatherapy with breast milk odors show less frequency of respiratory distress and high SpO2 levels compared to the control group on the third day of intervention.

On the other side, the finding of the current study was inconsistent with **lee (2019) & Rad., Aziznejadroshan., Amiri., Ahangar., Valizadehchari**. **(2021)** who found that there was insignificant difference between control and experiment group regarding oxygen saturation.

In relation to respiratory rate, there were highly statistically significant differences among control and olfactory group in the 3rd day of intervention and one day after intervention. This finding agreed with **El-hadary etal. (2022)** who discovered that there was statistically significance improvement regarding respiratory rate of preterm infants post intervention. Similarly **Rustaee etal. (2023)** mentioned that aromatherapy with breast milk odors could significantly reduce apnea in preterm infants This finding was contrary to **Asadinan, Shirinzadeh-Feizabadi., Amiri-Shadmehri., & Yaghoobi. (2023)** who declared that no significant relationship was found between mothers' milk odor and breathing variable. In addition to **Sheikh Shoaei et al. (2023)** who stated that respiratory rates did not show any significant difference between the intervention and control groups.

Pain profile of premature neonate is a set of measurable behavioral and physiological responses such as facial expression changes (squeezing eyes, raising eyebrows, wrinkling nasolabial groove) vigorous crying as well as vital signs changes (**Rustaee etal., 2023**).

Concerning behavioral response of newborns, there were highly statistically significant differences among preterm neonates of control and olfactory group in the 2nd, 3rd day of intervention and one day after intervention. This could be due to the fact that the breast milk smell has the unique chemical nature of the mothers' scent or the neonatal familiarity with their mothers. Olfactory stimulation restore the memory related to comfort that come from the mother to the neonate thus improving the behavioral status of newborn (**Asadian etal., 2023; Illmiasih & Juwitasari, 2022**). On the same way, **Fitri., Lusmilasari., Juffrie., & Bellieni. (2020)** who demonstrated that the intensity of pain and crying in the breast odor olfactory group was the lowest compared to the control group. Additionally, **De Clifford-Faugere et al. (2022)** who reported that familiar odors had calming influences that could diminish the irritable behaviors (motor and facial signs) and duration of crying during painful procedures.

Regarding to feeding, it was illustrated that the difference between olfactory and control groups according to the mean of amount of feeding was highly statistically significant in the 1st, 2nd and 3rd day of intervention. This might be due to olfactory stimulation with the

milk of mother triggers reflexes in the brain that enhance salivation, peristaltic movements, release of digestion hormones and enzymes, thereby starting the digestive process even before food reaches the stomach. These reflexes are referred to as the cephalic phase response and release appetite hormones in saliva.These salivary hormones are responsible for metabolism and increasing peristaltic movements (**Arafa., Radwan., & Mohammed, 2021; Schaal etal., 2020 ; Gellrich et al., 2018).**

This finding was supported by **Muelbert., Bloomfield., Pundir., Harding., & Pook. (2021)** who reported that exposure to odor of breastmilk enhanced sucking skills as more non-nutritive sucking bouts leading to great consumption of milk. Also, **Khakpour etal. (2022**) who discovered that there were increased frequency of sucking, mouth movement and the infant's ability to feed in preterm newborn exposed to fresh breast milk odor.

Conclusion: According to the findings of the current study, it can be concluded that breast odor smell improves physiological parameters and behaviors of preterm neonates. **Recommendations:** Considering the previous study findings, the current study recommends:

-Breast milk olfactory stimulation should be applied as a routine developmental appropriate care for all premature neonates at Neonatal Intensive Care Units.

- Health education programs should be conducted for nurses regarding breast milk olfactory stimulation to foster maturity and recovery of preterm neonates.

-The olfactory stimulations should be included in hospitals' policies and guidelines which focused on the care procedures of the premature newborns.

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