Impact of Colostrum Administration in the Oropharyngeal Mucosa of Very Low Birth Weight Infants on the Rate of Late-Onset Sepsis: A Randomized Blinded Trial

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Abstract. *Objectives:* To evaluate the effects of oropharyngeal administration of colostrum in very low birth weight infants on the rate of late-onset sepsis compared to placebo.

Methods: We conducted a double-blind, randomized (1:1), placebo-controlled trial involving 52 preterm infants born before 30 weeks gestation and with a birth weight < 1500 g. Subjects received 0.2 ml of their mother's colostrum or sterile water via oropharyngeal route every 3 hours for 3 days beginning at 48 to 96 hours of life. After therapy, the newborns were followed until hospital discharge or death.

Results: The sample consisted of 26 preterm infants born in the colostrum group and 26 preterm in the control group. Gestational age and birth weight (median and interquartile range (IQR)) were respectively [28.5 (8) vs 31 (11)] and [1.095 g (945) vs 1232.5 g (1.120)]. The results did not associate the administration of colostrum with the reduction of late sepsis [n = 13 (50%) vs n = 14 (53.8%)], as far as sepsis was confirmed by blood culture, the occurrence was higher. In the case group [n = 10 (38.5% vs 4 (15.4%)], there was also a higher mortality trend for the group receiving colostrum [n = 8 (30.8%) vs n = 3 (11.5%)].

Conclusions: This study suggests that oropharyngeal administration of colostrum does not decrease the rate of late-onset sepsis. These results may be associated with sample size, suggesting further studies.

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Keywords: oropharyngeal; colostrum; very low birth weight; sepsis; preterm

Background

Each year, approximately 15 million preterm infants are delivered all over the world (Liu et al., 2015), and significant increases in the survival of these preterm infants are associated with improvements in prenatal, obstetric and neonatal care. However, the survival of preterm infants is affected by high rates of morbimortality mainly due to neonatal sepsis, necrotizing

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enterocolitis (NEC), intraventricular haemorrhage (IVH), retinopathy of prematurity, hearing impairment and, primarily, sequelae in neurological, cognitive and motor development throughout life (Johnson et al., 2013; Stoll et al., 2002; Makhoul et al., 2005; Stoll et al., 2004; Cacho & Lawrence, 2017; Rodriguez et al., 2010; Rodriguez et al., 2009).

Neonatal sepsis, defined as an organic dysfunction caused by a systemic inflammatory response stemming from one or more sites of tissue injury by an infectious agent, is a cause of substantial morbidity and mortality (Shane, Sánchez, & Stoll, 2017; Jabandziev et al., 2014; Cuenca et al., 2013; Silva et al., 2015). Early-onset neonatal sepsis, presumably of maternal origin, can be seen up 72 hours after birth, while late-onset sepsis (L-OS), originating from the hospital, arises after the third day of life (Wynn et al., 2014). The incidence L-OS is inversely related to the degree of maturity of the infants and varies geographically from 25% to 75% among hospitalised newborns (NBs) (Gowda et al., 2017; Cailes et al. 2018; Romanelli et al., 2016; Cortese et al., 2016; Boghossian et al., 2013; Hammoud et al., 2017). Epidemiology data on very low birth weight (VLBW) infants shows that the predominant pathogens underlying L-OS are coagulase-negative staphylococci, followed by Gram-negative bacilli and fungi (Dong & Speer, 2015; Oeser et al., 2014; Shah & Padbury, 2014).

Maternal colostrum carries a diverse array of immunologic factors (Cacho & Lawrence, 2017; Lee et al., 2016; Pletsch et al., 2013; Obermajer et al., 2015) inversely proportional to the gestational age (GA), which is related to the maturity of the mammary gland (Rodriguez et al., 2010; Underwood & Sohn, 2017; Queiroz, Assis, & R Júnior, 2013). When administered in the oropharyngeal mucosa, colostrum stimulates an immunological cascade by interacting with lymphoid tissues (Jakaitis & Denning, 2014; Gephart & Weller, 2014; Snyder et al., 2017), which facilitate direct and rapid contact between external pathogens and immune cells, such as T lymphocytes and B monocytes (Rodriguez et al., 2010; Rodriguez et al., 2009; Rodriguez et al., 2015; Siggers & Hackam, 2011). Studies suggest that the early administration of colostrum can optimize the oral and intestinal microbiota of preterm infants, promoting a decrease in the risk of NEC, sepsis and death, as well as contributing to progression towards enteral diet and weight gain (Cacho & Lawrence, 2017; Rodriguez et al., 2010; Rodriguez et al., 2009; Lee et al., 2016; Snyder et al., 2017; Rodriguez et al., 2015; Rogier et al., 2014; Romano-Keeler et al., 2017; Toscano et al., 2017).

In summary, the oropharyngeal administration of maternal colostrum appears to be beneficial for preterm infants (Lee et al., 2016). Nevertheless, although emerging evidence is promising, small sample sizes and wide variations in the technique limit generalizability of this observation. Based on the evidence the objective this study to evaluate the effects of oropharyngeal administration of colostrum in very low birth weight infants on the rate of late-onset sepsis compared to placebo.

Objectives

In the present study, we tested the hypothesis that maternal colostrum when administered to the oropharyngeal mucosa of preterm infants reduce incidence of late-sepsis.

Methods

Study Design

A randomized, double-blind, placebo-controlled (1:1). The local institutional review board approved the protocol number 2012160. The entirety of this study was conducted in accordance with good clinical practice guidelines.

Participants

Neonates born before 30 weeks' gestation were enrolled. Newborns who were not followed up by their mothers during hospitalization, those with complex malformations, children of an HIV-positive mother, children of mothers who used illicit and twin drugs were excluded. After the researchers obtain the informed consent (IC) form.

Study Settings

The study took place at in the NICU of the Hospital Geral de Caxias do Sul (GHCS), Brazil, the intervention was conducted from May 2018 to February 2019.

Interventions

All participating mothers were encouraged to perform breast milk's hand expression in order to extract colostrum every 2 or 3 hours for a total of five to eight times during the first 24 hours. If any difficulty was brought up regarding with expression or with breast dry up, by the women who have just given birth, they were instructed to use an electrical pump available at the institution. The milk collections were taken place in the nursery collection room, where the mothers received information for the use of the disposable mask and hat and also hygiene of hand and breast, to ensure an aseptic procedure.

Thereafter, the mother was provided with a sterile vial for the extraction and storage of colostrum. Then, the vial was given to the professional in charge of the nursery, and it contents were divided into sterile syringes with a total capacity of 1 ml. In each syringe, only 0.2 ml of colostrum was aspirated to avoid wastage of material and interruption of therapy. The syringes received an adhesive tag featuring the patient's identification details, date and time to get milk. The syringes were immediately stored in an adequately refrigerated place according to the standard operating procedure of the sector. This process occurred whenever the mother expressed desire and the need to perform extraction of colostrum.

Therapy Allocation

Group 1 – Intervention group: NBs were received 0.2 ml of maternal colostrum applied with a syringe directly to the oropharyngeal mucosa, with three-hour intervals between each administration during a period of 72 consecutive hours; and

Group 2 – Control group: NBs were received 0.2 ml of sterile distilled water (placebo) with applied with a syringe directly to the oropharyngeal mucosa, with three-hour intervals between each administration during a period of 72 consecutive hours.

Oropharyngeal Administration Procedure

Approximately 10 minutes before the established time for colostrum administration in the oropharyngeal mucosa of NBs staying in the NICU, a syringe containing 0.2 ml of colostrum were obtained, protected by adhesive paper containing only data from the participant, so as not to enable the content of the syringe to be identified by the NICU team.

Application of Therapy

The syringe's cap was removed, and the syringe were gently placed in the infant's mouth, alongside the right and left buccal mucosal tissue. The syringe tip was directed posteriorly towards the oropharyngeal and the total volume (0.1 ml) and slowly administered. The stability time of the therapy contained in the syringe at room temperature is 20 minutes. This process was repeated every 3 hours for 72 consecutive hours.

Therapy Interval

It is important to emphasize that because this is a protocol where it is not possible to evaluate the interval between applications for the most effective therapy, we decided to maintain an interval of three hours for each application because the NICU has a minimal management protocol for extreme premature NBs based on the "Newborn Individualized Developmental Care and Assessment Program", where touch time occurs every 6 hours in the first week of life, consistent with the interval in a recently published study (Lee et al., 2016).

Monitoring and Assessments

During the administration of intervention agent (maternal colostrum) and placebo (sterilized distilled water), the infant's vitals signs was constantly monitored to observe any adverse events, such as alterations in oxygen saturation (SaO2), heart rate and respiratory rate. The data for the survey was collected on a prospective and consecutive basis.

Participants from both groups, following evaluation made by the medical team to determine the adequacy of clinical conditions, were received a trophic diet (mother's milk and/or infant formula for premature NBs through a gastric tube), according to the protocol already in place at the NICU/GHCS.

Throughout the study, vital signs of the infants were continuously monitored with the intention of identifying any adverse events, such as alterations in SaO2, heart rate and respiratory.

In the event of complications, such as tachycardia (>200 beats per minute), bradycardia (<100 beats per minute), tachypnoea (>60 breaths per minute) and/or a drop in SaO2 equal to or lower than 85%, therapy would be immediately interrupted, and a neonatologist notified to carry out an evaluation of the patient. Immediately, the authors of the study would be informed to review the procedure and determine whether therapy would continue. Data for the survey was collected on a prospective and consecutive basis, and the participants was divided into two groups.

For the purposes of this survey, clinical sepsis was defined as the presence of clinical signs of infection accompanied by concurrent antibiotic treatment occurring after 72 hours of life. For diagnosis, clinical sepsis will be defined as the presence of clinical signs of infection. (36) Participant should present at least one clinical sign from each one of the following three categories (Lee et al., 2016; Shane & Stoll, 2014):

a) General: fever, apnoea, tachypnoea, and respiratory distress;

b) Laboratory: leukopenia, leucocytosis, and increase in C-reactive protein; and

c) Haemodynamic: hypotension, tachycardia, and altered tissue perfusion.

Proven sepsis will be defined as bacterial growth in at least one blood culture and fulfilment of criteria for clinical sepsis (Satar & Özlü, 2012).

The TPN time (amount of time that total parenteral nutrition was used, in days); IVH incidence (using transfontanelle ultrasonography for the classification); length of time taken to return to weight at birth (in days), incidence NEC (diagnosis will be made according to the modified Bell's staging criteria by a neonatologist, based on clinical and radiographic signs, infants with stage II will be defined as the NEC (Mller, Paul, & Seeliger, 2016), and death.

Sample Size/Power Analysis

Literature data show that L-OS rate is 50% among PTNBs who received colostrum therapy and 92% among the others (Lee et al., 2016). Based on these data, the chi-squared test was used to calculate an expected difference of 0.42 in the incidence of sepsis between the two groups, considering an alternative bilateral, a level of statistical significance of 0.05 and a statistical power of 0.90. The number of patients needed to detect this magnitude in the sample will be 22 patients in each group. For calculating the sample size, software from the Laboratory

of Epidemiology and Biostatistics (www.lee.dante.br) was used. As such, allowing for 20% losses, a sample size of 27 patients was decided upon for the intervention group and a sample size of 27 patients was decided for the control group.

Randomisation

NBs were randomly allocated in a 1:1 ratio to the colostrum group or the placebo group via a randomization scheme developed by the website Randomization.com (http://randomization.com). Randomization was conducted using a computer-generated allocation sequence.

Blinding

Allocation was concealed from all investigators, nurses, doctors, and parents, with the exception of 5 unblinded independent research staff member, who prepared the colostrum and placebo syringes. The beginning of the enteral diet trophic each patient was decided by the team neonatology, if no contraindication was found. Both groups of neonates were fed breast milk or preterm formula.

Statistical Analysis

The obtained information was stored in a database created with Epi-data 3.1. Statistical analysis was performed using R Studio software, version 3.5.0. For testing the normality of variables, the Kolmogorov-Smirnov test was employed. For comparing the groups with normal distribution and independent samples, Student's t tests were used, and the Mann-Whitney U test will be used for non-parametric data. Categorical date will be compared with the chi-squared test or by Fisher's exact test. All values with $p \leq 0.20$ in bivariate analyses was included in a multivariate model with logistic regression to control for confounding factors. In the multivariate model, all values with $p \leq 0.05$ was considered statistically significant.

Missing Data

Regarding missing data, varying strategies were used to address the absence and unattainability of data and potentially erroneous data (depending on the level of quantification required for each variable). These strategies include considering patterns driven by demographic and behavioural factors to govern decisions about whether the missing values can be substituted with those generated through multiple allocations using the SAS PROC MI (SAS Inc., Cary, NC, USA) procedure if the absent data are MCAR (a complete set of data is missing randomly) or MAR (random data are missing). If the absent data are NMAR (not missing randomly), the "mixed patterns" approach was used to calculate the "weighted average" of the parameters associated with the missing data to estimate what the missing values.

Results

Were considered eligible for the study 61 preterm VLBW infants. Of these, 9 were excluded by the exclusion criteria, being 1 (congenital malformations) and 3 pairs of twins and 2 (did not receive therapy). A total of 52 participants were randomized, 26 for the Colostrum Group and 26 for the Placebo Group, as shown in Figure 1.

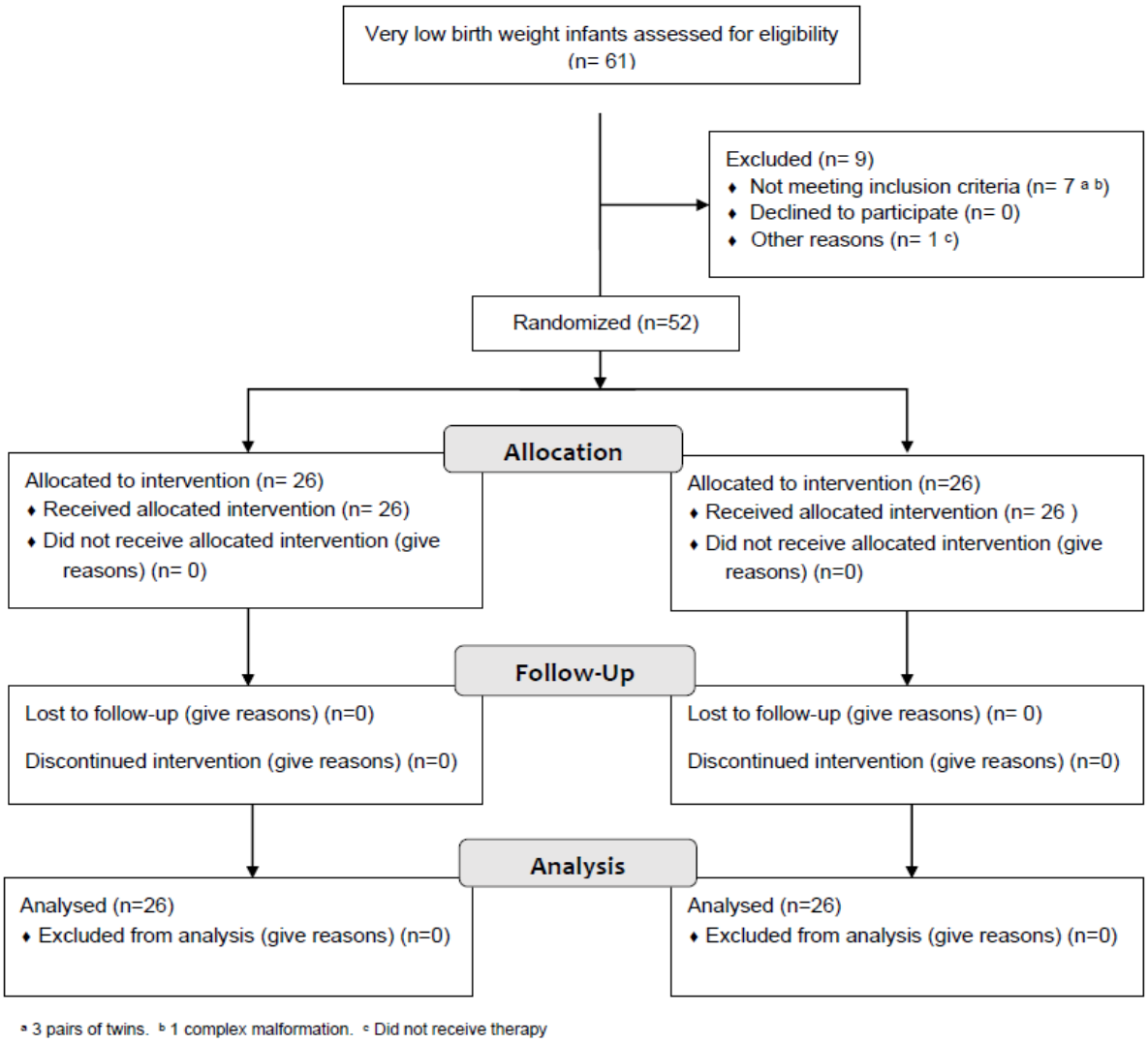

Figure 1. CONSORT Flow Diagram

The oropharyngeal administration was well tolerated by all preterm infants, there was no adverse effects during the treatment protocol, corroborating a previous study of Rodriguez et al. (2010) that evaluated safety in administration.

Table 1 shows the demographic characteristics of preterm infants included, according to the groups studied. No statistically significant differences were observed between them. It stands out among the data presented in this table, the weight.

Table 1. Distribution of characteristics of mothers and preterm infants from May/2017 to February/2019, at the NICU/GHCS

Variables	Colostrum Group (26)	Placebo Group (26)
Maternal characteristics		
Age (years, IQR) [2]	23.5 (24)	25.5 (27)
Drugs used during pregnancy, n (%)		
Corticosteroid complete dose [1]	16 (61.5)	17 (65.3)
Magnesium sulfate [1]	8 (30.7)	11 (42.3)
Neonate characteristics [1]		
Male gender, n (%)	13 (50)	12 (46.1)

Female gender, n (%)	13 (50)	14 (53.9)
Gestational age (median week, IQR) [1]	28.5 (8)	31 (11)
< 28 weeks	13 (50)	8 (30)
> 29 weeks	13 (50)	18 (70)
Birth weight (g, IQR) [2]	1.095 (945)	1.232.5 (1.120)
< 1.000g [1]	12 (46)	6 (23)
> 1.001g [1]	14 (54)	20 (77)

Note: The values are presented as median (IQR) or number (%).
[1] Mann-Whitney U test will be used for non-parametric; [2] Student's t test.

A large number of deaths were identified in Colostrum Group. Table 2 shows the evaluation of the clinical evolution of premature infants. There was no statistically significant difference in cases of late sepsis and NEC. Figure 2 shows the survival of both groups.

Table 2. Distribution of clinical outcomes of preterm infants from May/2017 to February/2019, at the NICU/GHCS

	Colostrum Group (n= 26)	**Placebo Group (n=26)**	***p* value**
Treatment (days, IQR) [1]			
Total parenteral nutrition	8.5 (16)	8.5 (31)	
Full diet	13.5 (19)	13 (29)	
Oxygen	10 (120)	9 (136)	
Non-invasive mechanical ventilation	2 (30)	2 (19)	
Invasive mechanical ventilation	0 (22)	0 (33)	
Diagnosis, n (%) [1]			
Positive blood culture	10 (38.5)	4 (15.4)	
Late-onset sepsis	13 (50)	14 (53.8)	
Necrotizing enterocolitis	6 (23)	5 (19.2)	
Intraventricular hemorrhage	10 (38.4)	7 (26.9)	
Length of stay (days, IQR) [2]	37 (171)	41 (134)	
Weight recovery time (days, IQR) [2]	9 (13)	8 (11)	
Weight at hospital discharge (g, IQR) [2]	2012.5 (4.950)	2.060 (4.640)	
Death, n (%) [1]	8 (30.8)	3 (11.5)	0.08
The values are presented as median (IQR) or number (%). **p*< 0.05 versus placebo group.			

Note: [1] Mann-Whitney U test will be used for non-parametric; [2] Student's t test.

The clinical outcomes are presented in Table 2, where there was no statistical difference in late sepsis among the groups studied. Regarding the number of positive blood culture, the highest occurrence was in the colostrum group. For ECN, there was no difference between the groups. As for mortality, a greater number occurred in the colostrum group, Figure 2 shows the survival of both groups.

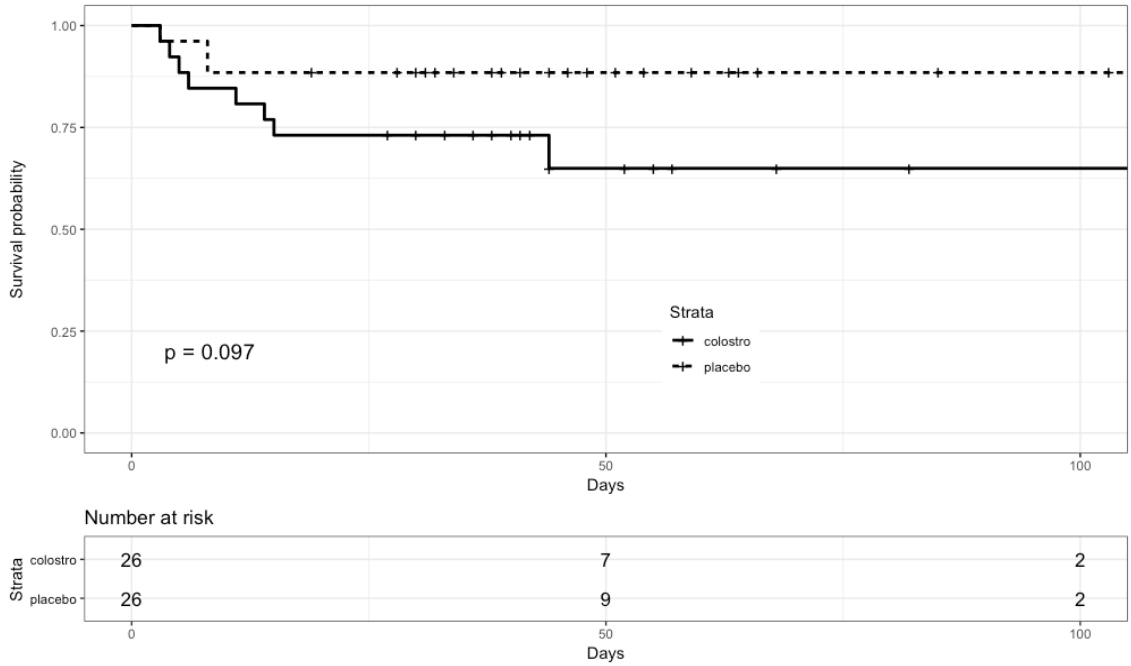

Figure 2. Kaplan-Meier curve showing the survival probability, expressed as a percentage, following therapy administration for very low birth weight preterm, stratified by Group Colostrum and Group Placebo

The study had evaluated 52 VLBW infants and found: 1) that is no association with decrease L-OS when they used colostrum; 2) tendency of mortality and positive blood culture in the intervention group; and 3) tend most altered laboratory tests confirming late sepsis in the intervention group.

In the intervention group, it was verified that there is no association with the reduction of L-OS when they used colostrum in compared to the control group (41.1% vs. 51.9%), similar to Romano-Keeler et al. (2017) (2% vs 6%) but contrary to what was suggested by Lee et al. (2016) (50% vs 92%).

When evaluating mortality, it can be seen that there is a tendency to increase in the intervention group compared to the control group (48.1%; p=0.06). A meta-analysis (Nasuf, Ojha, & Dorling, 2018) showed results that reinforce this outcome, where mortality not decreased when associated with colostrum use. Currently evidence shows an improvement in survival of extreme preterm infants (Ancel et al., 2015; Kusuda et al., 2012; Shah et al., 2012; Ishii et al., 2013; Su et al., 2015), however, we know that mortality is associated with gestational age of the newborn (Liu et al., 2015; Blencowe et al., 2012).

On a randomized, double-blind, placebo-controlled study with only preterm infants conducted by Lee et al. (2016) evaluated 42 preterm infants in which, after application of the protocol, demonstrated a significant reduction in the incidence of clinical sepsis. Regarding in blood culture proven sepsis did not obtain significant reduction (46% vs 58%; P = 0.56). In this clinical trial, the positive blood culture was tendentiously higher in the intervention group than in the control group (38% vs 18%; p = 0.09).

Although we found no differences in culture-proven sepsis or NEC some studies (Sadeghirad et al., 2018) have shown a positive effect trend. This effect, if present, may be related to the immunological effects of colostrum. Non-association may be related to the relatively low number of subjects studied. Additional clinical trials will increase the number of patients and may change the results for this results in laboratory tests confirming late sepsis in the intervention.

One of the strengths of this study is that the worldwide recommendations related to the application of colostrum therapy to oral mucosa of preterm newborns have been followed. However, this study has some limitations: (1) performed in a single center; (2) the sample of patients followed up was small (n = 52).

In conclusion, this study did not show an association between the reduction of the proven late sepsis rate between the groups, including a higher mortality and positive blood culture trend in the colostrum group. This result may be associated with sample size, this being one a limitation of the study. Further research should be conducted with larger groups.

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Conflict of Interest

The authors have no potential conflicts of interest to disclose.

Clinical Trial Registration

Registro de Ensaios Clínicos - Rebec: UTN: U1111-1203-3393 - http://www.ensaiosclinicos.gov.br/.

Abbreviations:

NEC: Necrotizing enterocolitis
GHCS: General Hospital of Caxias do Sul
IVH: Intraventricular haemorrhage
REBEC: Brazilian Registry of Clinical Trials
NBs: Newborns
VLBW: Very low birth weight
IC: Informed Consent
NICU: Neonatal intensive care unit
L-OS: Late-onset sepsis

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