CLINICAL RESEARCH ARTICLE

Gut priming with bovine colostrum and T regulatory cells in preterm neonates: a randomized controlled trial

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BACKGROUND: Necrotizing enterocolitis (NEC) and neonatal sepsis are still considered major problems, especially in formula-fed preterm neonates. This study aimed to investigate the effect of bovine colostrum on T regulatory cells, NEC, and late-onset sepsis in preterm neonates ≤34 weeks.
METHODS: This prospective double-blind randomized controlled trial was conducted on 80 preterm infants who were randomly assigned to either the bovine colostrum group ($n = 32$) or control group ($n = 48$). T lymphocytes and their subsets, necrotizing enterocolitis, late-onset sepsis (LOS) and its severity, feeding tolerance, growth, length of hospital stay, and mortality were documented.
RESULTS: The bovine colostrum group showed higher follow-up levels of CD4+CD25+ FOXP3+ T lymphocyte % (FOXP3 Tregs). FOXP3 Tregs and its difference in change levels between baseline and follow-up were considered as the most related factors to the bovine colostrum. Bovine colostrum group showed positive trends for reduction of sepsis severity and mortality with no significant difference in the incidence of NEC, LOS, and length of hospital stay.
CONCLUSIONS: Preterm neonates who received bovine colostrum showed a higher FOXP3 Treg level.

IMPACT:

- Bovine colostrum has no significant effect on the incidence of necrotizing enterocolitis.
- FOXP3 T regulatory cells and their increased level between baseline and follow-up is considered as the most influencing factors related to the bovine colostrum.
- Positive trends were noted for reduction of sepsis severity and concomitant mortality, but the study lacked the power to assess these outcomes.

INTRODUCTION

Human milk is the optimal and best nutrition for preterm infants. However, sometimes maternal or donor milk is not available during the first period of life. Thus an Infant formula may be given instead as the first diet especially in the absence of donor human milk banks in some countries.[1]

Bovine colostrum composition is similar to human colostrum in many constituents. In the absence of human colostrum, bovine colostrum could be a satisfactory replacement.[2] Bovine colostrum is a feasible and low-cost product that is present mostly in the form of dry powder or capsules. Processing includes pasteurization and a low-temperature spray drying. There is great variability in its bioactivity related to the degree of different proportions of heat exposure during the processing and the time of obtaining the colostrum from calves.[3] Intact bovine colostrum contains high amounts of protein, growth factors, and other immunoregulatory components that may stimulate intestinal structure and function and be involved in necrotizing enterocolitis (NEC) resistance.[4–6]

T regulatory cells (Tregs) are capable of regulating and suppressing the immune system. They regulate the innate and adaptive host responses and hence they are important for intestinal immune system homeostasis.[7] Tregs are considered as the primary mediators of the peripheral immune system tolerance by monitoring cell-mediated immunity extension and hindering the occurrence of excessive immune-mediated tissue damage.[8] High Treg levels have protective effects on nosocomial sepsis and NEC in preterm neonates.[9] FOXP3 expression is considered as a definitive Treg marker and is extremely important for its development and function.[10,11]

There is a paucity of literature investigating the effect of bovine colostrum on Tregs. Therefore, this study was conducted to test a hypothesis suggesting that the bovine colostrum could affect Tregs. The secondary aim was to evaluate its ability to reduce the NEC and late-onset sepsis (LOS) in preterm neonates.

PATIENTS AND METHODS

Study population

This prospective, double-blind randomized controlled clinical trial included 80 preterm neonates with gestational age ≤34 weeks. The included neonates were admitted to neonatal intensive care units of Ain Shams University hospitals during the period from

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Enrollment

Assessed for eligibility
(*n* = 108)

Excluded (*n* = 10)

- 5 with risk factors of early-onset sepsis

- 2 life-threatening congenital anomalies

- 1 perinatal asphyxia

- 1 inborn error of metabolism

- 1 chromosomal aberrations

Randomized
(*n* = 98)

Allocation

Allocated to bovine colostrum group (*n* = 49)

Allocated to non-bovine colostrum (*n* = 49)

Follow up

Lost to follow-up (*n* = 5); 2 died, 3 developed established early-onset sepsis (before the first dose of intervention)

Discontinued intervention (*n* = 10); available breast milk

Lost to follow-up (*n* = 0)

Discontinued intervention (*n* = 1); available breast milk

Analysis

Excluded from analysis (*n* = 2); insufficient blood sample

Analyzed (*n* = 32)

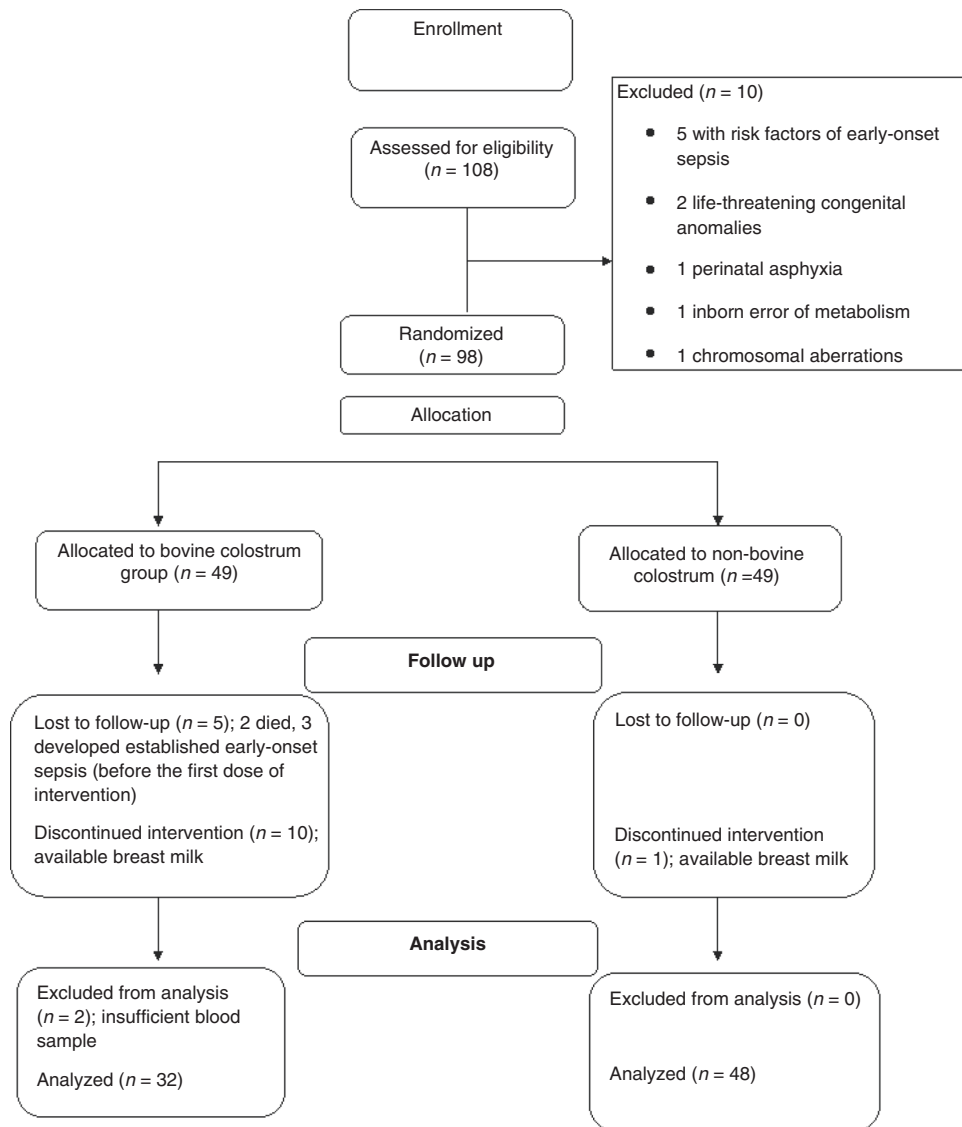Excluded from analysis (*n* = 0)

Analyzed (*n* = 48)

Fig. 1 CONSORT flow diagram of the studied neonates. CONSORT flow diagram demonstrating participant flow through the phases of the randomized controlled trial (enrollment, randomization, allocation, follow up and data analysis). *n* number.

September 2018 to September 2019. Preterm neonates with risk factors of early-onset sepsis, life-threatening congenital abnormalities, an inborn error of metabolism, chromosomal aberrations, neonates with underlying gastrointestinal problems that prevent enteral feeding, perinatal asphyxia, or presence of available breast milk were excluded from the study.

Informed consent was provided by parents or caregivers of each participant before enrollment. The study was approved by the Research Ethical Committee of Ain Shams University hospitals: IDFMASU MD50/2017, and it is in accordance with the Helsinki Declaration of 1975. This trial is registered at Clinical Trial.gov; identifier: NCT03926390. Reporting of the study conforms to the consort 2010 statement.[12]

Randomization and blinding
Out of 108 preterm neonates assessed for eligibility, 10 neonates met exclusion criteria. Thus 98 preterm neonates were enrolled and randomly assigned according to a predetermined schedule generated from random numbers based on a computer-generated randomization sequence into either the bovine colostrum group or control group. The bovine colostrum group included 49 preterm neonates: 32 preterm neonates who received the

allocated intervention and were analyzed, 10 discontinued the intervention as breast milk became available after the allocation, 2 died, 3 developed established early-onset sepsis, and 2 had insufficient blood samples. The control group included 49 preterm neonates: 48 were analyzed and 1 discontinued the intervention because of the availability of breast milk after the allocation (Fig. 1).

The doctors, nurses, laboratory staff, and the investigator measuring the outcome were blinded. To conceal allocation, the bottles were serially numbered on the outside, based on the random sequence.

Drug composition and dilution
Immuguard® (Dulex-Lab Pharmaceutical Co.) was used in the first 2 weeks of life in the bovine colostrum group. This product is the first 6 h bovine colostrum and it is present in the form of dry powder to be dissolved in distilled water (3 g in 66 mL). This dilution is considered safe to avoid osmolality disturbance. It consists of concentrated immunoglobulins (Igs; IgA, IgG, and IgM), lactoferrin, proline-rich polypeptide, interferons, lymphokines, interleukins, and growth factors, which include transforming growth factors (TGFs; TGF-α and TGF-β), and insulin-like growth

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factors (IGFs; IGF-1 and IGF-2), in addition to multiple essential minerals and vitamins.

Drug administration and feeding protocol
In absence of available breast milk, the feeding policy of the unit is to omit the routine oral care with human colostrum and to start early trophic feeding with 10 mL/kg/day of preterm formula, with an increment of feeding volume up to 20 mL/kg/day for as long as tolerated (judged by the attending neonatologist) till reaching the full intake (200 mL/kg/day).

The bovine colostrum group received bovine colostrum as a gut priming and gradually increased to a maximum of 20 mL/kg/day, then continued with a preterm formula to be subtracted from the total volume of the feed for the first 2 weeks of life. Then the preterm neonate continued on the preterm formula till discharge. The control group received preterm formula only from the start and continued on it till discharge. Both groups received the same feeding protocol and they were not changed during the study period. When maternal milk became available at any time, the study protocol was discontinued.

Feeding intolerance was defined as the presence of any of the following signs and symptoms for at least 3 consecutive days: emesis, gastric residuals exceeding 25% of feeding volume, diarrhea, visible non-explained blood in the stool, or abnormally enlarged bowel loops presented on physical examination or with an imaging study.[13] Definite NEC (stage ≥2) was diagnosed according to the modified Bell's criteria.[13]

Withholding feeding was indicated in cases of feeding intolerance, definitive NEC, heavily bile-stained vomiting denoting clear abdominal pathology, or marked cardiorespiratory instability.

Clinical and radiologic assessment
Detailed perinatal history was obtained for all included neonates, followed by a thorough clinical examination and standard neonatal care. The gestational age was determined by maternal last menstrual period and further confirmed by using the Ballard score.[14] The birth weight Z score was documented, and the weight was plotted twice weekly during admission.

LOS was diagnosed as sepsis after 72 h of life[15,16] with Töllner sepsis score ≥10[17] and/or hematologic scoring system (HSS) of Rodwell ≥3[18] with or without positive blood culture results. The duration of sepsis episode started with a diagnosis of sepsis and ended with Töllner sepsis score <10 and HSS of Rodwell ≤2.

Severe sepsis was defined as a case of sepsis associated with cardiac hemodynamic instability, acute respiratory dysfunction, or at least two organ dysfunction.[19,20] The severity of the illness was graded according to the score for neonatal acute physiology II (SNAPII score), which was plotted and followed up daily during admission and the maximum achieved score was documented.[21,22]

Flow cytometric analysis
Multicolor flow cytometry for the studied groups was done in the first 24 h (baseline levels) and at the end of the second week of life (follow-up levels; day 14).[23] It was performed on erythrocyte-lysed peripheral blood samples using fluorescein isothiocyanate-labeled anti-CD4, phycoerythrin (PE)-labeled anti-CD25 (Beckman Coulter®, Inc., Fullerton, CA), and PE-Cyanine 5 (PC5)-labeled anti-FOXP3 (eBioscience™, ThermoFisher Scientific, Waltham, MA).[23]

The total lymphocytes were gated depending on the forward scatter and side scatter characteristics, then the percentage of CD4$^+$CD25$^+$ T lymphocytes was expressed as a percentage of the total lymphocytes. Three subsets of CD4$^+$ T cells were defined according to the intensity of CD25 staining: CD25$^-$, CD25^{low}, and CD25^{high}. FOXP3 expression was detected on CD4$^+$CD25$^+$ T cells. Antigens were scored positive using a cut-off of ≥20% cells staining brighter than an isotype-matched negative control. Functionally active Treg cells were defined by expressing high levels of the interleukin-2 receptor α-chain (CD25) or by expressing CD25 and the FOXP3 transcription factor simultaneously.[23,24]

Outcome measures and endpoint of the study
The following data were recorded: duration to establish half enteral feeding (100 mL/kg/day) and full enteral feeding (200 mL/kg/day), duration of total parenteral nutrition, feeding intolerance, the incidence of definite NEC, LOS and its severity, inotrope use and their duration, respiratory support and their type, weight gain at the end of the second week (day 14), T lymphocytes levels and their subsets including T regulatory cells, length of hospital stay, mortality, and adverse effects of treatment (if any) for the duration of the study. Factors related to bovine colostrum intervention were documented. The bovine colostrum was discontinued in cases of withholding of feeding for >24 h, reported side effects of the drug, discharged, or death of preterm infants whichever came first.

Sample size and statistical analysis
The sample size was calculated using the PASS program, setting the type-1 error ($α$) at 0.05 and the power ($1 − β$) at 0.8. A result of a previous study showed that the incidence of definitive NEC among the control group was 20% compared to 0% of the treatment group.[9] Calculation according to these values produced a minimal sample size of 32 cases per group, raised to 40 cases per group taking into account a 20% dropout rate.

Statistical analysis
Data were analyzed using the Statistical Package for Social Science (IBM SPSS) version 23 (IBM© Corp., Armonk, NY). Normally distributed numerical variables were presented as mean ± standard deviation (SD). Quantitative non-parametric data were described in the form of the median and interquartile range. Qualitative data were described as frequency and percentage. Qualitative variables were compared using Chi-square (X^2) test or Fischer's exact test when frequencies were below five. Quantitative parametric variables were compared using the independent Student's sample t test while the non-parametric data were compared using Mann–Whitney test. Wilcoxon signed-rank test was used for comparison between two paired groups with quantitative non-parametric data. The logistic regression model was performed by estimating the odds ratio (OR) and 95% confidence interval (CI) to define factors related to bovine colostrum intervention. p value <0.05 was considered the cut-off value for significance in all analyses.

RESULTS
The maternal and neonatal clinical characteristics of the studied groups showed no significant differences (Table 1).

Clinical outcome measures are shown in Table 2; both groups showed no significant differences regarding time to achieve half enteral feeding or full intake. The incidence of definite NEC showed no significant differences between groups. It was noted that the severity of sepsis episodes and the need for adrenaline/noradrenaline was significantly higher in the control group ($p = 0.01$); they are second-line inotropes in our unit indicating sepsis severity, while there is no significant difference between both groups regarding the incidence of LOS, the need for dopamine/dobutamine, the first-line inotropes in our unit ($p = 0.2$), and the total inotrope duration ($p = 0.86$). The bovine colostrum group showed less mortality than the control group ($p = 0.01$). Pathogens of severe sepsis episodes were extended-spectrum B lactamase *Klebsiella pneumoniae* (4 cases), *Pseudomonas aeruginosa* (2 cases), and methicillin-resistant coagulase-negative staphylococcus (2 cases).

Notably, the absolute risk reduction (ARR) of feeding intolerance, sepsis severity, and mortality was 15, 11, and 16%,

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Table 1. Maternal and neonatal characteristics of the studied groups.

Variable	Control group ($n = 48$)	Bovine colostrum group ($n = 32$)	Test	p value
High-risk pregnancy (%)	15 (31.3%)	7 (21.9%)	0.84	0.35
Mode of delivery CS, n (%)	42 (87.5%)	28 (87.5%)	0.00	1.00
APGAR score, median (IQR)				
At 1 min	6 (5 to 6)	5 (5 to 7)	−0.07	0.94
At 5 min	8 (7.5 to 9)	8 (8 to 9)	−0.79	0.42
GA (weeks), mean ± SD	32.18 ± 1.58	32.6 ± 1.31	−1.23	0.21
Birth weight (g), mean ± SD	1494.13 ± 313.65	1562.63 ± 341.66	−0.92	0.35
Birth weight z score, median (IQR)	−1.18 (−1.6 to −0.29)	−1.11 (−1.58 to −0.46)	−0.26	0.79
Male gender, n (%)	24 (50.0%)	15(46.9%)	0.07	0.78
Multiple birth, n (%)	4 (8.3%)	6 (18.8%)	1.90	0.16
Age at admission (days), mean ± SD	1.58 ± 0.99	1.44 ± 0.8	0.69	0.48
Diagnosis on admission			0.00	1.00
Grower, n (%)	30 (62.5%)	20 (62.5%)		
RD, n (%)	18 (37.5%)	12 (37.5%)		

Data are expressed as the number and percentage (%) where Chi-square test (X^2) was used or mean ± SD where independent t test was used or median and IQR (interquartile range) where Mann–Whitney test used for comparison.
CS cesarean section, GA gestational age, RD respiratory distress.

Table 2. Clinical outcome measures of the studied groups.

Variables	Control group ($n = 48$)	Bovine colostrum group ($n = 32$)	Test	p value
Feeding characteristics				
Reached 100 mL/kg/day, n (%)	46 (97.9)	32 (100.0)	0.69	0.40
Time to achieve100 mL/kg (days), mean ± SD	8.09 ± 2.5	9.13 ± 5.24	−1.16	0.24
Reached full intake, n (%)	44 (91.7%)	32 (100.0%)	2.80	0.09
Time to achieve full intake (days), mean ± SD	12 ± 3.4	13 ± 6.52	−0.86	0.38
Time to discontinue TPN (days), median (IQR)	8.5 (4–10)	8 (5–10)	−0.20	0.83
Feeding intolerance, n (%)	9 (18.8)	1 (3.1)	4.28	0.03
Definite NEC, n (%)	5 (10.4)	0 (0.0)	3.55	0.059
Late-onset sepsis, n (%)	10 (20.8)	3 (9.4)	1.85	0.17
Number of sepsis episodes, n (%)			2.71	0.25
No	38 (79.2)	29 (90.6)		
One	7 (14.6)	3 (9.4)		
Recurrent (>1)	3 (6.3)	0 (0.0)		
Duration of sepsis episodes (days), median (IQR)	13 (7–20)	4 (3–30)	−0.34	0.73
Severe sepsis, n (%)	8 (16.7)	0 (0.0)	5.92	0.01
Maximum SNAPII score, n (%)			7.23	0.02
Mild	40 (83.3)	31 (96.9)		
Moderate	0 (0.0)	1 (3.1)		
Severe	8 (16.7)	0 (0.0)		
Need for inotropes				
Dopamine/dobutamine, n (%)	9 (18.8)	3 (9.4)	1.32	0.25
Adrenaline/noradrenaline, n (%)	8 (16.7)	0 (0.0)	5.92	0.01
Total duration of inotropes (days), median (IQR)	6.5 (2–10)	8 (2–10)	−0.17	0.86
Weight increment at the end of second week (g), median (IQR)	75.5 (50–90)	90 (65–100)	−1.74	0.08
Length of hospital stay (days), median (IQR)	14 (10–21)	15 (11.5–22.5)	−0.69	0.49
Mortality, n (%)	8 (16.7%)	0 (0.0%)	5.92	0.01

Data are expressed as number and percentage (%) where Chi-square test (X^2) was used or mean ± SD where independent t test was used or median and IQR (interquartile range) where Mann–Whitney test used for comparison.
NEC necrotizing enterocolitis, SNAPII score for neonatal acute physiology II.

respectively, and thus the number needed to treat (NNT) was 6.4, 8.8, and 6, respectively, in the bovine colostrum group (NNT = 1/ ARR = 1/0.1).

Additionally, air leaks, intracranial hemorrhage, the need for respiratory support, its type and duration, and baseline and follow-up hemoglobin levels were found to have no significant

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Table 3. Baseline and follow-up T lymphocytes levels and their subsets in the studied groups.

Variable	Control group (n = 48)	Bovine colostrum group (n = 32)	Test	p value
Baseline T lymphocyte levels and their subsets				
Lymphocyte % of TLC, mean ± SD	30.13 ± 13.93	30.9 ± 12.87	−0.25	0.80
CD4+CD25 T lymphocyte %, mean ± SD	11.71 ± 7.42	10.88 ± 5.2	0.55	0.58
CD4+CD25 HIGH T lymphocyte %, mean ± SD	4.71 ± 2.39	5.04 ± 2.52	−0.59	0.55
CD4+CD25+FOXP3+ T lymphocyte %, median (IQR)	2.64 (0.84 to 4.61)	2.6 (1.65 to 4.14)	−0.12	0.89
Active T regulatory cells %, median (IQR)	6.35 (4.69 to 8.59)	6.5 (4.5 to 9)	−0.07	0.94
Follow-up T lymphocyte levels and their subsets				
Lymphocyte % of TLC, mean ± SD	29.65 ± 14.91	34.38 ± 19.56	−1.22	0.22
CD4+CD25 T lymphocyte %, mean ± SD	15.08 ± 7.18	13.99 ± 8.01	0.63	0.52
CD4+CD25 HIGH T lymphocyte %, mean ± SD	6.99 ± 3.83	6.63 ± 3.42	0.430	0.66
CD4+CD25+FOXP3+ T lymphocyte %, median (IQR)	1.65 (0.53 to 3.09)	2.6 (1.75 to 4.81)	−2.43	0.01
Active T regulatory cells %, median (IQR)	7.75 (5.63 to 11.27)	8 (4.8 to 10)	−0.19	0.84
Difference of change of T lymphocytes and their subsets between baseline and follow-up samples				
Lymphocyte % of TLC, median (IQR)	0.3 (−9.7 to 11.15)	4.5 (−7.95 to 14.4)	−1.07	0.28
CD4+ 25% of lymphocyte, median (IQR)	2.9 (−1.7 to 9.8)	1.4 (−3.15 to 7.75)	−0.93	0.35
CD4+ 25 HIGH % of lymphocyte, median (IQR)	1.5 (−1 to 3.85)	1.15 (−0.4 to 4.15)	−0.09	0.92
CD4+CD25+FOXP3+ T lymphocyte %, median (IQR)	−0.93 (−2.1 to 0.5)	0.61 (−1.53 to 2.42)	−2.36	0.01
Active T regulatory cells %, median (IQR)	0.27 (−1.97 to 4.02)	0.7 (−2.05 to 4.13)	−0.24	0.80

Data are expressed as mean ± SD where independent t test was used or median and IQR (interquartile range) where Mann–Whitney test used for comparison. FOXP3 Tregs: CD4+CD25+FOXP3+ T lymphocyte %; active T regulatory cells: expressing high levels of the interleukin-2 receptor α-chain (CD25) or by expressing CD25 and the FOXP3 transcription factor simultaneously.

differences between both groups, with a significant change of hemoglobin levels (baseline–follow-up) in the bovine colostrum group (Supplementary Table 1).

Table 3 showed that follow-up CD4+CD25+FOXP3+ T lymphocyte % (FOXP3 Tregs) was significantly higher in the bovine colostrum group with a higher difference of its change between baseline and follow-up samples in both groups ($p < 0.05$ for all).

Regarding the difference between baseline and follow-up levels of T lymphocytic cells and their subsets in the bovine colostrum and control groups (each group separately), both bovine colostrum and control groups showed a significant rise of CD4+CD25+HIGH T lymphocytes % levels from 5.04 ± 2.25 to 6.63 ± 3.42 and from 4.71 ± 2.39 to 6.99 ± 3.83, respectively ($p < 0.05$), but on the other hand, the FOXP3 Tregs showed a significant drop in its level from 2.64 (0.84–4.61) to 1.65 (0.53–3.09) in the control group ($p = 0.005$) while it maintained its level without a significant change in the bovine colostrum group (p value = 0.31) (Fig. 2).

The most important outcomes affected by oral bovine colostrum were follow-up levels of FOXP3 Tregs and its difference of change between baseline and follow-up (p value = 0.027, 0.028, respectively) with an OR (95% CI) of 0.80 (0.66–0.97) and 0.82 (0.69–0.97), respectively (Table 4).

DISCUSSION

In the present study, both groups reached half and full enteral feeding at comparable timing, but later there was a reduction in feeding intolerance among the bovine colostrum group. It was noted that bovine colostrum improved the gut maturation in both intestinal proliferation and differentiation. Also, it improved the microscopic picture of the intestine acting as an enterocyte growth factor.[5,25] That might be related to its content of various growth factors including IGF, which was stated to be beneficial in improving the feeding tolerance.[4,26,27] However, Sadeghirad
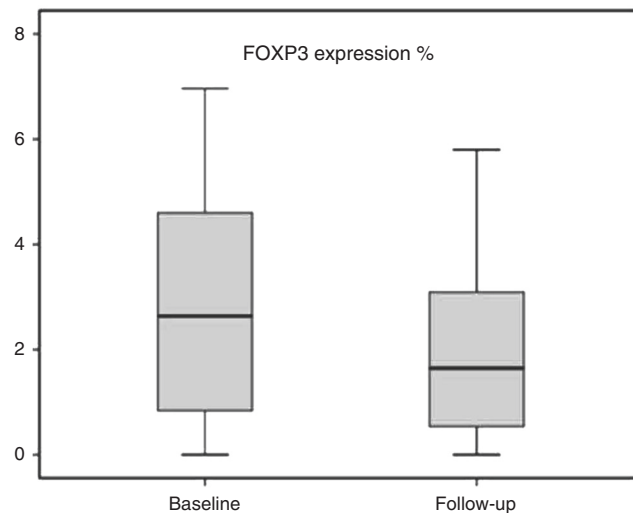
Fig. 2 FOXP3 expression% in the the nonbovine colostrum group. Change in FOXP3 Tregs % between basal and follow-up levels in the nonbovine colostrum group. FOXP3 Tregs:CD4+CD25+FOXP3+ Tlymphocyte%.

et al.[28] reported in a meta-analysis on both human and bovine colostrum that time to reach full enteral feeding had no significant difference, although infants receiving human colostrum reached full feed 3.5 days earlier.

In the context of NEC, our study showed that none of the neonates in the bovine colostrum group developed definite NEC vs. 5 cases (10.4%) in the control group; this did not express a statistically significant difference. Similarly, previous studies of bovine colostrum and/or lactoferrin did not show manifestations of definite NEC.[4,9,28] Moreover, Balachandran et al.[29] showed no significant difference in NEC but with a higher incidence of ileus

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Table 4. The most important outcomes affected by oral bovine colostrum.

Variable	B	S.E.	Wald	p value	Odds ratio (OR)	95% CI for OR	
						Lower	Upper
Feeding intolerance	1.96	1.08	3.31	0.06	7.15	0.85	59.54
Severe SNAPII score	1.33	0.77	2.98	0.08	3.81	0.83	17.42
Maximum respiratory support	0.09	0.23	0.16	0.68	1.09	0.69	1.73
Hb change (baseline–follow-up)	−0.04	0.02	3.00	0.08	0.96	0.91	1.00
CD4+CD25+FOXP3+ T lymphocyte % (follow-up level)	−0.21	0.09	4.87	0.02	0.80	0.66	0.97
CD4+CD25+FOXP3+ T lymphocyte % difference of change (baseline–follow-up)	−0.19	0.08	4.84	0.02	0.82	0.69	0.97

FOXP3 Tregs: CD4+CD25+FOXP3+ T lymphocyte %; Wald: statistical test.
B regression coefficient, *S.E.* standard error of regression coefficient, *SNAPII* score for neonatal acute physiology II, *OR* odds ratio, *CI* confidence interval.

and radiological signs of NEC in the bovine colostrum group ($p = 0 > 0.05$). They used different product with different dose and dilution, this product might not have effects similar to the used product in our study.

Upon studying the effect of bovine colostrum on LOS, we found that there were fewer sepsis episodes with the use of bovine colostrum as they were only 3 (9.4%) vs. 10 (20.8%) in the control group ($p = 0.17$). However, we found significantly lower severe sepsis episodes with bovine colostrum ($p = 0.01$) and none of their patients developed severe SNAPII score or required the use of adrenaline/noradrenaline inotropes. Similarly, Balachandran et al.[29] detected fewer cases with definite sepsis in the bovine colostrum group 7 (16.3%) than the control group 10 (23.5%) but it did not reach statistical significance ($p = 0.4$). Li et al.[4] showed that none of the infants who received bovine colostrum were diagnosed with sepsis. Sadeghirad et al.[28] reported that infants who received colostrum had a 22% less risk of sepsis development than those receiving a placebo or usual care. Additionally, other previous studies supported our findings.[9,28,30]

The explanation of this could be that bovine colostrum has both direct and indirect antimicrobial effects by modulation of the immune system,[31] reducing gut inflammation, and enhancing mucosal integrity and tissue repair.[32] It has a synergistic action with antimicrobial drugs and a great inhibitory effect on the growth of different microorganisms.[30] Moreover, bovine lactoferrin has an important role in promoting innate immunity against pathogens by its direct actions at the level of cell membranes to gain both a bactericidal and a bacteriostatic effect.[33]

In the current study, the baseline T lymphocytes and their subsets showed no significant difference between the study groups. However, the follow-up results showed that FOXP3 Tregs were higher in the bovine colostrum group with a higher difference of change between the two measurements. Additionally, FOXP3 Tregs and its difference of change levels were considered as the most related factors to the bovine colostrum.

Akin et al.[9] found that FOXP3 Treg frequency was higher at discharge in the lactoferrin group than the control group (4.02 ± 1.05 vs. 2.55 ± 0.81, respectively, $p = 0.001$). They estimated a higher percentage of change in FOXP3 Tregs in the lactoferrin group between baseline and discharge levels ($p = 0.002$), which signified the effect of the lactoferrin on the FOXP3 Tregs. Additionally, they found no significant difference when comparing the FOXP3 Tregs levels at birth and discharge in both the lactoferrin and control groups.

Lluis et al.[34] stated that bovine milk had higher Tregs with increased FOXP3 demethylation and expression. It enhanced the long-lasting differentiation and maintenance of Tregs and hence reducing the inflammation.[35] Moreover, Tregs are crucial for intestinal immune homeostasis. Relative lack or poor function of Tregs in intestinal lamina propria of premature infants might play

an important role in the NEC. Additionally, there was a significant decrease in CD3+, CD4+, and CD8+ cells and lower levels of FOXP3+ cells in NEC patients reported in previous studies.[36,37] Furthermore, the infusion of FOXP3 Tregs could prevent and treat colitis in experimental models.[38]

As regards weight increment, the bovine colostrum group had a higher weight increment at the end of the second week than the control group, but it did not reach statistical significance. Similarly, previous studies support this finding.[4,27,39]

In the context of the duration of hospitalization, the present study showed no significant differences. Sadeghirad et al.[28] reported that the duration of hospitalization did not reveal any significance between the groups (mean difference 1.3 days, 95% CI: −13.7 to 16.3). Notably, we found that the use of bovine colostrum significantly decreased mortality without any recorded deaths in the bovine colostrum group compared to 8 deaths (16.7%) in the control group. No difference as regard mortality between bovine colostrum and control groups were noted in other studies.[4,27–29] In this study, we did not find any side effects for bovine colostrum, which is the same result as most of the studies done on bovine colostrum in preterm neonates.[4,27,28]

The limitation of this study is that we did not include breastfed preterm infants because we considered it unethical to deprive neonates of available maternal milk to give bovine colostrum in the early days of life. We could not measure bovine colostrum osmolarity because of financial issues, which could be beneficial. Only a few preterm infants had a birth weight of <1000 g.

In conclusion, bovine colostrum could be used safely in preterm neonates as a gut priming and might be a relevant alternative to human milk when it is unavailable. Bovine colostrum has a significant effect on CD4+CD25+FOXP3+ T lymphocytes and hence the immune system homeostasis. Larger studies with long-term observations on the immunomodulatory effect of bovine colostrum would give a promise of its preventive and therapeutic role in preterm infants, especially those of extremely low birth weights.

DISCLAIMER
This work is not previously published or under editorial consideration in another journal.

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colostrum intervention. N.M.B. drafted the initial manuscript and analyzed and interpreted the data.

ADDITIONAL INFORMATION

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