

The Effect of Breast Milk Cells on the Clinical Outcomes of Neonates With Birth Weight Equal to or Less Than 1800 Grams: a Randomized Controlled Trial

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Research

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Abstract

Background: Most premature and very low birth weight infants cannot tolerate feedings in the first few days of life and are deprived of breast milk's beneficial effects. This study aims to evaluate the breast milk cells' effects on neonates' clinical outcomes with a birth weight of ≤ 1800 grams.

Methods: This research is a randomized controlled trial conducted on 156 infants in the neonatal intensive care unit of Mahdiah maternity Hospital in Tehran, Iran, from May 2019 to April 2020. All neonates with a birth weight of ≤ 1800 grams were enrolled and randomly divided into intervention and control groups. During the first 6-12 hours of delivery, neonates in the intervention group received the extracted breast milk cells (BMCs) provided by centrifuging their own mothers' breast milk for one time. Demographic data and clinical outcomes were compared between the two groups. We also had a subgroup analysis in neonates with birth weight less than 1500 grams.

Results: A total of 156 neonates entered the final analysis. We divided participants into two groups by using a computer-generated block randomization sequence, including 75 patients in the intervention group and 81 neonates in the control group. The mean birth weight of neonates was 1390.1 ± 314.4 grams, with a total mortality rate of 12.2% (n=19). We found that in-hospital mortality was significantly lower in neonates who received BMCs (6.7% vs. 17.3%; P-value: 0.043) compared to the control group, and it was more prominent in neonates with birth weight less than 1500 grams (9.5% vs. 30.2%; P-value: 0.017). We did not find any other significant differences in major complications such as retinopathy of prematurity, necrotizing enterocolitis, and bronchopulmonary dysplasia between the two groups.

Conclusion: Our research demonstrated a significantly lower mortality rate in neonates (with a birth weight of ≤ 1800 grams) who received breast milk cells on the first day of life. Since this is a novel method with minimal intervention, we are looking forward to developing and evaluating this method in larger studies, with more frequent use of BMCs in very low birth weight infants.

Trial registration: Iranian Registry of Clinical Trials, IRCT20190228042868N1. Registered 25 May 2019, <https://irct.ir/trial/38230>.

Background

Breast milk is the best source of nutrition for all babies, especially premature infants, and it has a significant and sensitive role in improving the function of their immune systems (1). It contains lipids, proteins, carbohydrates, and bioactive molecules, such as vitamins, immunomodulatory factors, and several different types of mediators (2)(3). Breast milk's nutritional content is completely compatible with the neonatal requirements, while the immunological properties of breast milk change during the breastfeeding period. According to recent studies, breast milk components can change in response to neonatal infection (4)(5). Breast milk is effective in preventing diseases related to free oxygen radicals such as necrotizing enterocolitis (NEC), retinopathy of prematurity (ROP), and bronchopulmonary dysplasia (BPD) due to its very strong antioxidant property (6)(7)(8).

In preterm and immature babies, we cannot initiate feeding in the first few days of life due to some limitations like respiratory distress, poor sucking, swallowing, low gastrointestinal motility, and lack of digestive enzymes (9)(10)(11). Despite the strong recommendation for early feeding and minimal enteral nutrition in the first days of life, initiation of effective enteral nutrition of these high-risk neonates postpone several days or weeks afterbirths, and most preterm infants are deprived of this miraculous food. Regardless of feeding tolerance, we can decline the consequences of delayed-onset breastfeeding by minimizing the tolerable amount of breast milk to preterm infants (10).

Breast milk has different phases; the watery portion of breast milk contains many bioactive factors and is mostly absorbed in the oral cavity (12). In this trial, we decided to separate the creamy layer from mothers' breast milk and let the neonates receive cellular and immune components of breast milk earlier and compare them with other babies who receive routine care in the ward.

Methods

Study design and participants

This randomized controlled trial was conducted at Mahdiah maternity Hospital affiliated with Shahid Beheshti University of Medical Sciences in Tehran, Iran, from May 2019 to April 2020. The inclusion criteria were all inborn neonates with a birth weight of ≤ 1800 grams. The exclusion criteria were significant congenital anomalies, severe birth asphyxia (APGAR score < 3 at the first minute), and other lethal diseases (such as severe metabolic diseases, massive intracranial hemorrhage, etc.).

breast milk cells sampling and intervention

We asked all of the mothers to express their breast milk within the first six hours after delivery. Then we used a refrigerated centrifuge to separate the fresh expressed breast milk components at a speed of 600 rounds per minute for 5 minutes at 4 °C temperature (Fig. 1). After centrifugation by "Universal 320 R Hettich Zentrifugen" and formation of two separate layers (Fig. 2), the top layer of the cream removed with the Pasteur pipette and sterile swab, then 1–2 milliliter of the lower watery part and cream-colored strings at the bottom of the tube, was dropped in the oral cavity of the neonates participated in the intervention group during the first 6–12 hours of delivery. We considered hygiene protocols in all steps of milk preparation. Both groups received all neonatal intensive care unit routine care, and breast milk initiation was based on the neonates' general condition. Blinding was not possible due to the nature of the intervention, but the person who gave the BMCs was not part of the treatment staff and acted completely independently.

Data collection and definitions

Data were collected through hospital medical records. Demographics and clinical outcomes were recorded using a pre-prepared checklist. We observed all participants for mortality during the hospitalization, hospital length of stay, sepsis (positive blood culture), NEC, BPD, and ROP.

NEC was defined using Bell's criteria (13). Bronchopulmonary dysplasia (BPD) was defined as per the National Institute of Health consensus definition (14). ROP was defined according to the international classification of retinopathy of prematurity (15).

Sample size and statistical analysis

The sample size was calculated to detect a 20% difference in NEC mortality rate after using breast milk cells, assuming a 35% NEC mortality rate (16). We used the sample size calculation for comparing proportions (17) considering a 5% alpha-type error rate and a statistical power of 80%. After correcting the 15% sample volume loss in each group, a sample size of 80 participants was needed for each group. Neonates were randomly assigned to the case and control group using a computer-generated block randomization sequence of variable block-sized (18), stratified for birth weight of 1800 grams. According to the classified sampling method, we randomly divided patients into two equal groups: a) neonates with birth weight less than 1500 grams and b) neonates with birth weight 1500–1800 grams. During the study, 6 infants in the intervention group were excluded due to lack of cooperation (Fig. 3).

Categorical variables presented as number (percentage) and compared using chi-square and Fisher exact test. Continuous variables reported as mean \pm standard deviation, and we used the Kolmogorov-Smirnov test to evaluate the distribution. Means of continuous variables were compared using an independent group T-test if the data were normally distributed; otherwise, the Mann–Whitney U-test was used. Data were analyzed using SPSS version 23, and P-value < 0.05 is considered statistically significant.

Results

Based on the study protocol, 156 neonates with a birth weight of ≤ 1800 grams entered the final analysis, including 75 neonates in the intervention group who received BMCs and 81 neonates who received routine care as the control group. The birth weight means was 1390.1 ± 314.4 grams. Also, 69(44.2%) infants were female, and 87(55.8%) infants were male. The mode of delivery in 32(20.5%) births was normal vaginal delivery (NVD), and in 123(78.8%) births was a cesarian section (C/S). The overall rate of mortality in our population was 12.2% (19 out of 156).

The baseline characteristics, in-hospital outcomes, and neonatal complications between intervention and control groups are presented in (Table 1). There was a significantly lower mortality rate in the intervention group compared to the control group (5(6.7%) vs. 14(17.3%); P-value: 0.043). There was no significant difference in demographics and baseline characteristics except for higher C/S birth in the intervention group (65(86.7%) vs. 58(71.6%); P-value: 0.026). We observed a trend for lower incidence rate of NEC (4(5.3%) vs. 7(8.8%); P-value: 0.408), less positive blood cultures (4(5.4%) vs. 6(7.6%); P-value: 0.584), and shorter intubation period (3.2 ± 7.1 vs. 4.0 ± 6.6 ; P-value: 0.175) in neonates in the intervention group, but the difference was not significant. The in-hospital complications were similar in both groups.

Table 1

Baseline characteristics and clinical outcomes in neonates who received BMCs and the control group.

Characteristic*		Total (N = 156)	Received BMCs (N = 75)	Control (N = 81)	P†
Demographics					
Gestational age (week)		30.79 ± 2.47	31.00 ± 2.61	30.59 ± 2.34	0.184
Birth weight (grams)		1390.1 ± 314.4	1392.0 ± 325.6	1388.3 ± 305.7	0.847
Sex	Female	69 (44.2%)	34 (45.3%)	35 (43.2%)	0.790
	Male	87 (55.8%)	41 (54.7%)	46 (56.8%)	
Delivery parameters					
Delivery mode	NVD	32 (20.5%)	9 (12.0%)	23 (28.4%)	0.026
	C-Section	123 (78.8%)	65 (86.7%)	58 (71.6%)	
1-min APGAR		8.0 [6.0–8.0]	9.0 [8.0–10.0]	9.0 [8.0–9.0]	0.529
5-min APGAR		9.0 [8.0–9.75]	9.0 [8.0–10.0]	9.0 [8.0–9.0]	0.421
Gravidity		2.0 [1.0–3.0]	2.0 [1.0–3.0]	2.0 [1.0–3.0]	0.913
Parity		1.0 [1.0–2.0]	1.0 [1.0–2.0]	1.0 [1.0–2.0]	0.525
Abortion		0 [0–1.0]	0 [0–1.0]	0 [0–1.0]	0.502
Live birth		2.0 [1.0–2.0]	2.0 [1.0–2.0]	2.0 [1.0–2.0]	0.507
In-hospital outcomes					
Hospital length of stay (day)		28.0 ± 22.9	29.8 ± 23.8	26.3 ± 21.9	0.482
Discharge weight (grams)		1694.6 ± 212.6	1679.1 ± 195.2	1710.8 ± 229.7	0.604
Mortality		19 (12.2%)	5 (6.7%)	14 (17.3%)	0.043
Intubation period (day)		3.6 ± 6.9	3.2 ± 7.1	4.0 ± 6.6	0.175
NIV period (day)		8.1 ± 10.9	9.4 ± 11.9	6.9 ± 9.7	0.210
NEC		11 (7.1%)	4 (5.3%)	7 (8.8%)	0.408

* Data are presented as mean ± standard deviation, number (%), or median [interquartile range].

† Statistically significant P-values are bolded.

BMCs: breast milk cells; BPD: bronchopulmonary dysplasia; C-Section: Cesarean-section; NEC: necrotizing enterocolitis; NIV: non-invasive ventilation; NVD: normal vaginal delivery; ROP: retinopathy of prematurity.

Characteristic*	Total (N = 156)	Received BMCs (N = 75)	Control (N = 81)	P†
BPD	24 (15.4%)	14 (18.7%)	10 (12.3%)	0.274
ROP	41 (26.3%)	21 (28.4%)	20 (24.7%)	0.603
Positive blood culture	10 (6.4%)	4 (5.4%)	6 (7.6%)	0.584
* Data are presented as mean ± standard deviation, number (%), or median [interquartile range].				
† Statistically significant P-values are bolded.				
BMCs: breast milk cells; BPD: bronchopulmonary dysplasia; C-Section: Cesarean-section; NEC: necrotizing enterocolitis; NIV: non-invasive ventilation; NVD: normal vaginal delivery; ROP: retinopathy of prematurity.				

In a subgroup analysis, we separately compared the in-hospital outcomes of both intervention and control groups in two categories: a) neonates with a birth weight of less than 1500 grams and b) neonates with a birth weight of 1500–1800 grams (Table 2). In neonates with a birth weight < 1500 grams, there was a significantly lower mortality rate in the intervention group compared to the control group (4(9.5%) vs. 13(30.2%); P = 0.017). However, in neonates with a birth weight of 1500–1800 grams, the mortality rate was similar between the two groups (P-value: 0.919). In terms of in-hospital complications, there was no statistically significant difference between the two groups. Nevertheless, there was a trend for the lower incidence of NEC, less positive blood cultures, and shorter intubation periods in the intervention group, in both subgroups, which is in line with our prior results.

Table 2

In-hospital outcomes according to the birth weight in neonates who received BMCs and the control group.

In-hospital outcomes*	Total (N = 156)	Birth weight \geq 1500gr		P†	Birth weight < 1500gr		P†
		Received BMCs (N = 33)	Control (N = 38)		Received BMCs (N = 42)	Control (N = 43)	
Hospital length of stay (day)	28.0 \pm 22.9	14.7 \pm 7.1	18.3 \pm 13.6	0.355	41.6 \pm 25.7	33.4 \pm 25.4	0.092
Discharge weight (grams)	1694.6 \pm 212.6	1683.7 \pm 113.6	1683.6 \pm 166.5	0.383	1675.1 \pm 245.4	1744.3 \pm 289.1	0.150
Mortality	19 (12.2%)	1 (3.0%)	1 (2.6%)	0.919	4 (9.5%)	13 (30.2%)	0.017
Intubation period (day)	3.6 \pm 6.9	1.2 \pm 1.5	1.5 \pm 2.8	0.819	4.7 \pm 9.0	6.2 \pm 8.2	0.066
NIV period (day)	8.1 \pm 10.9	3.5 \pm 2.7	4.2 \pm 4.0	0.636	14.1 \pm 14.3	9.3 \pm 12.4	0.059
NEC	11 (7.1%)	1 (3.0%)	2 (5.3%)	0.641	3 (7.1%)	5 (11.9%)	0.457
BPD	24 (15.4%)	0	2 (5.3%)	0.181	14 (33.3%)	8 (18.6%)	0.121
ROP	41 (26.3%)	1 (3.0%)	3 (7.9%)	0.375	20 (48.8%)	17(39.5%)	0.394
Positive blood culture	10 (6.4%)	1 (3.0%)	1 (2.7%)	0.935	3 (7.3%)	5 (11.9%)	0.479
* Data are presented as mean \pm standard deviation, number (%).							
† Statistically significant P-values are bolded.							
BMCs: breast milk cells; BPD: bronchopulmonary dysplasia; NEC: necrotizing enterocolitis; NIV: non-invasive ventilation; ROP: retinopathy of prematurity.							

Discussion

In this randomized controlled trial, we showed a significantly lower mortality rate in neonates with a birth weight of \leq 1800 grams who received BMCs on the first day of life. In subgroup analysis in neonates with a birth weight of < 1500 grams, the mortality rate was more than 3x times lower in the BMCs group (9.5% vs. 30.2%). In addition, there was a trend for a lower incidence rate of NEC, less positive blood cultures, and shorter intubation period in neonates in the intervention group. However, the difference was not significant in any major complications.

Despite the increased survival rate of premature neonates in recent years, some complications are the most leading cause of death in premature neonates (19). As one of the most severe complications in preterm infants, NEC has a mortality rate ranging from 15–30%, followed by an increased risk of low long-term growth and neurodevelopmental impairment in survived infants (20). In recent decades, many discoveries have been made about BMCs. Breast milk contains a myriad of cell types, including leukocytes, epithelial cells, stem cells, and probiotic bacteria (21)(22). In a study by Indumathi et al. (23), after identifying the cell surface markers in human breast milk, myoepithelial progenitors, immune cells, growth factors, and cell adhesion molecules were demonstrated the major constituents of BMCs. Some studies presented mesenchymal stem cells as the most multipotent stem cells in breast milk (24)(25). These cells can potentially differentiate into chondrogenic, osteogenic, adipogenic cells and can differentiate into astrocytes and oligodendrocytes as well as neurons (24)(26) (27).

In this controlled trial study, we centrifuged the neonates' own mother breast milk (Fig. 2), and the lower cream-colored watery part at the bottom of the tube extracted and used for pouring into the mouth by using a syringe. A study by Maffei et al. (28) investigated early oral colostrum administration in preterm neonates; Infants who received the oral colostrum by syringe had significantly higher urinary secreted immunoglobulin A and lactoferrin comparing to using a swab. Many studies have suggested early breastfeeding initiation (< 24 h), which leads to a decrease in the mortality rate in the newborn (28)(29). At the same time, there are few controlled trials about the early progressive feeding in preterm infants.

In our study, early use of extracted BMCs during the first 6–12 hours of delivery reduced the risk of in-hospital mortality in neonates with a birth weight of ≤ 1800 grams, while other major complications including NEC, BPD, and positive blood culture were similar between groups. The difference was more significant after sub-group analysis in neonates with a birth weight of less than 1500 grams (9.5% vs. 30.2%; P-value: 0.017). There was no difference regarding gestational age and sex between these groups. In a study by Modi et al. (9), 131 neonates with a birth weight of 750–1250 grams were evaluated for early enteral feeding. All-cause mortality was lower in infants with early aggressive regimes than routine regimes (33.3% vs. 43.1%; P-value: 0.25), but there was no significant difference in mortality or major morbidities. In another study by Salas et al. (11), they investigated early feeding in 60 preterm infants (< 28 weeks). Early progressive feeding reduced the need for parenteral feeding, while in-hospital outcomes, including mortality or NEC, were similar between groups. These differences may be explained by a) we exclusively used their own mothers' breast milk rather than any formula. b) in this new method, we extracted and used precipitated BMCs and 1–2 millimeters of the watery phase. In comparison, other studies used the whole components of breast milk. c) different inclusion criteria regarding gestational age and birth weight can potentially affect these studies' results.

We would like to emphasize that our study has some limitations. First, the low sample size and the lack of double-blinding may have influenced the study results. Second, the prescription of only a single dose of BMCs in the intervention group is another limitation of the study. The repetitive use of BMCs in the first days of life may change this research results, especially in infants who are not allowed to start enteral feeding due to an underlying disease. Third, since it is a single-center study on the Iranian population,

future multicenter studies on different ethnicities are needed. Nevertheless, according to our knowledge, this is the first randomized clinical trial that prescribes BMCs to neonates with a birth weight of ≤ 1800 grams. We hope this new method is followed by further research with a larger sample size with repetitive use of BMCs in very low birth weight infants.

Conclusions

Our research demonstrated a significantly lower mortality rate in neonates (with a birth weight of ≤ 1800 grams) who received breast milk cells on the first day of life. Since this is a novel method with minimal intervention, we are looking forward to developing and evaluating this method in larger studies, with more frequent use of BMCs in very low birth weight infants.

Abbreviations

BMCs: Breast milk cells, BPD: Bronchopulmonary dysplasia, C/S: Cesarean section, NEC: necrotizing enterocolitis, ROP: Retinopathy of prematurity, NVD: Normal vaginal delivery.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the ethics committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.RETECH.REC.1397.1287) and the Iranian clinical trial ethical committee (IRCT20190228042868N1) in May 25, 2019, and we obtained informed consent from all the parents.

Consent for publication

Not applicable.

Availability of data and materials

Data are available upon a reasonable request to the corresponding author.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable.

Authors' contributions

Concept and design: MF, SMS, MK. Acquisition, analysis, or interpretation of data: SMS, NTT, MKS, SN, FP. Statistical analysis: SK, MH, MK. Drafting of the manuscript: MF, SMS, SK, MK. Critical revision of the manuscript: MF, NTT, SN. Supervision: MK. All authors have read and approved the manuscript and are responsible for its content.

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Figures



Figure 1

Refrigerated centrifuge" Universal 320 R Hettich Zentrifugen"

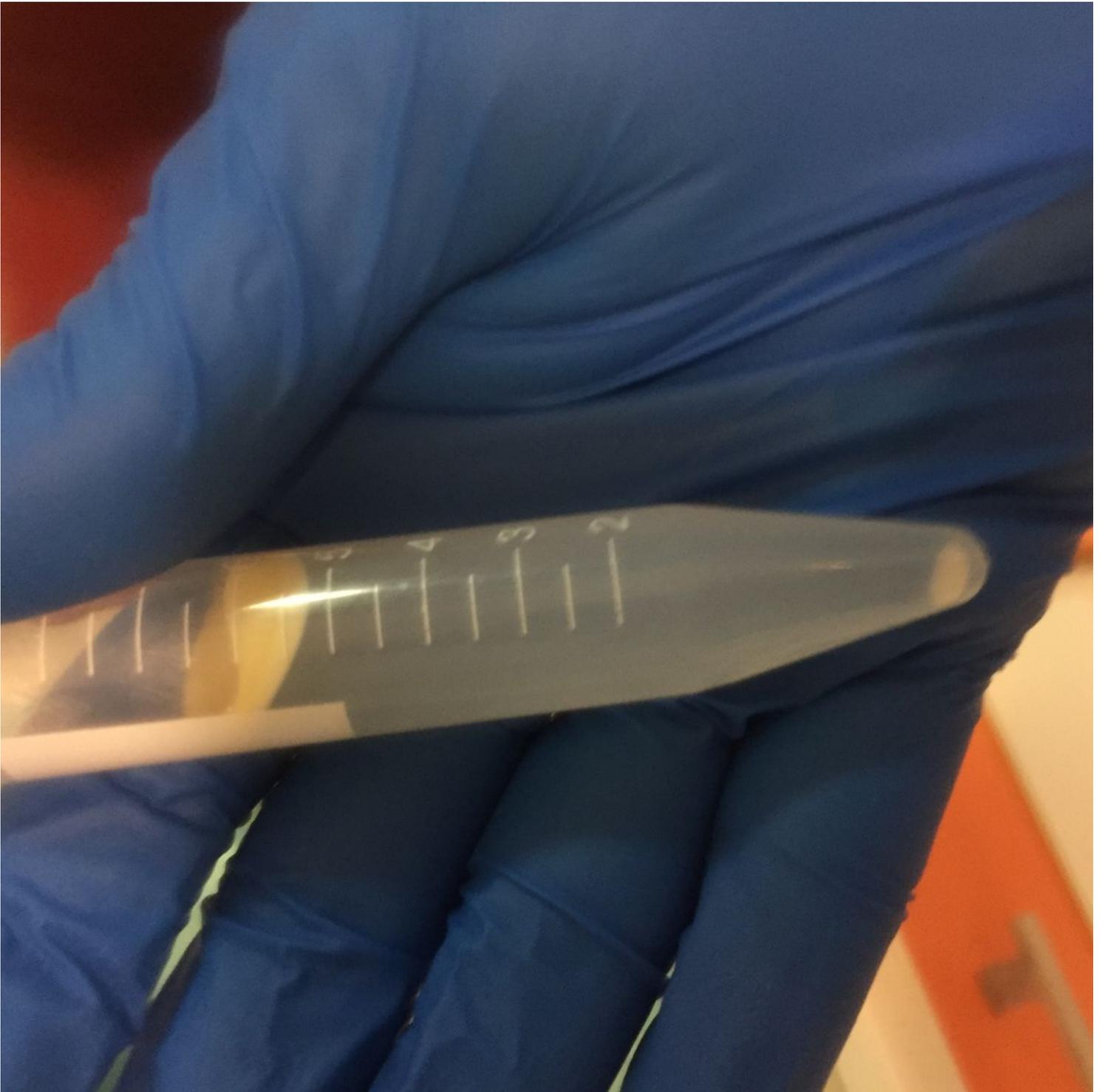


Figure 2

Centrifuged breast milk sample. A creamy layer on the top followed by a watery phase with cream-colored strings at the bottom of the sample that contains most of the cells.

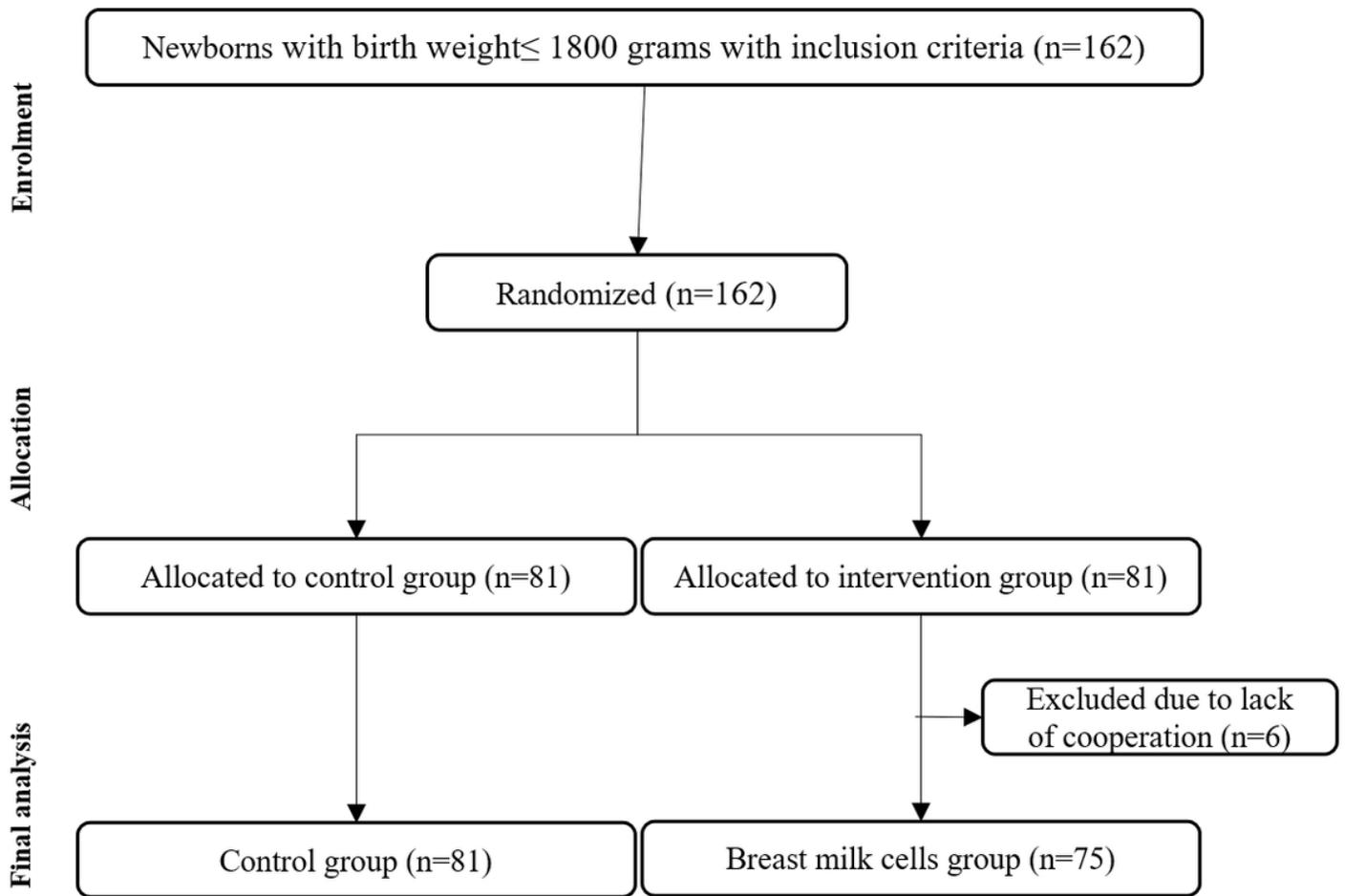


Figure 3

Participants flow chart