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Research Article / Araştırma

Effect of breast milk/formula temperature on the vital signs of preterm infants

Anne sütü/formül süt ısısının preterm bebeklerin yaşam bulguları üzerine etkisinin değerlendirilmesi

Eda Aktaş[1], Ayşe Ferda Ocakçı[2], Güner Karatekin[3]

[1] Sağlık Bilimleri Üniversitesi Hamidiye Hemşirelik Fakültesi, Çocuk Sağlığı ve Hastalıkları Hemşireliği Anabilim Dalı, eda.aktas@sbu.edu.tr. 0000-0003-1424-9678

[2] Koç Üniversitesi Hemşirelik Fakültesi, aysocakci@gmail.com, 0000-0002-4501-1913

[3] Sağlık Bilimleri Üniversitesi/İstanbul Zeynep Kamil Kadın ve Çocuk Hastalıkları Sağlık Uygulama ve Araştırma Merkezi, gunerkaratekin@yahoo.com, 0000-0001-7112-0323

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Corresponding Author/Sorumlu Yazar:
Sağlık Bilimleri Üniversitesi Hamidiye Hemşirelik Fakültesi, Çocuk Sağlığı ve Hastalıkları Hemşireliği Anabilim Dalı, eda.aktas@sbu.edu.tr. 0000-0003-1424-9678

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ABSTRACT

Purpose: The aim of this study was to evaluate the impact of breast milk/formula milk at different temperatures prepared and given to preterm infants in accordance with a standard on the vital signs of the infant. **Methods**: This pretest-posttest controlled experimental study was conducted with 150 preterm infants in the NICU of a training and research hospital. Infants in experimental group 1 received breast milk/formula at 24 °C and those in experimental group 2 received breast milk/formula at 37 °C. Infants in the control group received breast milk/formula at the temperature prepared by the nurses as per routine practice with no specific instruction or intervention. The infants' vital signs (body temperature, heart rate, and oxygen saturation) were measured and recorded immediately before feeding and at 5 minutes and 30 minutes after the start of feeding. **Results**: The study sample comprised 44% female and 56% male infants with a mean gestational age of 31.3±3.0 weeks. The groups were similar in terms of descriptive characteristics ($p>0.05$). The vital signs of infants in experimental group 2 measured at 5 and 30 minutes after the start of feeding tended to be better and more stable than those of the infants in experimental group 1 and the control group. **Conclusion**: Breast milk/formula given at 37 °C improved preterm infants' vital signs. Based on these results, it can be recommended to administer breast milk/formula at 37 °C to preterm infants fed enterally.

ÖZ

Amaç: Araştırma preterm bebeklere bir standart doğrultusunda hazırlanarak verilen farklı ısılardaki anne sütü/formül sütün bebeğin yaşam bulguları üzerine etkisini değerlendirmek amacı ile gerçekleştirilmiştir. **Yöntem**: Araştırma İstanbul ilindeki bir eğitim araştırma hastanesinin YYBÜ'nde, ön test-son test kontrol gruplu deneysel tipte, 150 preterm bebek ile gerçekleştirilmiştir. Veriler, araştırmacı tarafından geliştirilen preterm bebeği tanıtıcı bilgi formu ve preterm bebek takip çizelgesi aracılığı ile toplanmıştır. Anne sütü/formül sütler Deney-1 grubu bebeklere 24 °C'de, Deney-2 grubu bebeklere 37 °C'de verilmiştir. Kontrol grubu bebeklere verilen süt ısısına herhangi bir müdahale yapılmamış, hemşirelerin hazırladığı ısıda verilmiştir. Farklı ısılardaki anne sütü/formül sütler preterm bebeklere verilmeden hemen önce, verildikten 5 dakika ve 30 dakika sonra bebeğin yaşam bulguları (vücut ısısı, KTA ve SpO2) ölçülmüş ve kayıt altına alınmıştır. **Bulgular**: Araştırma kapsamındaki preterm bebeklerin %44'ü kız, %56'sı erkektir ve ortalama gestasyon haftası 31,31±3,02 olarak saptanmıştır. Gruplar tanımlayıcı özellikleri açısından benzerdir ($p>0,05$). Deney-2 grubu bebeklerin beslenme başladıktan sonra 5. ve 30. dakikalarda ölçülen yaşam bulgularının Kontrol ve Deney-1 grubuna göre fizyolojik stabilizasyonu daha etkili sağladığı saptanmıştır ($p<0,05$). **Sonuç**: Preterm bebeklere 37 °C'de verilen anne sütü/formül sütler bebeğin yaşam bulgularını olumlu yönde etkilemiştir. Bu sonuçlara göre enteral beslenen preterm bebeklere anne sütü/formül sütün 37 °C'de verilmesi önerilebilir.

INTRODUCTION

Today, high-risk newborns have a higher chance of survival because of the increasing importance given to of maternal and infant health and the application of technological, scientific, and medical engineering advances in neonatal intensive care units (NICUs) (Lee et al., 2022; Mohammed et al., 2018). In addition to keeping them alive, NICUs aim to reduce preterm infants' morbidity rates and ensure that they live a healthy life both in childhood and adulthood (Filippa et al., 2020). Adequate and balanced nutrition from birth has an important role in achieving this goal (Belfort & Ramel, 2019; Gökçe, 2017). When feeding preterm infants, various factors such as gestational age, diagnosis, food amount, feeding route, and frequency are taken into account (Kültürsay et al., 2018). The infant's position and feeding route and method are variables that affect nutrition (Kültürsay et al., 2018; Lawlor-Klean et al., 2013). Food temperature may be another relevant factor in feeding, but this parameter is often not mentioned or

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considered when discussing preterm infants' feeding tolerance and development (Lawlor-Klean et al., 2013).

The temperature of breast milk/formula given to the infant is important in terms of both nutritional value of the food and the infant's vital signs and gastrointestinal findings (Dumm et al., 2013; Uygur et al., 2019). Breastfed babies receive breast milk at the ideal temperature. However, attention should also be paid to the temperature of formula or expressed breast milk being prepared and given to infants. It is essential that the breast milk or formula fed to infants is not degraded (Çamur & Erdoğan, 2023; Gabrielski & Lessen, 2015; Samancı, 2017; Uygur et al., 2019). Giving breast milk/formula at the appropriate temperature to preterm infants accelerates the development of gastrointestinal tract functions and the growth process, thus shortening the length of hospital stay (Lawlor-Klean et al., 2013).

There are a small number of studies on the appropriate temperature of breast milk/formula given to preterm infants (Anderson & Berseth, 1996; Blumenthal et al., 1980; Çamur & Erdoğan, 2023; Eckburg et al., 1987; Gonzales et al., 1995; Holt et al., 1962; Uygur et al., 2019). Holt et al. (1962) reported a decrease in body temperature in infants fed cold milk. In a study by Blumenthal et al. (1980), there was no difference in the gastric emptying times of preterm infants fed cold or warm milk. Two other studies demonstrated better tolerance and less gastric residual in preterm infants given milk at body temperature (37 °C) (Eckburg et al., 1987; Gonzakes et al., 1995). In another study, infants fed milk heated to 32-34 °C had lower gastric residual volume than those fed milk heat at 22-24 °C (Uygur et al., 2019). Several studies show that not measuring the temperature of milk with a standard measurement method can lead to problems in preterm infants (Anderson & Berseth, 1996; Dumm et al., 2013; Eckburg et al., 1987; Gonzales et al., 1995; Holt et al., 1962; Lawlor-Klean et al., 2013; Uygur et al., 2019).

Feeding preterm infants with breast milk/formula at the appropriate temperature is among healthcare professionals' duties to promote and improve the health of these infants (Çamur & Erdoğan, 2023). However, it was observed that NICU nurses did not have sufficient information about the proper temperature for milk given to infants and did not use any standard for milk heating and temperature measurement (Çövener Özçelik & Aktaş, 2019).

The temperature of milk given to infants may have an impact on their vital signs. Therefore, the present study was conducted to evaluate the effect of breast milk/formula at different temperatures, prepared according to a standard and given to preterm infants, on the vital signs of preterm infants.

METHODS

Study Design

This pretest-posttest controlled experimental study evaluated the effect of breast milk/formula prepared according to a standard protocol and given to preterm infants at different temperatures on their vital signs.

Setting and Sample

This study was conducted with 150 preterm infants in the NICU of a training and research hospital between March 23, 2016 and November 1, 2016. The NICU in which the study was conducted provides level I-III care with 63 beds. To allow the use of parametric tests in statistical evaluation, increase the reliability of the results, and account for possible data loss, the sample group was planned to consist of 150 preterm infants, 50 in each group: experimental group 1 (24 °C), experimental group 2 (37 °C), and the control group. After recruiting 150 infants, power analysis was performed using the G*Power (v3.1.9) program based on the standard deviation of the mean body temperature of the infants in the sample. According to this evaluation, the effect size was calculated as W=0.624 and at least 42 subjects in each group were necessary to achieve 80% power at an α level of 0.05. Power of 80% or greater indicates an adequate sample size (Malone et al., 2016). As 50 preterm infants were already included in each group, there was no need to continue recruitment and the study was completed with 150 preterm infants.

To ensure the experimental and control groups were similar in terms of characteristics that may affect body temperature, oxygen saturation (SpO2), and heart rate (HR), inclusion criteria for the study sample were birth weight of 1000 g or more, Apgar score higher than 6, being stable for the first 24 hours after birth, and being fed enterally by orogastric tube (OGT), while exclusion criteria were cranial hemorrhage, peripheral circulatory disorder, congenital anomaly or malformation, developmental brain abnormality, endotracheal intubation, hyperbilirubinemia requiring blood exchange, using a pacifier to reduce stress symptoms, and undergoing phototherapy or any invasive interventions during the study.

Data Collection Tools and Instruments

The data of the study were collected with a preterm infant information form, preterm infant observation chart, standard operating procedure (SOP) for preparing and heating breastmilk/formula for preterm infants, and a nurse breast milk/formula preparation and heating assessment checklist. The tools used in the data

collection process included a bedside monitoring device, thermometer, thermostat-controlled milk warmer, liquid thermometer, and thermal milk carrying bag.

Preterm infant information form

This form was prepared by the researchers based on literature data (Dumm et al., 2013; Lawlor-Klean et al.,2013). It consists of 13 questions (5 closed-ended and 8 open-ended) about the infant's diagnosis, gestational age, date of birth, Apgar score, sex, weight, height, head circumference, place of follow-up, and breast milk/formula intake. The information was obtained from the patients' records and entered into the form by the researcher.

Preterm infant follow-up chart

This chart was created by the researcher and used to record the preterm infant's weight, the amount of milk given to the infant, the temperature of the breast milk/formula when prepared in the kitchen and at the time of feeding, and the infant's body temperature, oxygen saturation (SpO2), and heart rate measured before feeding, 5 minutes after the start of feeding, and 30 minutes after the start of feeding.

Standard operating procedure for preparing and heating breast milk/formula for preterm infants

The SOP chart was prepared by the researcher in line with the literature (Başkale & Serçekuş, 2014; Kayhan Tetik, 2016; Turkish Public Health Institution, 2015a; Turkish Public Health Institution, 2015b; White et al., 2013). It comprises three sections listing framework, process, and outcome criteria. The framework criteria consist of 16 items regarding the physical conditions of the breast milk/formula preparation kitchen and considerations pertaining to the kitchen and the staff working there. The process criteria consist of 26 items related to breast milk/formula storage, preparation, and transfer to the unit, and the outcome criteria consist of 2 items about the overall quality of the feeding and the expected changes in the baby after feeding.

Nurse breast milk/formula preparation and heating assessment checklist

The checklist includes all items from the SOP for preparing and heating breast milk/formula for preterm infants. Two observers (the researcher and a neonatal nurse) used the checklist to assess whether the nurses preparing the breast milk/formula and feeding the infants adhered to the items in practice. In addition to the SOP items, there are 3 open-ended questions about how and for how long the nurse warmed the breast milk/formula and how they measured the temperature of the milk. The assessment checklist was used only for the control group.

Bedside monitor

A Covidien Nellcor bedside monitor was used to determine the preterm infants' SpO2 and HR values.

Thermometer

A Moms Lap thermometer was used to determine the body temperature values of the preterm infants.

Thermostat-controlled milk warmer

A Sümer SM-ASB bottle warmer was used to warm the milk/formula given to preterm infants to the appropriate temperature and keep it at the set temperature. The device is waterless, with an aluminum heating compartment that can hold up to 25 bottles.

Fluid thermometer

A digital food and liquid thermometer was used to measure the temperature of the prepared breast milk/formula. The liquid thermometer has a long stainless-steel sensor and a wide measurement range (-50 °C to 300 °C). From the device's memory, it can display the last measured value and maximum and minimum measurements.

Thermal milk carrying bag

A Milkdot brand insulated thermal bag was used to transport and maintain the temperature of the prepared breast milk/formula.

Procedures

Before study initiation, the researcher met with the NICU nurses and physicians and explained the purpose of the study. The researcher was in the unit between 8:00 and 16:00 every weekday for 3 weeks before starting the data collection process to observe the breast milk/formula preparation kitchen nurse's use of the kitchen, preparation practices, and storage of expressed milk brought by mothers. In addition, the researcher followed nurses providing level I, II, and III care to observe their OGT insertion, how they checked milk temperature, how they fed the infants, and their post-feeding approach. Through this 3-week observation process, the researcher became a part of the clinical operations, and the nursing staff began to see her as one of the unit staff. This prevented the clinical nurses from behaving differently from normal during data collection for the study, thus increasing the reliability of the results.

To avoid changes in the nurses' practice resulting from the influence of the study, data collection was completed with the control group before the study groups.

Stage 1: Control Group Data Collection:

The researcher met with the breast milk/formula preparation kitchen nurse daily and learned which infants were fed by OGT. The charts of all infants fed with by OGT were examined and those who met the study criteria were included in the study. Infants in the study group were fed 8 times a day at 3-hour intervals. Data from three consecutive feedings done between 08:00 and 16:00 were used in the study.

A total of 50 infants were included in the control group. The researcher observed how the breast milk or formula given to control infants was prepared and to what temperature it was heated, but did not intervene in any way if these practices did not comply with the standard criteria developed for the experimental groups. Two observers (the researcher and a neonatal nurse) simultaneously and independently completed the nurse breast milk/formula preparation and heating assessment checklist. The researcher measured the temperature of the prepared milk using a liquid thermometer before it was taken from the milk preparation kitchen to be given to the infants and recorded this data in the preterm infant observation chart. The preterm infant information form and preterm infant observation chart were completed for each infant in the control group. The infant's vital signs (HR, body temperature, and SpO2) measured by the researcher immediately before feeding and breast milk/formula temperature measured at the start of feeding were recorded in the preterm infant observation chart. The same form was used to record vital signs measured by the researcher at 5 and 30 minutes after the start of feeding.

After completing control group data collection, the assessment checklists completed independently by the two observers were evaluated for interobserver agreement to determine the reliability of the developed SOP for preparing and heating breast milk/formula for preterm infants (Table 1).

Both observers gave exactly the same answers for the Framework standards, indicating 100% agreement. For the Process criteria, agreement was 0.912 (excellent) for the item "If breast milk is to be refrigerated, it is stored on the middle shelf toward the back of a refrigerator at +4 °C", 0.790 (high) for the item "The thermostat-controlled heater is never set above 40 °C as this would disrupt enzyme activity in the milk", and 0.920 (excellent) for the item "The syringes and containers used to hold breast milk/formula are disposable and are not reused." Interobserver agreement was 100% for all other items in this section. For the Outcome standards, both observers gave exactly the same answers (100% agreement). An interobserver reliability value above 0.60 is required to be acceptable (Landis & Koch, 1977). The similar results and agreement values over 0.60 indicate good interobserver agreement and demonstrate reliability of the assessment results.

Stage 2: Data Collection for Experimental Groups 1 and 2:

The milk given to preterm infants in the experimental groups was prepared in accordance with the breast milk/formula preparation and heating SOP as specified in the relevant source, and compliance with these standards was ensured in both groups (Aktaş, 2018).

For each infant in the experimental groups, a random number starting from one was written on their preterm infant information form. Infants assigned odd numbers were included in experimental group 1 (room temperature, 24 °C) and those who received even numbers were included in experimental group 2 (body temperature, 37 °C).

Breast milk/formula for infants in experiment group 1 was left at room temperature for 30-35 minutes, and that for infants in experimental group 2 were placed in a heater set to 37 °C. Before the warmed milk was removed from the kitchen to be given to the infants, its temperature was measured with a liquid thermometer and recorded. To prevent heat loss from the milk for experimental group 2 infants, the milk was placed in a thermal milk carrying bag for transfer to the ward. The prepared breastmilk/formula was not removed from the thermal bag until the time of feeding to prevent cooling.

Just before feeding, the infants' vital signs and the temperature of the breast milk/formula were measured and recorded by the researcher. Before giving the prepared milk, the feeding syringe was wrapped in aluminum foil to preserve the heat (HTMM Aluminium Foil, 2016). The temperature of the milk in the thermal bag was measured using a liquid thermometer and recorded by the researcher before feeding. Milk at the appropriate temperature was poured into the aluminum-wrapped syringe and fed via OGT to the infant by free drainage. The infant's vital signs were measured and recorded by the researcher again at 5 and 30 minutes after the start of feeding.

Data Analysis

Statistical analyses were performed using NCSS (Number Cruncher Statistical System) 2007 software (Kaysville, Utah, USA; License No: 1675948377483;

Table 1. Evaluation of Interobserver Agreement in the Assessment of Nurses' Compliance with Standard Procedures for Milk Preparation and Heating in the Control Group

No.	Standard		Observer 1		Observer 2		Cohen's Kappa Coefficient of Agreement
			Conforms	Does Not Conform	Conforms	Does Not Conform	
			n (%)	n (%)	n (%)	n (%)	
1	*Framework Standards*	Breast milk/formula is prepared 8-12 times a day for preterm infants fed enterally in the NICUs.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
2		There should be a separate kitchen in the unit used exclusively for breast milk/formula preparation.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
3		The breast milk/formula preparation kitchen should be suitable for aseptic conditions.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
4		In units without a breast milk/formula preparation kitchen, there should be a separate area suitable for aseptic conditions.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
5		There should be a guideline in the breast milk/formula preparation kitchen outlining aseptic conditions (e.g., hand hygiene, cleaning of tools and equipment, cleaning of the kitchen).	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
6		There should be a separate sink in the breast milk/formula preparation kitchen used for hand washing.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
7		The kitchen entrance should be designed such that unauthorized persons cannot enter.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
8		There should be ventilation in the breast milk/formula preparation kitchen.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
9		The working surfaces of the breast milk/formula preparation kitchen should be made of a cleanable material.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
10		There should be a closed cabinet containing materials used exclusively for cleaning the breast milk/formula preparation kitchen.	0 (0.0)	50 (100)	0 (0.0)	50 (100)	1.000
11		The lighting fixture(s) in the breast milk/formula preparation kitchen should be easy to clean and provide sufficient light.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
12		Breast milk/formula preparation kitchen should have a sufficient number of electrical outlets.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
13		Breast milk/formula preparation should be performed by a trained neonatal nurse (received a neonatal nursing certificate).	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
14		The neonatal nurse who prepares breast milk/formula should not have any infectious disease.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
15		The neonatal nurse who prepares breast milk/formula should only wear a clean uniform of the type used in their unit.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
16		The neonatal nurse who prepares breast milk/formula should have meticulous personal hygiene.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
1	*Process Standards*	Breast milk can be stored for 3 hours at room temperature (22-26 °C), 3 days in the refrigerator (+4 °C), and 3 months in the freezer (≤ -18 °C).	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
2		If breast milk is to be refrigerated, it is stored on the middle shelf toward the back of a refrigerator at + 4 °C.	6 (12.0)	44 (88.0)	7 (14.0)	43 (86.0)	0.912
3		If breast milk is to be frozen, it is stored in the back of the freezer to prevent warming as a result of opening and closing the freezer door.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
4		Thawed milk can be stored in the refrigerator for up to 24 hours, but is never refrozen.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
5		Frozen milk is thawed in the refrigerator or in warm water.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
6		Each infant's breast milk is stored in a closed container with a waterproof label indicating the name and surname of the infant and the mother and the date and time of milk expression.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
7		Formula is stored in a clean, dry, and cool place.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
8		Formula and milk that is past the expiration date or has spoiled is destroyed.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
9		Formula is prepared as per the instructions written on the box, paying attention to aseptic techniques.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000

Table 1.(Continued) Evaluation of Interobserver Agreement in the Assessment of Nurses' Compliance with Standard Procedures for Milk Preparation and Heating in the Control Group

No.	Standard		Observer 1		Observer 2		Cohen's Kappa Coefficient of Agreement
			Conforms n (%)	Does Not Conform n (%)	Conforms n (%)	Does Not Conform n (%)	
10		Powdered formula is prepared with bottled water that has been boiled and cooled for at least 10 minutes.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
11		If a fortifier is added to breast milk or formula, it is measured according to the instructions written on the box. Attention is paid to aseptic techniques during this procedure.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
12		During the preparation of breast milk/formula, the doors of the kitchen are kept closed with only authorized personnel inside.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
13		Breast milk/formula is never heated in a microwave or on a stove.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
14		The thermostat-controlled heater used to warm breast milk/formula should be calibrated.	0 (0.0)	50 (100)	0 (0.0)	50 (100)	1.000
15		The nurse labels used syringe or containers with the name of the baby and the content of the prepared milk (breast milk or formula).	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
16		As microorganisms thrive at temperatures below 60 °C, the neonatal nurse preparing formula should wear a mask covering the mouth and nose.	42 (84.0)	8 (16.0)	42 (84.0)	8 (16.0)	1.000
17	*Process Standards*	The hands are washed with soap and dried with a clean towel or disposable paper towel.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
18		The physician-prescribed amount of breast milk (if available) or formula for each infant is placed in a container or syringe for heating.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
19		The containers or syringes of breast milk/formula are placed in a thermostat-controlled heater and the heater is operated.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
20		The thermostat-controlled heater is never set above 40 °C as this would disrupt enzyme activity in the milk.	2 (4.0)	48 (96.0)	3 (6.0)	47 (94.0)	0.790
21		The temperature of warmed milk is measured with a liquid thermometer and recorded.	0 (0.0)	50 (100)	0 (0.0)	50 (100)	1.000
22		Warmed breast milk/formula is placed in a thermal bag and transported to the unit to prevent cooling.	0 (0.0)	50 (100)	0 (0.0)	50 (100)	1.000
23		The syringes and containers used to hold breast milk/formula are disposable and are not reused.	24 (48.0)	26 (52.0)	22 (44.0)	28 (56.0)	0.920
24		Before feeding, the nurse checks the label on the syringe/container and amount of milk and the prescription from the infant's attending physician.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
25		Breast milk/formula that is leftover after feeding is never used.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
26		After feeding, the amount of breast milk/formula taken and by what route are recorded on the nurse observation sheet.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
1	*Outcome Standards*	The preterm infant is provided breast milk/formula in a correct and safe manner.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000
2		The vital signs and body functions of the preterm infant remain stable after feeding.	50 (100)	0 (0.0)	50 (100)	0 (0.0)	1.000

Serial No: N7H5-J8E5-D4G2-H5L6-W2R7). For the analysis of quantitative variables, Mann-Whitney U test was used for comparisons of non-normally distributed data between two groups. One-way ANOVA was used for the comparison of normally distributed data between three or more groups, with post-hoc Tukey's HSD test for variables with homogeneous variances and Games-Howell test for those with non-homogeneous variances. Comparison of non-normally distributed data between three or more groups was performed using Pearson's chi-square test. Significance was accepted at $p<0.05$.

Ethical Considerations

Approval from the ethics committee of XXX training and research hospital was obtained prior to the start of the study (11.12.2015/190). Written permission was obtained from the institution where the study was conducted and informed consent was obtained from the parents of the preterm infants included in the study.

RESULTS

Sample characteristics

The study included a total of 150 preterm infants, 50 in each group. The groups showed no statistically significant difference in any demographic characteristic ($p>0.05$; Table 2).

Preterm infants' vital signs according to breast milk/formula temperature

The comparison of body temperatures before and during feeding between the study groups is shown in Table 3. In the first feeding, there was no statistically significant difference in body temperature between the groups at any time point ($p>0.05$). In the second feeding, a significant difference in body temperature was detected between the groups at 30 minutes after the start of feeding ($p=0.040$). The results of pairwise Mann-Whitney U tests indicated that control group infants had significantly higher body temperature 30 minutes after the start of feeding compared to infants in experimental group 2 ($p=0.008$). In the third feeding, there was also a statistically significant difference between the groups in body temperature measured before and at 5 and 30 minutes after the start of feeding ($p=0.003$; $p=0.001$; $p=0.001$). Mann-Whitney U tests indicated that body temperatures in the control group infants were significantly higher than those of the experimental group 2 group infants at all time points ($p=0.001$; $p=0.023$; $p=0.010$).

The comparison of SpO2 values in the study groups before and during feeding is shown in Table 4. There was a statistically significant difference between the groups in SpO2 values measured immediately before the first feeding ($p=0.002$). SpO2 values before feeding were significantly higher in experimental group 1 than in experimental group 2 and the control group ($p=0.001$; $p=0.012$). There was also a significant difference in SpO2 values measured 5 and 30 minutes after the start of the first feeding ($p=0.004$; $p=0.001$). This difference was due to the control group, which showed significantly lower SpO2 values compared to both experimental groups at 5 minutes ($p=0.040$; $p=0.002$) and compared to experimental group 1 at 30 minutes ($p=0.005$).

In the second feeding, SpO2 values did not differ between the groups just before feeding or at 30 minutes after the start of feeding ($p>0.05$) but showed a significant difference at 5 minutes after the start of feeding ($p=0.001$). SpO2 values measured 5 minutes after the start of feeding were significantly higher in experimental group 2 than in experimental group 1 and the control group ($p=0.005$; $p=0.001$).

In the third feeding, SpO2 values measured just before feeding were statistically comparable ($p>0.05$), whereas significant differences were observed between the groups at 5 and 30 minutes after the start of feeding ($p=0.001$; $p=0.014$). Again, this difference arose from the control group, which had significantly lower SpO2 values compared to experimental group 2 at 5 minutes ($p=0.001$) and both experimental groups 1 and 2 at 30 minutes ($p=0.007$; $p=0.021$).

The comparison of HR values before and during feeding between the study groups is shown in Table 5. HR values were statistically comparable before all three feedings ($p>0.05$) but showed significant differences between the groups during feeding. In the first feeding, the significant differences at 5 minutes ($p=0.045$) and 30 minutes ($p=0.005$) were due to higher HR values in the control group compared to experimental group 2 at 5 minutes ($p=0.043$) and compared to experimental groups 1 and 2 at 30 minutes ($p=0.018$; $p=0.011$).

In the second feeding, the differences among groups ($p=0.002$ for both time points) were the result of significantly lower HR values in experimental group 2 compared to experimental group 1 and the control group at 5 minutes ($p=0.015$; $p=0.002$) and higher HR values in the control group compared to both experimental groups at 30 minutes ($p=0.003$; $p=0.021$).

As in the first feeding, the differences in HR among the groups during the third feeding ($p=0.001$) were due to significantly higher HR values in the control group compared to experimental group 2 at 5 minutes ($p=0.001$) and both experimental groups 1 and 2 at 30 minutes ($p=0.001$ for both).

Table 2. Descriptive Characteristics of the Preterm Infants

		Experimental Group 1 (n=50) *Mean±SD (Minimum-Maximum)*	Experimental Group 2 (n=50) *Mean±SD (Minimum-Maximum)*	Control Group (n=50) *Mean±SD (Minimum-Maximum)*	Test statistic; p
Gestational Age		31.94±2.63 (24-35.86)	30.97±3.3 (24.71-36)	31±3.06 (24.14-36)	F:1.667 [a]***0.192***
At time of intervention	**Weight (g)**	1708.96±395.98 (1015-2480)	1707.74±510.26 (1015-2890)	1657.18±460.97 (1060-3100)	F:0.208 [a]***0.812***
	Height (cm)	42.18±3.89 (32-48)	42.16±3.67 (36.5-50)	41.42±3.78 (35-53)	F:0.649 [a]***0.524***
	Head Circumference (cm)	30.03±1.85 (26-34)	29.88±2.39 (25-34)	29.51±2.3 (25-37)	F:0.741 [a]***0.478***
		Median (minimum-maximum)	*Median (minimum-maximum)*	*Median (minimum-maximum)*	
APGAR, 1 min		6 (5-9)	6 (5-8)	6 (5-8)	χ^2=5.614 [b]***0.060***
APGAR, 5 min		8 (6-10)	8 (6-9)	8 (6-9)	χ^2=3.585 [b]***0.167***
		n (%)	**n (%)**	**n (%)**	
Sex	**Female**	20 (40.0)	20 (40.0)	26 (52.0)	χ^2:1.948
	Male	30 (60.0)	30 (60.0)	24 (48.0)	[c]***0.378***
Breast milk	**Yes**	30 (60.0)	28 (56.0)	23 (46.0)	χ^2:2.093
	No	20 (40.0)	22 (44.0)	27 (54.0)	[c]***0.351***
Formula	**Yes**	21 (42.0)	22 (44.0)	27 (54.0)	χ^2:1.661
	No	29 (58.0)	28 (56.0)	23 (46.0)	[c]***0.436***

[a]One-way ANOVA; [b]Kruskal-Wallis test; [c]Pearson's chi-square test; Experimental Group 1: 24 °C; Experimental Group 2: 37 °C

Table 3. Comparison of Body Temperature Before and During Feeding by Group

Body Temperature (°C)		[1]Experimental Group 1 (n=50) *Median (minimum-maximum)*	[2]Experimental Group 2 (n=50) *Median (minimum-maximum)*	[3]Control Group (n=50) *Median (minimum-maximum)*	Test statistic; p	[bb]Post-hoc test
Feeding 1	**Before feeding**	36.8 (36.4-37.8)	36.8 (36.5-37.2)	36.8 (36.3-37.4)	χ^2:4.054 [b]*0.132*	NS
	5 minutes after start of feeding	36.8 (36.4-37.8)	36.8 (36.5-37.2)	36.8 (36.3-37.4)	χ^2:5.291 [b]*0.071*	NS
	30 minutes after start of feeding	36.8 (36.3-37.7)	36.8 (36.5-37.2)	36.8 (36.3-37.4)	χ^2:5.878 [b]*0.053*	NS
Feeding 2	**Before feeding**	36.8 (36.4-37.6)	36.8 (36.4-37.1)	36.8 (36.3-37.4)	χ^2:1.676 [b]*0.432*	NS
	5 minutes after start of feeding	36.8 (36.4-37.6)	36.8 (36.4-37.1)	36.8 (36.4-37.4)	χ^2:3.866 [b]*0.145*	NS
	30 minutes after start of feeding	36.8 (36.4-37.5)	36.8 (36.4-37)	36.9 (36.4-37.5)	χ^2:6.443 [b]***0.040****	1<3; 2<3
Feeding 3	**Before feeding**	36.8 (36.3-37.8)	36.8 (36.4-37)	36.9 (36.4-37.3)	χ^2:11.545 [b]***0.003****	2<3
	5 minutes after start of feeding	36.8 (36.3-37.8)	36.8 (36.4-37)	36.9 (36.4-37.3)	χ^2:16.685 [b]***0.001****	1<3; 2<3
	30 minutes after start of feeding	36.8 (36.3-37.8)	36.8 (36.4-37)	36.9 (36.4-37.2)	χ^2:17.551 [b]***0.001****	1<3; 2<3

[b]Kruskal-Wallis test; [bb]Mann-Whitney U test with Bonferroni correction; NS: not significant; *p<0.05; **p<0.01; 1Experimental Group 1: 24 °C; 2Experimental Group 2: 37 °C

Table 4. Comparison of SpO2 Measurements Before and During Feeding by Group

SpO_2 (%)		[1]Experimental Group 1 (n=50) *Median (minimum-maximum)*	[2]Experimental Group 2 (n=50) *Median (minimum-maximum)*	[3]Control Group (n=50) *Median (minimum-maximum)*	Test statistic; p	[bb]Post-hoc test
Feeding 1	Before feeding	98.5 (95-100)	97.5 (87-100)	98 (90-100)	χ^2:12.012 [b]***0.002****	1>2;1>3
	5 minutes after start of feeding	97.5 (88-100)	98.5 (88-100)	96 (87-100)	χ^2:10.915 [b]***0.004****	1>3; 2>3
	30 minutes after start of feeding	98 (92-100)	98 (90-100)	97 (90-100)	χ^2:8.154 [b]***0.017****	1>3
Feeding 2	Before feeding	98 (92-100)	98 (93-100)	98 (90-100)	χ^2:2.183 [b]*0.336*	NS
	5 minutes after start of feeding	97 (87-100)	99 (92-100)	96 (88-100)	χ^2:14.268 [b]***0.001****	2>1; 2>3
	30 minutes after start of feeding	99 (95-100)	98.5 (93-100)	97 (90-100)	χ^2:5.855 [b]*0.054*	NS
Feeding 3	Before feeding	98 (94-100)	98 (94-100)	98 (90-100)	χ^2:1.100 [b]*0.577*	NS
	5 minutes after start of feeding	97 (82-100)	98.5 (94-100)	96 (89-100)	χ^2:13.299 [b]***0.001****	2>3
	30 minutes after start of feeding	98 (92-100)	99 (93-100)	97 (91-100)	χ^2:8.506 [b]***0.014****	1>3; 2>3

[b]Kruskal-Wallis Test; [bb]Mann-Whitney U test with Bonferroni correction; NS: not significant; *p<0.05; **p<0.01; 1Experimental Group 1: 24 °C; 2Experimental Group 2: 37 °C

Table 5. Comparison of Heart Rate Measurements Before and During Feeding by Group

Heart Rate		[1]*Experimental Group 1 (n=50)* *Mean±SD (Minimum-Maximum)*	[2]Experimental Group 2 (n=50) *Mean±SD (Minimum-Maximum)*	[3]Control Group (n=50) *Mean±SD (Minimum-Maximum)*	Test statistic; p	[aa]Post-hoc test
Feeding 1	Before feeding	144.26±13.62 (120-168)	146.12±12.42 (124-182)	149.44±13.71 (124-180)	F:1.956 [a]*0.145*	NS
	5 minutes after start of feeding	149.36±11.8 (120-180)	145.16±10.67 (124-172)	150.84±12.57 (120-174)	F:3.169 [a]***0.045****	3>2
	30 minutes after start of feeding	145.16±12.2 (120-172)	144.76±10.88 (124-168)	151.7±12.43 (120-178)	F:5.399 [a]***0.005****	3>1; 3>2
Feeding 2	Before feeding	142.76±11.97 (120-172)	142.4±10.83 (124-160)	147.0±12.1 (120-172)	F:2.450 [a]*0.090*	NS
	5 minutes after start of feeding	149.56±13.05 (124-184)	142.8±10.39 (124-164)	151.04±12.46 (124-180)	F:6.678 [a]***0.002****	2<1; 2<3
	30 minutes after start of feeding	142.86±13.11 (120-169)	145.36±9.57 (124-160)	150.84±10.55 (132-172)	F:6.437 [a]***0.002****	3>1; 3>2
Feeding 3	Before feeding	144.54±11.01 (120-166)	145.52±11.17 (124-168)	146.08±12.55 (120-170)	F:0.226 [a]*0.798*	NS
	5 minutes after start of feeding	149.44±10.65 (124-172)	144.92±9.47 (124-164)	153.92±12.62 (122-180)	F:8.380 [a]***0.001****	3>2
	30 minutes after start of feeding	144.36±11.7 (120-172)	144.48±9.63 (120-160)	152.64±12.3 (132-196)	F:8.872 [a]***0.001****	3>1; 3>2

[a]One-way ANOVA; [aa]Tukey HSD & Games-Howell's test; NS: not significant; *p<0.05; **p<0.01; 1Experimental Group 1: 24 °C; 2Experimental Group 2: 37 °C

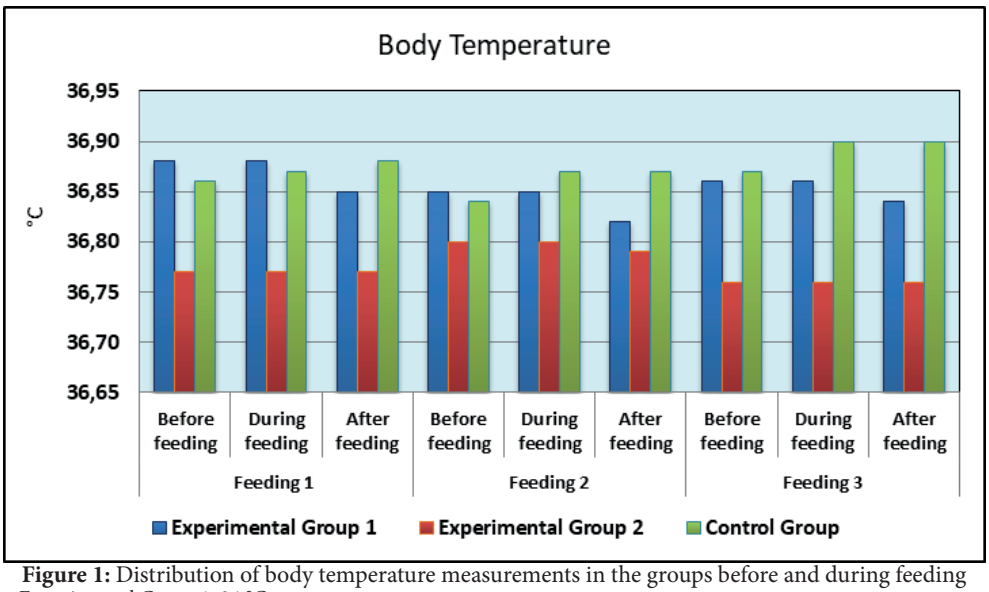

Figure 1: Distribution of body temperature measurements in the groups before and during feeding
Experimental Group 1: 24 °C
Experimental Group 2: 37 °C

DISCUSSION

The preterm infants in our study groups showed no statistical differences in terms of gestational age, sex, current weight, height, and head circumference, 1- and 5-minute Apgar scores, and rates of breastfeeding/formula feeding ($p>0.05$; Table 2). Having comparable descriptive characteristics is important in evaluating the effect of breast milk/formula temperature on the vital signs of preterm infants, whose vital signs are susceptible to influence. Homogeneity between the experimental and control groups allows a clear demonstration of the effect of breast milk/formula temperature on the vital signs of preterm infants.

In the present study, body temperature remained the same at 5 and 30 minutes after the start of feeding in infants fed breast milk/formula at temperatures of 37 °C and 24 °C, while there was a significant increase in body temperature in the control group ($p<0.05$; Table 3). Knobel (2014) stated that the temperature of food given to infants can cause a change in body temperature (Knobel, 2014). Dumm et al. (2013) reported that the temperature of breast milk given to infants varied between 21.8 and 36.2 °C and that the body temperatures of preterm infants increased between 0.1 and 0.4 °C during feeding. In an early study by Holt et al. (1962), a decrease in body temperature was observed in infants fed cold milk. Animal studies have also demonstrated mucosal injury, decreased body temperature and shivering, slowed gastric and intestinal blood flow, and reduced growth in newborn mice fed cold milk (Dvorak et al., 2002; Dvorak et al., 2003; Halpern et al., 2002; Lawlor-Klean et al., 2013; Sangild et al., 2002). In another early study by Eckburg et al. (1987), it was reported that giving preterm infants room-temperature formula caused a decrease of 0.2 °C in rectal temperature and 0.6 °C in skin temperature. In our study, body temperature measurements at 5 and 30 minutes after the start of feeding were increased compared to before feeding in the control group infants (Figure 1). We believe this is because breast milk/formula preparation in the control group did not comply with the appropriate standard protocol and as a result the infants received food at high temperatures. This suggests that in combination with the effect of environmental factors, giving breast milk/formula at high temperatures can lead to an increase in the body temperature of preterm infants. In experimental groups 1 and 2, body temperatures remained constant, indicating physiological stability.

In terms of SpO2 values, there was no difference between the groups before feeding except for a lower value in experimental group 2 before the first feeding. However, SpO2 values at 5 minutes after the start of feeding were higher in experimental group 2 compared to the other groups in all three feedings ($p<0.01$; Table 4). In the study by Dumm et al. (2013), the temperature of milk given to preterm infants was not associated with a change in their SpO2 values (Dumm et al., 2013). However, this study included a limited number of preterm infants (n=33). The present study included 150 preterm infants and our findings suggest that breast milk/formula temperature affected the infants' SpO2 values. To our knowledge, there are no other studies in the literature examining the relationship between milk temperature and SpO2. The minimum and maximum SpO2 values measured 5

minutes after the start of feeding were in the range of 87-100% in the control group and 82-100% in experimental group 1. Both groups had SpO2 values during feeding that could be considered a threat to health (below 90%). This may be related to the temperature of the breast milk/formula given to the infants. These results indicate that breast milk/formula warmed to 37 °C has a favorable effect on preterm infants' SpO2 values.

There was no difference between the groups in terms of HR before feeding, whereas HR values measured at 5 and 30 minutes after the start of feeding were found to be more stable in experimental group 2 compared to experimental group 1 and the control group in all three feedings ($p<0.05$; Table 5). In their study examining the effects of milk temperature on preterm infants, Dumm et al. (2013) observed no significant change in HR measured at 5 and 30 minutes after the start of feeding, unlike our results (Dumm et al., 2013). In the literature, there are no other studies analyzing infants' HR values during feeding based on milk temperature. In Table 5, it is seen that HR values in the control group infants ranged from 120 to 196 beats per minute. The higher HR values were seen at 5 and 30 minutes after the start of feeding. This may be a result of the preterm infants' physiological response to breast milk/formula given at room temperature or unregulated high temperatures. Furthermore, an increase in HR was observed in experimental group 1 and control group infants after receiving breast milk/formula at different temperatures, whereas no change in HR was observed in experimental group 2 infants. The administration of breast milk/formula prepared at 37 °C according to the SOP developed for NICUs seems effective in promoting physiological stability in terms of HR.

LIMITATIONS

Conducting the study with data from a single center is the main limitation, and the results of the study cannot be generalized to the whole population.

CONCLUSIONS

The results of this study show that the temperature of breast milk/formula given to preterm infants affected their vital signs, with a favorable effect observed in preterm infants who received breast milk/formula at body temperature (37 °C) by OGT. Therefore, standardized heating of breast milk/formula to 37 °C and measuring its temperature before feeding to infants in the NICU are important. A larger study including different sample groups should be conducted to further evaluate the effects of breast milk/formula temperature on the vital signs of preterm infants, and neonatal intensive care nurses should be informed about the relevance of milk temperature and advised to heat milk in accordance with standard procedures.

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