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Randomized Control Trials

Bovine colostrum to supplement the first feeding of very preterm infants: The PreColos randomized controlled trial

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SUMMARY

Background & aims: Gut immaturity leads to feeding difficulties in very preterm infants (<32 weeks gestation at birth). Maternal milk (MM) is the optimal diet but often absent or insufficient. We hypothesized that bovine colostrum (BC), rich in protein and bioactive components, improves enteral feeding progression, relative to preterm formula (PF), when supplemented to MM. Aim of the study is to determine whether BC supplementation to MM during the first 14 days of life shortens the time to full enteral feeding (120 mL/kg/d, TFF120).

Methods: This was a multicenter, randomized, controlled trial at seven hospitals in South China without access to human donor milk and with slow feeding progression. Infants were randomly assigned to receive BC or PF when MM was insufficient. Volume of BC was restricted by recommended protein intake (4–4.5 g/kg/d). Primary outcome was TFF120. Feeding intolerance, growth, morbidities and blood parameters were recorded to assess safety.

Results: A total of 350 infants were recruited. BC supplementation had no effect on TFF120 in intention-to-treat analysis [n (BC) = 171, n (PF) = 179; adjusted hazard ratio, aHR: 0.82 (95% CI: 0.64, 1.06); P = 0.13]. Body growth and morbidities did not differ, but more cases of periventricular leukomalacia

Abbreviations: list: BC, Bovine colostrum; BPD, bronchopulmonary dysplasia; BUN, blood urea nitrogen; CRP, C-reactive protein; DSMB, data safety monitoring board; DM, human donor milk; EN, enteral nutrition; EOS, early-onset sepsis; GA, gestational age; HR, hazard ratio; IQR, interquartile range; ITT, intent-to-treat; IVH, intraventricular hemorrhage; LOS, late onset sepsis; MM, maternal milk; MRI, magnetic resonance imaging; NEC, necrotizing enterocolitis; NICU, neonatal intensive care unit; OR, odds ratio; PDA, patent ductus arteriosus; PF, preterm formula; PMA, post menstrual age; PN, parenteral nutrition; PP, per-protocol; PVL, periventricular leukomalacia; RCT, randomized controlled trial; RDS, respiratory distress syndrome; ROP, retinopathy of prematurity; TGF-β, transforming growth factor-β; SD, standard deviation; TFF, time to full enteral feeding; WBC, white blood cells.

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were detected in the infants fed BC (5/155 vs. 0/181, P = 0.06). Blood chemistry and hematology data were similar between the intervention groups.
Conclusions: BC supplementation during the first two weeks of life did not reduce TFF120 and had only marginal effects on clinical variables. Clinical effects of BC supplementation on very preterm infants in the first weeks of life may depend on feeding regimen and remaining milk diet. Trial Registration: http://www.clinicaltrials.gov: NCT03085277.

1. Introduction

Preterm infants, especially those born very preterm (gestational age, GA, <32 weeks) and/or with very low birth weight (VLBW, <1500 g), are often difficult to feed because of the immaturity of the gastrointestinal tract with regards to motility, digestion and immunity. Maternal milk (MM) is considered the optimal nutrition for very preterm infants (VPIs) and reduces feeding intolerance, relative to human donor milk or preterm formula (PF) [1]. However, MM is often absent or insufficient in the first weeks after preterm birth, and access to human donor milk may be limited. Therefore, PF remains the first enteral feed for VPIs in many countries, despite increased risk of feeding intolerance and necrotizing enterocolitis (NEC), prolonged time to full enteral feeding (TFF) and therefore hospitalization length [2–4]. It is important to find an alternative to PF that induces gut maturation and improves feeding intolerance, when used as the first feed for VPIs with limited or no access to human milk.

Bovine colostrum (BC) contains high levels of protein, growth factors and bioactive components [5,6] that may improve gut maturation across species in early life [7]. In preterm pigs, used as a model for preterm infants, exclusive or partial BC feeding protects against NEC and improves gut function after birth [8–12]. Benefits of BC were associated with improved gut growth, barrier function, food passage rate, digestive enzyme activities, mucosal immunity and protection from gut epithelial pathogen attachment [8,11,13–15]. Recently, safety and feasibility of feeding BC to preterm infants were tested in pilot human studies in Denmark and China [16,17]. BC supplementation tended to reduce TFF, compared with exclusive PF feeding, in a setting of slow feeding advancement and limited access to MM [16]. A small randomized controlled trial (RCT) on moderately preterm infants (mean GA 32 weeks, n = 80) showed that BC supplementation for two weeks reduced incidences of feeding intolerance, NEC, sepsis and mortality [18]. Another study (n = 86, mean GA 30 weeks) showed a trend to negative effects when high amounts of a concentrated BC product was added to MM and PF [19]. There is a need to determine the safety and efficacy of BC as a milk supplement in the first weeks of life after preterm birth, using a larger sample size with detailed nutrition and clinical recordings.

We hypothesized that BC supplementation to MM for VPIs within the first two weeks of life is safe and reduces TFF in clinical settings of slow feeding advancement and variable access to MM [20,21]. Based on a pilot study at one hospital [16], TFF at 120 mL/kg/d was chosen as the primary outcome. Secondary outcomes, indirectly or directly related to TFF, were included to further explore effects of BC supplementation and assess clinical safety. Considering the novelty and complexity of the intact BC and its possible effects within and beyond the gut, a range of in-hospital morbidities, growth and blood parameters of clinical well-being were recorded and assessed on days 7 and 14.

2. Materials & methods

2.1. Study design and participants

This PreColos study was a parallel two-armed, randomized, controlled trial, conducted at seven neonatal intensive care units (NICUs) in South China (Shenzhen People's Hospital, Shenzhen Luohu Maternal Child Health Hospital, Shenzhen Nanshan Maternity and Child Healthcare Hospital, Longgang District Central Hospital of Shenzhen, The Sixth Affiliated Hospital of Sun Yat-sen University in Guangzhou, Foshan Maternal and Child Health Hospital, Dongguan Women and Children's Hospital). Shenzhen Guangming People's Hospital was a part of the study design, but no participants were enrolled at this site. Participating hospital is referred to by an arbitrarily assigned letter (A to G) in the following text, tables and figures. Infants with GA 26 + 0 to 31 + 6 weeks were enrolled within 48 h of birth at a study site, if eligible and written informed consent were obtained from parents. Exclusion criteria were major congenital anomalies or birth defects, congenital infection defined as suspected TORCH (toxoplasmosis, rubella, cytomegalovirus, herpes, hepatitis, coxsackie, syphilis, varicella zoster, human immunodeficiency virus, parvo B19), perinatal asphyxia (umbilical or first neonatal blood pH < 7.0), extremely small for gestational age (birth weight z-score < −3), no realistic hope of immediate survival, or receiving PF prior to randomization. Provision of MM was allowed before randomization. Outborn infants admitted to the participating NICUs within 24 h of birth and fulfilling the inclusion criteria were also eligible for recruitment. The trial was approved by the ethics committees of all participating hospitals, registered at ClinicalTrials.gov (NCT03085277) and monitored by an international data safety monitoring board (DSMB).

2.2. Randomization

Infants at each NICU were randomized to an intervention group receiving BC or a control group receiving PF, both as a supplement to MM. Randomization was stratified by birth weight (≥1000 and < 1000 g) and randomly permuted blocks of size 4 and 6. A random sequence list was generated for each hospital and corresponding sequence numbers were obtained for each infant to be enrolled using a web-based system (open.rsyd.dk).

2.3. Nutrition and feeding protocol

Enrolled infants were fed MM whenever available. When MM was insufficient, infants received supplementary feed with either BC or PF until postnatal day 14 (see details below). After the intervention period, the participants in both groups received MM with or without supplemental PF. Infants were followed until postmenstrual age (PMA) 37 weeks or discharge, whichever came first. Blinding of clinical personnel to the intervention groups was

not possible because the reconstituted BC and PF products differed in color, odor and viscosity.

The BC powder used was from the first or second milking of Danish dairy cows and provided by Biofiber-Damino (Gesten, Denmark). The intact, unmodified BC product was supplied as sterile powder (10 g sachets) following gentle, low-temperature pasteurization (63 °C, 30 min), gamma irradiation and spray-drying. This processing has limited effects on the bioactivity of the BC product, as documented *in vitro* and in preterm pigs [22–24]. Before use, the BC powder was reconstituted to an energy density similar to the PF used for control infants (10 g BC mixed into 50 mL sterile warm water, approximately 800 kcal/L). The nutritional composition of the reconstituted BC is provided in Supplementary Table 1.

Enteral (EN) and parenteral nutrition (PN) were given according to the targeted daily fluid, energy and protein levels following the ESPGHAN [25] and CSPEN guidelines [26,27]. Enteral feeding was encouraged to be initiated within 24–48 h after birth with an initial feeding volume of 5–10 mL/kg/d and advancement rates of 5–20 mL/kg/d until 150–160 mL/kg/d. MM was the first choice of EN and supplementation with BC or PF was only conducted when MM was insufficient. The maximum daily volume of BC was calculated based on the volume of available MM with estimated protein concentrations (MM: 1.5 g/100 mL and BC: 8.0 g/100 mL) according to literature [28] to keep the total protein intake lower than 4.5 g/kg/d. Infants in the BC group also received PF as supplement when protein intake with the desired feeding volume of BC and MM would exceed the limit. The amount of amino acids provided by PN was adjusted accordingly to allow higher proportion of protein from BC within a controlled total fluid volume. Apart from adjusting protein provision, local routine practices were maintained at each NICU site. Gastric residuals were assessed routinely in five participating hospitals (Sites B, C, E, F and G) prior to each feeding, but not at two hospitals (Sites A and D). Volume of PN was provided according to local standards at each hospital. Separate amino acid infusion for PN was used at Site B, while mixtures of amino acids, lipids and glucose were used at other hospitals. The PF group was fed according to the local feeding routine, using one of two main PF products. The PF feeding details were not known for specific infants (PF compositions listed in Supplementary Table 1). After the intervention period of maximum two weeks, enteral feeding in the BC group was transitioned over 1–2 days to standard feeding (MM supplemented with PF when required), similar to the PF group.

2.4. Study outcomes

The primary outcome was the days to the first day receiving 120 mL/kg/d enterally for a consecutive 72 h (TFF120). Only 39% of the first 175 infants reached 150 mL/kg/d and local neonatologists generally considered 120 mL/kg/d as full enteral feeding, thus TFF120 was chosen as the primary outcome, despite that 150–160 mL/kg/d is suggested by CSPEN for full enteral feeding. Secondary outcomes included feeding intolerance, gastric residual volumes, mortality, incidences of NEC, sepsis and meningitis, days on PN, days of hospitalization and various growth parameters. All these endpoints were considered closely related to nutrition, gut and immune functions in VPIs, thus of relevance to record for our BC intervention. To further assess clinical safety, clinical outcomes with speculative or indirect relation to nutrition, gut and TFF120 were recorded, including occurrence of respiratory distress syndrome (RDS), bronchopulmonary dysplasia (BPD, as defined by the individual NICU), lung bleeding, patient ductus arteriosus (PDA, which required treatment), retinopathy of prematurity (ROP), intraventricular hemorrhage (IVH, diagnosed by ultrasonography and/or magnetic resonance imaging, MRI), periventricular leukomalacia (PVL, diagnosed by MRI), metabolic acidosis (base treatment required) and cow's milk allergy (by clinical diagnosis). Diagnoses were made based on local procedures and standards at each NICU. Furthermore, blood gas, biochemistry and hematology were collected on postnatal day 7 and 14 from the clinical records to provide indications of potential benefits or harms of the intervention.

Feeding intolerance was defined as withholding at least one full meal by the attending neonatologist, either during the period 1–7 days or 8–14 days after birth. Volumes of gastric residuals were assessed. NEC was defined as Stage II or III according to modified Bell's criteria [29]. Early-onset sepsis (EOS) was defined as clinical symptoms of sepsis occurring in the first three days of life and a positive blood culture. Late-onset sepsis (LOS) was based on clinical record plus a positive blood culture after the first three days of life. Clinical sepsis was defined as septic symptoms with a negative bacterial culture from blood antibiotic treatment for at least five days (or shorter than five days if the patient died) and fulfilling at least two of the following criteria: 1) decreased white blood cell count (WBC, $<5 \times 10^9$/L), increased WBC (<3 d, $>25 \times 10^9$/L; >3 d, $>20 \times 10^9$/L), 2) immature/total neutrophils >0.16, 3) CRP >8 μg/mL, 4) procalcitonin >2 ng/mL, 5) platelets $<100 \times 10^9$/L. Meningitis was defined as positive clinical symptoms and isolation of a causative organism from cerebrospinal fluid. Body weight, body length and head circumference were recorded at birth and weekly afterwards according to local standards, using digital anthropometric scales. Body weight z-scores were calculated with reference to the Fenton growth chart for preterm infants [30].

2.5. Sample size calculation and statistical analysis

The sample size of 350 was calculated based on an expected hazard ratio (HR) of 1.6, comparing TTF120 in BC and PF, with 80% probability of event in the intervention group, with a power of 90% and $\alpha = 0.05$ [31]. An expected HR of 1.6 corresponds to detecting a difference of 5 days between groups assuming a standard deviation (SD) of TFF of 11 days (power of 80% and $\alpha = 0.05$). The calculation also accounted for an inflation correction for enrollment of multiple births [32] and reduced effect of nutritional intervention when large proportions of MM (>50%) were fed [33]. Original calculations were based on surveys of participating sites, and results from previous observational and pilot interventional studies [16,21]. The sample size estimate was re-assessed based on the blinded data from the first 175 infants enrolled.

For both primary and secondary endpoints, statistical analyses were performed on both intention-to-treat (ITT) and per-protocol (PP) basis, where the PP analysis was performed on data collected from infants who received any dose of BC or PF during the 14-day intervention period. Time-to-event outcomes were analyzed using Cox proportional hazard model with HR with 95% confidence interval (CI) calculated. For other outcomes, complete case analyses were performed with the cases lost to follow-up excluded. Dichotomous outcomes were analyzed using logistic regression models with odds ratio (OR) with 95% CI presented. Continuous data were analyzed using linear regression model with mean difference and 95% CI calculated. Log-transformation was applied to achieve normality as appropriate. Comparison of treatment groups were obtained from both unadjusted and adjusted models including predefined baseline variables (site, GA, birth weight and sex). In the adjusted models, multiple birth was adjusted for by including the mother as a random factor in the models. Supplementary analyses were performed to assess the impact of MM proportion on the primary outcome by grouping infants into those receiving ≥50% MM and those receiving <50% MM during the first

14 days. Ancillary subgroup analyses within and between specific NICUs were performed to assess possible site-specific intervention effects.

Continuous outcomes were presented as mean and SD or median and interquartile range (IQR), depending on data normality. Dichotomous outcomes were presented as counts or percentages. In case of right-censored time-to-event outcomes (*e.g.,* TFF), median and IQR were presented. Qualitative variables were compared using Chi-square test with continuity correction. An additional Benjamini-Hochberg False Discovery Rate (FDR) correction was conducted for all secondary outcomes and blood results within each dataset (morbidities, blood chemistry, hematology) for P value correction. Data were analyzed in R (version 4.0.2).

3. Results

3.1. Enrollment and baseline characteristics

In total, 463 VPIs were screened, and 350 were randomized to BC (n = 171) and PF groups (n = 179) in the study period from 1st July 2017 to 23rd October 2020 with the follow-up ended on 18th November 2020 (Fig. 1). Complete case analyses were performed for the outcomes other than the primary outcome with infants not available for follow up excluded (n = 48). Baseline characteristics of enrolled infants were shown in Table 1. Baseline characteristics at each site are shown in Supplementary Table 2.

3.2. Nutrition

In this study, 155 out of the 171 infants randomized to the BC group did receive BC, while 173 out of the 179 infants randomized to the PF group received PF. Eight infants in the BC group were given PF only (separated randomization of two of triplets, n = 2; unknown reasons, n = 6) and were included in the PF group in the PP analyses. A total of 336 infants were included in the PP analysis, excluding infants that did not receive any supplementary feeding (Fig. 1). Nutrition information from each NICU site and for individual subjects are shown in Supplementary Figs. 1 and 2. The following results are from ITT analysis unless otherwise specified.

In the ITT analysis, infants in the BC group were fed 12 ± 16 MM, 13 ± 12 BC and 6 ± 14 PF (mL/kg/d, mean ± SD) during the first two weeks, comprising 35, 55 and 10% of total EN, respectively (Supplementary Table 3). In comparison, infants in the PF group were fed 14 ± 19 and 20 ± 22 mL/kg/d MM and PF (31 and 69% of total EN), respectively. The PP analysis showed infants fed BC received 12 ± 15 mL/kg/d MM, 14 ± 12 mL/kg/d BC and 5 ± 13 mL/kg/d PF (mean ± SD) that comprised 34, 60 and 6% of total EN, respectively (Fig. 2). The infants fed PF only were fed 14 ± 19 mL/kg/d MM and 21 ± 22 mL/kg/d PF that comprised 29 and 71% of total EN, respectively (Supplementary Table 3). The proportion of MM tended to be higher in infants fed BC versus the ones fed PF only [35 vs. 31%; adjusted mean difference: 6% (95% CI: −1, 12); P = 0.09, Supplementary Table 3]. There was high site variation in feeding volume and BC was fed at highest level at Site B (19 ± 14 mL/kg/d, 71 ± 35% of EN) and lowest at Site E (0.7 ± 0.8 mL/kg/d, 15 ± 22% of EN, Supplementary Fig. 1).

3.3. Primary outcome and nutrition-related endpoints

Within the study period, no significant effect of the intervention was found on the primary outcome, TFF120, in the ITT analysis [adjusted hazard ratio, aHR = 0.82 (95% CI: 0.64, 1.06); P = 0.13; Table 2, Fig. 3]. The incidence of infants that reached the enteral feeding of 120 mL/kg/d by the end of the study tended to be lower in the BC group [91 vs. 95%, adjusted odds ratio (aOR): 0.43 (95% CI: 0.16, 1.15); P = 0.08]. Infants fed BC were 34% less likely to reach the enteral feeding volume of 120 mL/kg/d than the infants fed PF only throughout the study period [aHR: 0.76 (95% CI: 0.59, 0.98); P = 0.04; PP analysis]. Site appeared to be an effect modifier (P < 0.01, Supplementary Fig. 3). Individual NICUs with large number of enrollments (Sites A, B, E and F) indicated site-specific effects of BC on TFF120 with lower possibility of reaching TFF120 at Site F [n = 159; median: 43 vs. 33 days; aHR: 0.57 (95% CI: 0.39, 0.82); P = 0.003] and higher possibility at Site B [n = 60; median: 24 vs. 27 days; aHR: 2.18 (95% CI: 1.2, 3.9); P = 0.01]. No significant effect was seen at Site E [n = 47; median: 25 vs. 21 days; aHR: 0.75 (95% CI: 0.40, 1.40); P = 0.37] or Site A [n = 46; median: 13 vs. 17 days; aHR: 0.90 (95% CI: 0.45, 1.81); P = 0.77] (Supplementary Fig. 3 showing weighted aHRs for all NICU sites).

To assess effects of BC supplementation on infants receiving different proportion of MM, subgroup analyses were performed for infants fed no more than 50% MM (BC: n = 103, PF: n = 127) or more than 50% MM (BC: n = 52, PF: n = 54) during the intervention period (Supplementary Table 4). The difference in TFF120 between the infants fed BC and the ones fed PF were less pronounced in the low MM subgroup [aHR: 0.80 (95% CI: 0.58, 1.10); P = 0.17] than the high MM subgroup [aHR: 0.67 (95% CI: 0.45, 1.02); P = 0.06; Supplementary Table 4].

3.4. Secondary outcomes, safety assessment and adverse events

The incidences of feeding intolerance were 42% and 48% in infants in the BC group vs. the ones in the PF group in week 1 [aOR: 0.82 (95% CI: 0.50, 1.33); P = 0.39], decreasing to 26% and 36% in week 2 [aOR: 0.64 (95%CI: 0.39, 1.07); P = 0.09], respectively. Length of hospitalization was 52 ± 21 days for the BC group and 49 ± 18 day for the PF group [adjusted mean difference: 1.83 (95% CI: −0.28, 5.06); P = 0.08]. Mean duration of PN, hospitalization, use and days of antibiotics did not differ between the treatment groups in the ITT analysis (Table 3). Infants fed BC tended to have higher body weight z-scores at PMA 37 weeks than the ones fed PF (−1.79 ± 0.96 vs −1.96 ± 0.83, adjusted P = 0.10, Table 3). No significant difference was found in the days on PN, the days on antibiotics, time to regain birth weight or body weight z-scores at PMA 37 wks. Large proportions of infants received antibiotics (99%), postnatal steroids (32%) and mechanical ventilation (50%), but only a few infants developed NEC (2–3%) and LOS (6–7%). There was no significant effect of BC supplementation on any of these outcomes in ITT analysis (Table 3). A trend to higher body weight z-score at PMA 37 weeks was found in the infants fed BC versus PF only [adjusted mean difference: 0.2 (95% CI: −0.03, 0.43); P = 0.09; PP analysis]. In the NICU-specific analyses, BC reduced the number of infants with feeding intolerance at Site B [week 2; n = 57; aOR: 0.2 (95% CI: 0.05, 0.78); P = 0.02]. At Site A, gastric residual volumes were significantly lower in the infants of the BC group [n = 44; adjusted mean difference: −0.59 (95% CI: −1.17, −0.02); P = 0.049].

Table 4 shows incidence of morbidities in each group. There were no significant group differences for RDS (incidence across groups: 91%), BPD (31%), PDA (28%), metabolic acidosis (27%) and IVH (8%). The incidence of ROP showed a trend to be higher for the BC group [24 vs. 18%; aOR: 1.77 (95% CI: 0.87, 3.59); P = 0.10). Lung bleeding was seen in four and three infants in the BC and PF group, respectively. Cow's milk allergy was diagnosed in three and one infant from the BC and PF group, respectively. Regarding PVL, five cases were found in the BC group and none in the PF group (P = 0.06). Details on birth characteristics, nutrition and comorbidities of the five infants with PVL are available in **Supplementary Details**. Three of these infants with PVL were fed only small amounts of BC (<10 mL/kg/d, Supplementary Fig. 2 and **Supplementary Details**), two infants with PVL received higher volumes

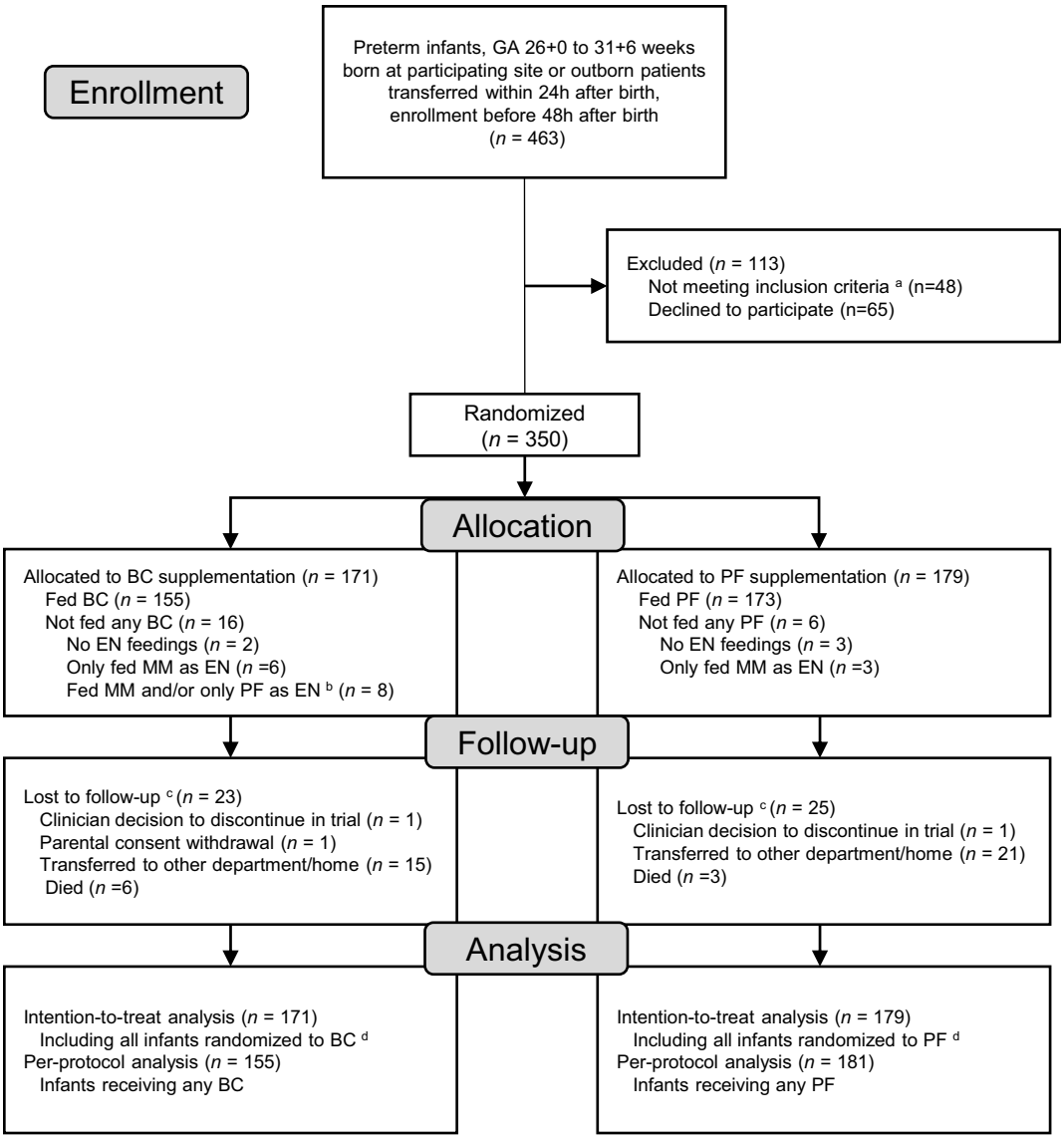

Fig. 1. A CONSORT flow diagram of participants included into the PreColos clinical trial. [1]Exclusion criteria were major congenital anomalies or birth defects, congenital infection or suspected TORCH (toxoplasmosis, rubella, CMV, herpes, hepatitis, coxsackie, syphilis, varicella zoster, HIV, parvo B19), perinatal asphyxia (umbilical or neonatal blood pH < 7.0), extremely small for gestational age (birth weight z score < −3), no realistic hope of survival, or if infants received PF prior to randomization. [2]Discordant with protocol or unknown reasons for withholding BC, cases classified as infants received PF only in the PP analysis. [3]Granted permission to use data acquired prior to discontinuation. [4]Assessment of TFF included cases lost to follow-up as censored data in time-to-event analyses. BC: bovine colostrum; EN: enteral nutrition; MM: maternal milk; PF: preterm formula.

of BC (19–35 mL/kg/d) together with high PF volumes (40–49 mL/kg/d). Two of the three infants fed low BC amount (<10 mL/kg/d) were also found with IVH (a related brain morbidity, Supplementary Fig. 2 and Details). The diagnostic criteria of IVH and PVL were similar for the BC and PF groups as indicated by review of the MRIs performed at the NICUs (**Supplementary Details**).

For 12 infants, BC supplementation was stopped or switched to PF within the intervention period due to safety concerns (metabolic acidosis, n = 4; frequent distention and gastric residuals, n = 8). The DSMB concluded that the adverse clinical signs were not likely to be related to the study or the BC intervention and that it was safe to continue the study as planned. Two infants received BC with amounts higher than planned (46–49 mL/kg/d across the two weeks of intervention, see B036, B037, Supplementary Fig. 2). The clinical data from these two infants were reviewed by the DMSB and no adverse reactions were found.

Supplementary Tables 5 and 6 show the blood gases, chemistry and hematology of the enrolled infants on day 7 and 14 of life, respectively. Most recorded variables did not show significant differences between the BC and PF groups and the mean values were within normal value range. However, in both ITT and PP analyses, the BC group showed higher partial oxygen pressure and lower base excess, bicarbonate and white blood cells (WBC) counts than the PF group (all $P < 0.05$). There was significant reduction in alkaline phosphatase levels [ALP; adjusted mean difference: −47.11 IU/L (95% CI: −69.23, −24.15); $P < 0.001$], together with increased blood phosphorus levels in the BC group [adjusted mean difference: 0.29 mmol/L (95% CI: 0.18, 0.40); $P < 0.001$]. On day 14 (Supplementary Table 6), differences in WBCs and lymphocytes were no longer present. In addition to ALP levels and blood phosphorus, blood urea nitrogen (BUN) levels were higher in the BC group versus the PF group [adjusted mean difference: 0.98 mmol/L (95% CI: 0.62, 1.57); $P < 0.001$]. The above effects of BC on base

Table 1
Baseline characteristics of mothers and infants at very preterm birth.

Characteristic	BC	PF
Number of infants	171 (100%)	179 (100%)
Site A, n (%)	23 (13.4%)	23 (12.8%)
Site B, n (%)	32 (18.7%)	28 (15.6%)
Site C, n (%)	3 (1.8%)	8 (4.5%)
Site D, n (%)	6 (3.5%)	9 (5.0%)
Site E, n (%)	22 (12.9%)	25 (14.0%)
Site F, n (%)	78 (45.6%)	81 (45.2%)
Site G, n (%)	7 (4.1%)	5 (2.8%)
Birth weight (g), mean ± SD	1336.93 ± 280.81	1346.28 ± 266.22
Birth length (cm), mean ± SD	38.75 ± 3.46	38.65 ± 3.43
Head circumference (cm), mean ± SD	27.32 ± 2.04	27.31 ± 2.05
Birth weight (z-score), mean ± SD	0.28 ± 0.77	0.37 ± 0.72
Birth length (z-score), mean ± SD	0.28 ± 1.22	0.30 ± 1.17
Head circumference (z-score), mean ± SD	0.43 ± 1.28	0.48 ± 1.18
Birth GA (weeks), mean ± SD	29.87 ± 1.37	29.75 ± 1.41
Apgar 5, median (IQR)[a]	10 (10–10)	10 (9–10)
Male gender, n (%)	107 (62.6%)	104 (58.1%)
Multiple birth, n (%)	62 (36.3%)	59 (33.0%)
Antenatal steroid treatment, n (%)	133 (77.8%)	125 (69.8%)
Caesarean section, n (%)	107 (62.6%)	104 (58.1%)
Chorioamnionitis, n (%)[a]	6 (3.6%)	15 (8.4%)
Abruptio placenta, n (%)[a]	7 (4.1%)	7 (3.9%)
Placenta praevia, n (%)[a]	7 (4.1%)	4 (2.2%)
Preterm premature rupture of membranes > 24 h, n (%)[a]	45 (26.6%)	43 (24.0%)
Preeclampsia or eclampsia, n (%)[a]	18 (10.6%)	17 (9.5%)

[a] Two infants were born at home, thus baseline information missing for some variables for these infants.

excess, alkaline phosphatase and phosphorus levels were also detected when analyzed separately for the subgroups of Site A, B and F (data not shown).

After FDR correction for multiple testing, alkaline phosphatase levels, blood phosphorus (weeks 1 + 2) and base excess, bicarbonate and BUN (week 2) remained significant (FDR-corrected $P < 0.05$).

4. Discussion

In this trial, supplementing MM with BC during the first two weeks of life did not reduce the time to full enteral feeding, relative to exclusive PF supplementation. Across the NICUs, there was even a tendency for BC supplementation to increase TFF120, length of parenteral nutrition and hospitalization (PP analyses). The results were unexpected considering the clear trend to shorter TFF120 in our pilot study [16] and the consistent gut maturational effects of BC across many pre-clinical studies in preterm pigs [7]. Analyses also found effect modification by trial site, shown as significant interaction between intervention and site. Analyses of data from Site B showed three days shorter TFF120 in the BC group ($P = 0.01$, $n = 60$, ITT analyses), indicating that BC effects on TFF120 vary among NICUs (Supplementary Fig. 3). This conclusion remains speculative as our trial was not adequately powered to detect site-specific effects of BC supplementation. TFF is a common outcome in clinical trials on feeding preterm infants but the present data, together with our previous studies across five continents [34], suggest that TFF is highly dependent on hospital conditions, care traditions, nutrition procedures and clinical assessment of infants. The observed large site variation suggests that effects of BC supplementation may not be generalizable across NICUs and feeding practices. Thus, further studies are required to determine the safety and clinical efficacy of BC supplementation under different feeding conditions in the first weeks after preterm birth. After this immediate neonatal period, BC may potentially be used as a protein fortifier to human milk (MM or donor milk), as investigated in trials in Denmark (NCT03537365) and China (NCT03822104). The studies in Denmark showed no clinical benefits (growth, morbidities, blood biochemistry) until 35 weeks' postmenstrual age [35,36]. Possibly, BC supplementation has the highest potential to improve immature

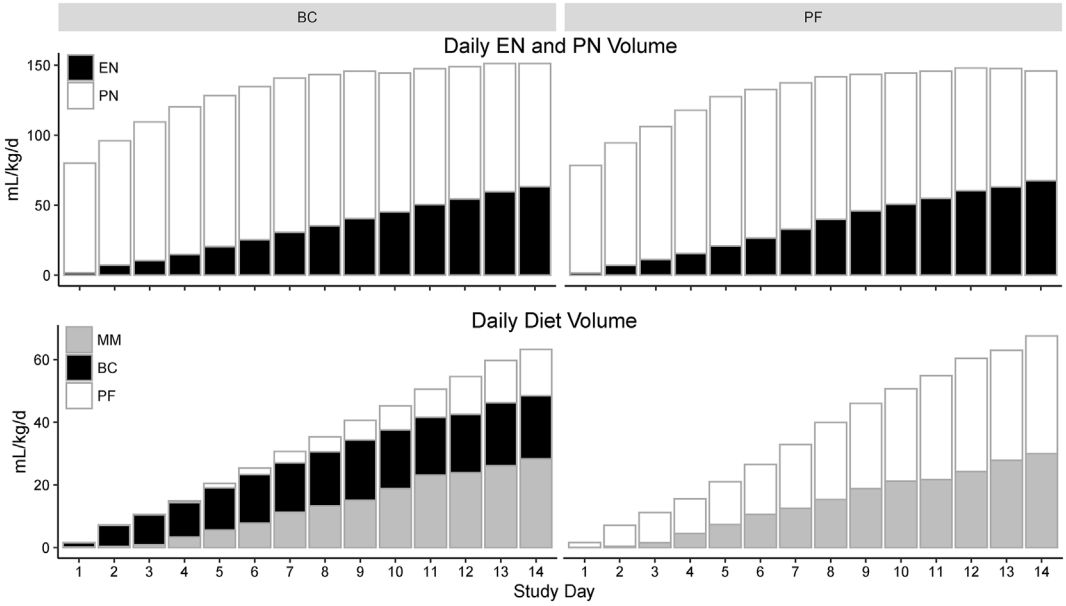

Fig. 2. Volumes parenteral nutrition (PN), total enteral nutrition (EN), -maternal milk (MM), preterm formula (PF) or bovine colostrum (BC) to infants after very preterm birth (per-protocol analyses). The bars show the mean value of daily EN and PN volume (upper panel) or daily enteral diet volume (lower panel) during the intervention period of two weeks. BC group, n = 155; PF group, n = 181.

Table 2
Primary outcome and feeding tolerance-related outcomes for very preterm infants fed mothers milk supplemented with preterm formula (PF) alone, or bovine colostrum (BC) plus PF.

	BC n	PF n	BC outcome	PF Outcome	Effect size (95% CI)	Adjusted effect size (95% CI)[a]	P	Adj. P[a]
ITT analysis								
TFF120, median (IQR)	171	179	28 (20–44)	27 (20–42)	0.89 (0.71, 1.12)[b]	0.82 (0.64, 1.06)[b]	0.33	0.13
Reached 120 mL/kg/d, n (%)	148	154	134 (90.5%)	147 (95.4%)	0.46 (0.18, 1.16)[c]	0.43 (0.16, 1.15)[c]	0.09	0.08
Feeding intolerance (week 1), n (%)	159	171	67 (42.1%)	82 (48.0%)	0.79 (0.51, 1.22)[c]	0.82 (0.50, 1.33)[c]	0.29	0.39
Feeding intolerance (week 2), n (%)	156	167	41 (26.3%)	60 (35.9%)	0.64 (0.39, 1.02)[c]	0.64 (0.39, 1.07)[c]	0.06	0.09
Days withholding feeds, median (IQR)	156	167	1.00 (0.00–2.00)	1.00 (0.00–4.00)	NA	NA	0.03[d]	NA
Ratio of withheld meals, median (IQR)	156	167	0.01 (0.00–0.05)	0.02 (0.00–0.16)	NA	NA	0.04[d]	NA
Gastric residual (mL), median (IQR)	156	167	0.50 (0.00–1.21)	0.57 (0.04–1.89)	NA	NA	0.33[d]	NA
PP analysis								
TFF120, median (IQR)	155	181	30 (21–45)	26 (18–42)	0.84 (0.66, 1.06)[b]	0.76 (0.59, 0.98)[b]	0.15	0.04
Reached 120 mL/kg/d, n (%)	135	160	122 (90.4%)	152 (95.0%)	0.49 (0.2, 1.23)[c]	0.47 (0.18, 1.23)[c]	0.12	0.12
Feeding intolerance (week 1), n (%)	145	176	62 (42.8%)	84 (47.7%)	0.82 (0.53, 1.27)[c]	0.86 (0.53, 1.41)[c]	0.37	0.52
Feeding intolerance (week 2), n (%)	142	173	37 (26.1%)	63 (36.4%)	0.62 (0.38, 1.00)[c]	0.59 (0.35, 1.00)[c]	0.05	0.05
Days withholding feeds, median (IQR)	142	173	1.00 (0.00–2.00)	1.00 (0.00–4.00)	NA	NA	0.03[d]	NA
Ratio of withheld meals, median (IQR)	142	173	0.01 (0.00–0.04)	0.01 (0.00–0.16)	NA	NA	0.05[d]	NA
Gastric residual (mL), median (IQR)	142	173	0.50 (0.02–1.21)	0.64 (0.07–1.93)	NA	NA	0.32[d]	NA

NA, not available, *i.e.*, -regression analysis could not be performed due to unmet assumptions.
[a] Adjusted for site, GA, birth weight and sex as fixed effects and mother as a random effect.
[b] Hazard ratio (95%CI).
[c] Odds ratio (95% CI).
[d] Wilcoxon rank sum test.

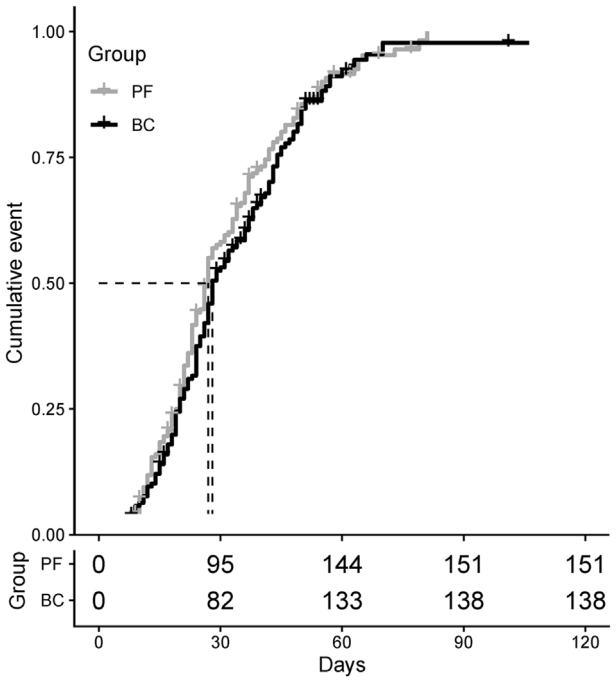

Fig. 3. Time to full enteral feeding at 120 mL/kg/d (TFF120) for very preterm infants fed MM supplemented with preterm formula alone (PF) or bovine colostrum (BC) plus PF. The presented Kaplan–Meier plot was based on the ITT analyses (upper panel), showing the proportion of infants reaching enteral feeding at 120 mL/kg/day relative to postnatal age. Dotted lines indicate the median time corresponding to a 50% probability of event. The number of infants reaching enteral feeding at 120 mL/kg/day at different time points is shown (lower panel).

gut functions and immunity in the first week(s) after preterm birth when the risks of feeding intolerance and NEC are highest.

Two previous small RCTs investigating BC for preterm infants (less than 50 infants in each arm) were underpowered to detect differences in TFF or feeding intolerance [18,19], but one of the studies with blinding showed only suggestion of lower incidence of feeding intolerance in BC infants [18]. Potential differences in the BC products used and feeding protocols may contribute to the varying results in different studies. Our open-label pilot trial showed shorter TFF120 in the infants that received BC in one Chinese hospital [16], consistent with the results from Site B in the current trial (Supplementary Fig. 3). Clinical decisions regarding feeding advancement rate, feeding intolerance and gastric residuals are highly subjective and vary widely around the world [21], even among clinical staff within the same NICU [37]. Blinding was deemed not possible in this trial because of the color difference between the BC and PF feed, which may have influenced the local practice of feeding management and assessment of feeding intolerance. Increased caution when feeding BC could have led to differences in feeding advancement at Sites B and F (Supplementary Fig. 1) and the associated difference in the primary outcome (TFF120, Supplementary Fig. 3). Performing routine control of gastric residuals before each meal may create undue concern for feeding intolerance and thereby delay TFF [38–40]. Two NICUs in this study (Site A and D) did not perform routine aspiration control of gastric residuals and our supplementary analysis showed shorter TFF120 than the NICUs performing routine control of gastric residuals (16 vs. 31 days, aHR: 5.04, $P < 0.001$). Feeding intolerance tended to be less for infants fed BC vs. PF, despite their longer TFF120, suggesting that TFF120 was affected by factors other than feeding intolerance in the first weeks. Similarly, BC effects on EN intake in the first two weeks and TFF120 failed to correlate with PN duration, indicating that these parameters were not causally connected, as analyzed across NICUs.

Our study did not have statistical power to detect differences in morbidities, but infants fed BC showed more cases of cow's milk allergy, metabolic acidosis, ROP and PVL (PP analyses). For the five PVL cases in the BC group, additional clinical details were retrieved and no difference in number of MRI scans performed was found, and the five cases with PVL were not fed particularly high amounts of BC. The incidence of PVL and other morbidities in the BC group did not appear higher than those reported for VPIs in the same region [41] or other regions in China [42]. Nevertheless, it cannot be excluded that intact BC, relative to PF, is deficient in certain micronutrients, such as docosahexaenoic acid, which has been related to elevated ROP and PVL incidence [43]. The trend to higher incidence of metabolic acidosis in the BC group is consistent with

Table 3
Clinical outcomes for very preterm infants fed mothers milk supplemented only with preterm formula (PF) alone, or with bovine colostrum (BC) plus PF.

Outcomes	BC n	PF n	BC Outcome	PF Outcome	Effect size (95% CI)	Adjusted effect size (95% CI)[a]	P	Adj. P[a]
ITT analysis								
Days of hospitalization, mean ± SD	148	154	51.51 ± 21.01	49.16 ± 17.54	1.76 (−2.16, 5.67)[d]	1.83 (−0.28, 5.06)[d]	0.38	0.08
Days on parenteral nutrition, mean ± SD	148	154	30.89 ± 15.77	29.39 ± 15.70	4.03 (−2.3, 10.35)[d]	4.25 (−2.27, 10.85)[d]	0.21	0.20
Days of antibiotics, mean ± SD	148	154	18.73 ± 15.59	18.91 ± 14.33	−1.46 (−7.12, 4.21)[d]	−1.73 (−8.39, 5.39)[d]	0.61	0.67
Use of antibiotics, n (%)	148	154	146 (98.6%)	152 (98.7%)	0.96 (0.13, 6.91)[c]	1.52 (0.18, 12.62)[c]	0.97	0.70
NEC stage II or III, n (%)	148	154	5 (3.4%)	4 (2.6%)	1.31 (0.35, 4.98)[c]	1.34 (0.34, 5.36)[c]	0.69	0.67
Early-onset sepsis, n (%)	148	154	2 (1.4%)	2 (1.3%)	1.04 (0.14, 7.49)[c]	1.20 (0.16, 9.15)[c]	0.97	0.86
Late-onset sepsis, n (%)	148	154	10 (6.8%)	10 (6.5%)	1.04 (0.42, 2.58)[c]	0.86 (0.31, 2.35)[c]	0.93	0.77
Clinical sepsis, n (%)	148	154	8 (5.4%)	5 (3.2%)	1.11 (0.57, 2.14)[c]	1.03 (0.51, 2.06)[c]	0.76	0.94
Meningitis, n (%)	148	154	1 (0.7%)	0 (0.0%)	NA	NA	0.98[e]	NA
Clinical meningitis, n (%)	148	154	3 (2.0%)	1 (0.6%)	3.17 (0.33, 30.77)[c]	3.44 (0.25, 47.86)[c]	0.29	0.33
Time to regain birth weight, median (IQR)	171	179	9 (7–14)	10 (7–14)	1.09 (0.88, 1.36)[c]	1.07 (0.86, 1.34)[c]	0.43	0.55
Body weight z-score at PMA 37 weeks, mean ± SD	57	51	−1.79 ± 0.96	−1.96 ± 0.83	0.17 (−0.17, 0.52)[d]	0.2 (−0.03, 0.43)[d]	0.32	0.10
PP analysis								
Days of hospitalization, mean ± SD	135	160	51.85 ± 20.85	48.71 ± 18.08	2.74 (−1.23, 6.7)[d]	2.85 (−0.34, 5.11)[d]	0.18	0.09
Days on parenteral nutrition, mean ± SD	135	160	31.64 ± 15.96	28.99 ± 15.58	4.9 (−1.59, 11.39)[d]	5.18 (−1.68, 11.82)[d]	0.14	0.14
Days of antibiotics, mean ± SD	135	160	19.61 ± 15.95	18.59 ± 14.20	−1.03 (−6.9, 4.83)[d]	−1.24 (−8.58, 5.83)[c]	0.73	0.71
Use of antibiotics, n (%)	135	160	133 (98.5%)	158 (98.8%)	0.84 (0.12, 6.06)[c]	1.20 (0.15, 9.69)[c]	0.86	0.86
NEC stage II or III, n (%)	135	160	5 (3.7%)	4 (2.5%)	1.5 (0.39, 5.70)[c]	1.52 (0.38, 6.08)[c]	0.55	0.55
Early onset sepsis, n (%)	135	160	2 (1.5%)	2 (1.2%)	1.19 (0.17, 8.55)[c]	1.32 (0.17, 10.31)[c]	0.86	0.79
Late onset sepsis, n (%)	135	160	9 (6.7%)	11 (6.9%)	0.97 (0.39, 2.41)[c]	0.89 (0.33, 2.43)[c]	0.94	0.82
Clinical sepsis, n (%)	135	160	8 (5.9%)	5 (3.1%)	1.08 (0.56, 2.11)[c]	1.06 (0.52, 2.14)[c]	0.81	0.88
Meningitis, n (%)	135	160	1 (0.7%)	0 (0.0%)	NA	NA	0.93	NA
Clinical meningitis, n (%)	135	160	3 (2.2%)	1 (0.6%)	3.61 (0.37, 35.14)[c]	4.17 (0.33, 53.28)[c]	0.23	0.24
Time to regain birth weight, median (IQR)	155	181	10 (7–14)	10 (7–14)	1.06 (0.85, 1.32) b	1.02 (0.81, 1.29)[b]	0.63	0.85
Body weight z-score at PMA 37 weeks, mean ± SD	54	52	−1.81 ± 0.97	−1.93 ± 0.85	0.12 (−0.22, 0.47)[d]	0.2 (−0.03, 0.43)[d]	0.49	0.09

NA, not available, *i.e.*, regression analysis could not be performed due to unmet assumptions.
[a] Adjusted for site, GA, birth weight and sex as fixed effects and mother as a random effect.
[b] Hazard ratio.
[c] Odds ratio.
[d] Mean difference.
[e] Fisher's exact test.

Table 4
Safety outcomes and morbidities of very preterm infants fed mothers milk supplemented with preterm formula (PF) alone, or bovine colostrum (BC) plus PF.

Morbidities	BC n	PF n	BC Outcome	PF Outcome	OR (95% CI)	Adjusted OR (95% CI)[a]	P	Adj. P[a]
ITT analysis								
Respiratory distress syndrome, n (%)	148	154	137 (92.6%)	137 (89.0%)	1.55 (0.70, 3.42)	1.56 (0.64, 3.82)	0.28	0.31
Bronchopulmonary dysplasia, n (%)	148	154	46 (31.1%)	49 (31.8%)	0.97 (0.59, 1.57)	0.81 (0.41, 1.58)	0.89	0.53
Lung bleeding, n (%)	148	154	4 (2.7%)	3 (2.0%)	1.40 (0.31, 6.36)	2.64 (0.40, 17.54)	0.66	0.30
Patient ductus arteriosus, n (%)	148	154	45 (30.4%)	40 (26.0%)	1.25 (0.75, 2.06)	1.41 (0.78, 2.52)	0.39	0.23
Retinopathy of prematurity, n (%)	148	154	36 (24.3%)	28 (18.2%)	1.45 (0.83, 2.52)	1.77 (0.87, 3.59)	0.19	0.10
Intraventricular hemorrhage, n (%)	148	154	12 (8.1%)	13 (8.4%)	0.96 (0.42, 2.17)	1.03 (0.44, 2.42)	0.92	0.95
Periventricular leukomalacia, n (%)	148	154	5 (3.4%)	0 (0.0%)	NA	NA	0.06[b]	NA
Cow's milk allergy, n (%)	148	154	3 (2.0%)	1 (0.6%)	3.17 (0.33, 30.77)	7.80 (0.36, 168.58)	0.29	0.14
Metabolic acidosis, n (%)	148	154	42 (28.4%)	39 (25.3%)	1.17 (0.70, 1.94)	1.39 (0.73, 2.62)	0.55	0.30
PP analysis								
Respiratory distress syndrome, n (%)	135	160	125 (92.6%)	142 (88.8%)	1.58 (0.71, 3.56)	1.52 (0.61, 3.79)	0.26	0.35
Bronchopulmonary dysplasia, n (%)	135	160	42 (31.1%)	48 (30.0%)	1.05 (0.64, 1.73)	0.80 (0.40, 1.57)	0.84	0.51
Lung bleeding, n (%)	135	160	4 (3.0%)	3 (1.9%)	1.6 (0.35, 7.27)	2.92 (0.43, 19.94)	0.54	0.26
Patient ductus arteriosus, n (%)	135	160	37 (27.4%)	41 (25.6%)	1.1 (0.65, 1.84)	1.13 (0.63, 2.05)	0.73	0.66
Retinopathy of prematurity, n (%)	135	160	32 (23.7%)	29 (18.1%)	1.4 (0.8, 2.47)	1.41 (0.69, 2.89)	0.24	0.33
Intraventricular hemorrhage, n (%)	135	160	12 (8.9%)	13 (8.1%)	1.1 (0.49, 2.51)	1.17 (0.49, 2.77)	0.81	0.73
Periventricular leukomalacia, n (%)	135	160	5 (3.7%)	0 (0.0%)	NA	NA	0.05[b]	NA
Cow's milk allergy, n (%)	135	160	3 (2.2%)	1 (0.6%)	3.61 (0.37, 35.14)	13.68 (0.54, 345.27)	0.23	0.07
Metabolic acidosis, n (%)	135	160	39 (28.9%)	37 (23.1%)	1.35 (0.8, 2.28)	1.59 (0.83, 3.04)	0.26	0.15

OR, odds ratio; NA, not available, *i.e.*, regression analysis could not be performed due to unmet assumptions.
[a] Adjusted for site, GA, birth weight and sex as fixed effects and mother as a random effect.
[b] Fisher's exact test.

the findings in the pilot studies [16,17]. Like for PF supplement, the clinical benefits of supplemental BC may be less when MM feeding is dominating [33]. As MM intake varied markedly among NICUs and infants (Supplementary Figs. 1 and 2), subgroup analyses based on this parameter has high risk of bias. This may explain why the tendency to longer TFF120 in BC infants was most pronounced at NICUs with high MM proportion. Conversely, the finding that BC shortened TFF120 at Site B (Supplementary Fig. 3) suggests that BC could be most effective to improve gut maturation when MM feeding is limited or absent. Our protocol required detailed nutrition calculations to determine when it was necessary to switch from BC to PF and adjust amino acids supply via PN to prevent

exceeding the set protein limits. Beyond these requirements, the NICUs were allowed to use local standards for feeding progression and comorbidity diagnosis. Hence, potential bias and heterogeneity among sites cannot be excluded, including those related to a non-blinded study design.

The mechanisms behind the clinical benefits of BC feeding or supplementation in previous studies are not clear but may include modulation of the gut microbiota and binding of luminal bacteria via colostral IgG and other bioactive components, such as lactoferrin and lactoperoxidase. This may inhibit detrimental mucosal responses to bacterial–mucosal interaction, directly promote gut growth via growth factors [5,44] or changes to the gut microbiota as shown in infants [44] and preterm pigs [10,21]. The lacking clinical benefit of BC supplementation in the present study may be explained by the limited amount of BC fed to most infants, and with addition of PF to avoid exceeding protein limits. Studies in preterm pigs show that the gut-protective effects of BC effects are less prominant when BC amount is reduced from 50 to 25% of total EN [12]. A protein supply of 3–4.5 g/kg/d is considered optimal for VPI growth in the first weeks [45] and there may be no further benefit of additional protein [46], and even concerns of amino acid toxicity and metabolic acidosis [47]. Partly intact passage of immunoglobulin G through the immature gut of infants [48,49] may secure the local bioactivity of BC but also reduce dietary amino acid availability. However, the tendency to more cases of metabolic acidosis among infants fed BC in this trial suggests that intake levels should not be further increased, although moderate excess of protein intake over metabolic requirements are unlikely to cause detrimental effects [47]. High plasma levels of certain amino acids (*e.g.*, tyrosine) in preterm infants fed exclusively BC in the first week after birth in our pilot trial [16] suggest there is good reason to avoid excessive BC feeding at this time. In future studies, a casein-reduced BC product may be relevant to test as a diet supplement for VPIs.

In conclusion, this multi-center, randomized trial showed that BC supplementation to VPIs during the first two weeks of life did not reduce TFF. The effects appeared highly dependent on nutritional practices at each NICU, influencing the amount of BC supplementation. No definite signs of adverse events or harm were observed across the NICUs, but further studies are required to assess the safety and efficacy of intact BC, or modified fractions of BC, in different clinical and nutritional settings.

Data share Statement

Data described in the manuscript, code book, and analytic code will be made available upon request pending application and approval. For future use, the collected dataset has been anonymized and stored in secured environments at Shenzhen People's Hospital, Shenzhen, China and University of Copenhagen, Denmark. The data handling and storage are in compliance with the European General Data Protection Regulation (GDPR).

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Author contributions

Xudong Yan, Investigation and Data curation. **Xiaoyu Pan**, Data curation, Formal analysis, Writing - Original draft. **Lu Ding**, Investigation. **Yiheng Dai,** Investigation**. Jun Chen,** Investigation. **Yong Yang,** Investigation. **Yuefeng Li,** Investigation. **Hu Hao,** Investigation. **Huixian Qiu,** Investigation. **Zhenzhi Ye,** Investigation. **René Liang Shen,** Data curation, Formal analysis and Writing - original draft. **Yanqi Li,** Conceptualization, Project administration. **Christian Ritz,** Methodology and Formal analysis. **Yueming Peng,** Investigation. **Ping Zhou,** Investigation. **Fei Gao,** Data curation and Formal analysis. **Ping–Ping Jiang,** Data curation, Formal analysis and Writing - review & editing. **Hung-Chih Lin,** Conceptualization. **Gitte Zachariassen,** Conceptualization and Data curation. **Per Torp Sangild,** Conceptualization, Funding acquisition, Supervision and Writing - review & editing. **Benqing Wu**, Supervision and Writing - review & editing.

Conflict of Interest

The study received support from a Danish company, Biofiber-Damino, which donated the bovine colostrum used for the study. University of Copenhagen holds a patent (Eur. Patent no. EP2858653A1) on the use of bovine colostrum for preterm infants. Per Torp Sangild is listed as the sole inventor but has declined any share of potential revenue arising from commercial exploitation of such a patent. All other authors have no conflicts of interest. All funding bodies had no role in the design, analysis or writing of this article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2023.06.024.

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