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Review Article

Effects of oropharyngeal colostrum and minimal enteral nutrition on respiratory morbidity and necrotizing enterocolitis in preterm infants: A systematic review of randomized trials

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ABSTRACT

Background & Aim: Necrotizing enterocolitis is one of the most severe gastrointestinal complications in premature infants, characterized by acute intestinal inflammation and progressive mucosal damage. This systematic review aimed to evaluate the effects of minimal enteral nutrition and oropharyngeal or buccal colostrum administration on respiratory morbidity and the incidence of necrotizing enterocolitis in preterm neonates.

Materials & Methods: A comprehensive search was conducted using PubMed, Web of Science, Scopus, ProQuest, and Google Scholar databases. From 10,460 identified records, twelve randomized controlled trials published between 2015 and 2024 met inclusion criteria and were incorporated into the qualitative synthesis. Data were extracted regarding early growth, feeding tolerance, immune biomarkers, and safety outcomes.

Results: Minimal enteral nutrition was associated with improved early growth and reduced feeding intolerance. Oropharyngeal or buccal exposure to colostrum increased mucosal immune biomarkers, including immunoglobulin A, immunoglobulin M, and lactoferrin, and promoted early colonization of beneficial intestinal microorganisms. Some trials reported reductions in late-onset sepsis and retinopathy of prematurity. However, neither minimal enteral nutrition nor colostrum-based interventions significantly reduced the incidence of necrotizing enterocolitis ($p > 0.05$). Both approaches showed favorable safety profiles without increased adverse effects or clinical instability.

Conclusion: Minimal enteral nutrition and oropharyngeal or buccal colostrum administration appear to be safe, feasible, and biologically plausible strategies for enhancing early nutrition and mucosal immunity in premature infants. Larger, multicenter, and standardized clinical trials are warranted to determine their definitive impact on necrotizing enterocolitis prevention.

Introduction

Necrotizing enterocolitis (NEC) remains a paramount clinical challenge and a leading cause of morbidity and mortality in preterm infants, particularly those with a birth weight below 1500 g (1-3). Affecting approximately 7-10% of very low birth weight newborns, the risk is disproportionately higher in infants born before 28 weeks' gestation (4, 5). The clinical progression of NEC is often unpredictable and devastating, manifesting through abdominal distension, vomiting, and bloody stools, which can rapidly escalate to septic shock or coagulopathy in severe cases (6). Despite significant advancements in neonatal intensive care, mortality rates remain alarmingly high (15-30%), reaching as much as 50% in surgical cases (7). Furthermore, the impact extends far beyond the acute phase; survivors frequently face life-altering long-term complications, including short bowel syndrome,

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growth impairment, and neurodevelopmental delays (8, 9). Beyond the clinical burden, the substantial healthcare costs associated with prolonged hospitalization underscore the urgent and critical need for effective preventive strategies (10, 11).

To mitigate the devastating risks associated with NEC, early nutritional and immunomodulatory interventions have become a cornerstone of neonatal management. Minimal enteral nutrition (MEN) (12), also termed trophic feeding, involves administering sub-nutritional volumes of milk to 'prime' the gastrointestinal tract and facilitate early maturation (13). By enhancing gut motility and gastric emptying, MEN limits stasis and prevents harmful bacterial overgrowth (13). Mechanistically, this strategy strengthens the intestinal barrier through improved mucin production and tight-junction integrity, thereby reducing the risk of bacterial translocation (14). Immunologically, MEN activates local gut defenses, such as Peyer's patches and secretory IgA, while promoting beneficial microbial colonization to modulate inflammation (15, 16). In parallel with MEN, the administration of oropharyngeal/buccal colostrum (OBC) has emerged as a complementary strategy, providing high concentrations of bioactive factors and cytokines directly to the neonate's immune system (17). Together, these interventions represent a biologically plausible approach to enhancing gut maturation and reducing NEC risk in vulnerable preterm infants (17, 18).

"Complementary to the benefits of MEN, oropharyngeal/buccal colostrum (OBC) administration has emerged as a potent immunomodulatory strategy during the first hours of life. OBC involves applying small volumes of maternal colostrum directly to the infant's buccal mucosa, facilitating immune activation even before full enteral feeding is established (19, 20). This approach leverages the rich composition of colostrum-abundant in sIgA, lactoferrin, cytokines, and growth factors-to strengthen mucosal immunity and modulate systemic inflammatory pathways by reducing pro-inflammatory markers such as TNF-α and IL-6 (21, 22). Beyond its immunological role, the bioactive factors in colostrum promote early beneficial microbial colonization and enhance the structural integrity of the intestinal barrier (23). Given its safety and biological potency, OBC represents a promising non-invasive intervention to bolster early neonatal defense and potentially mitigate the risk of NEC in vulnerable preterm populations (22, 24, 25).

Despite the clinical significance of early nutritional interventions in the NICU, the current body of evidence remains fragmented. While previous systematic reviews have examined either MEN (26, 27) or OBC (19, 20, 28) as isolated strategies, there is a notable absence of synthesized data evaluating these two distinct yet complementary immunomodulatory approaches within a single, integrated framework. Furthermore, the existing literature has reported inconsistent findings regarding the effects of these interventions on NEC incidence and respiratory morbidity, including dyspnea, pneumonia, and respiratory tract infections. Given the publication of several high-quality randomized controlled trials (RCTs) between 2022 and 2024, an updated and comprehensive synthesis is needed to reconcile these discrepancies. This review systematically addresses this gap by evaluating MEN and OBC-based care, offering a more integrated perspective on early neonatal management and supporting evidence-based clinical decision-making.

Methods

Protocol registration

The protocol for this systematic review is registered with PROSPERO (CRD420251108441).

Data sources

A comprehensive search was conducted across PubMed, Scopus, Web of Science, and ProQuest, supplemented by a targeted search of

grey literature, including clinical trial registries (e.g., ClinicalTrials.gov) and the first ten pages of Google Scholar, to ensure broad coverage of relevant studies (Table S1). In addition, the reference lists of included studies and relevant systematic reviews were manually screened to minimize publication bias. All identified records (n= 10,460) were imported into Zotero, where 908 duplicates were removed. The remaining 9,552 records were screened by title and abstract to identify potentially relevant studies. Full-text reports of potentially eligible articles were then retrieved and assessed against the predefined inclusion and exclusion criteria. During the full-text stage, four retracted papers were identified and excluded from the review. The search, screening, and selection process was conducted in accordance with PRISMA guidelines, with full documentation provided in Table S2 and Figure 1.

Study selection

We included English-language studies that evaluated the effects of MEN or OBC interventions on NEC and/or other clinically relevant outcomes in preterm infants (<37 weeks' gestation). Particular attention was given to high-risk populations, including VLBW infants (<1500 g) and extremely preterm infants (<28 weeks' gestation), as these groups represent the highest clinical need for NEC prevention. No date restrictions were applied. Studies were excluded if they were unrelated to the review question, lacked sufficient outcome data, or were publication types such as abstracts, brief reports, book chapters, letters, or reviews. Eligible studies included randomized and comparative clinical studies assessing MEN versus standard feeding protocols or control and/or OBC versus placebo or standard care; a direct head-to-head comparison between MEN and OBC was not required. Although some included articles did not focus primarily on NEC prevention, relevant outcomes were extracted to address the aims of this systematic review. After title and abstract screening, full texts of potentially eligible reports were independently assessed by two reviewers according to the predefined criteria. Reasons for exclusion at the full-text stage were recorded and are presented in the PRISMA flow diagram. Any disagreements between reviewers were resolved through discussion, and if necessary, by consultation with a third reviewer. Inter-rater agreement was assessed using the Kappa statistic, with a minimum acceptable agreement level of 0.80.

Data abstraction and risk of bias assessment

Ultimately, 12 studies met the inclusion criteria and were included in the final qualitative synthesis. Data were extracted using a standardized Excel form, capturing key study characteristics, participant demographics, intervention protocols, and reported outcomes, including NEC incidence, feeding progression, growth, inflammatory markers, and sepsis. Both primary and secondary outcomes, together with statistical methods and significance measures, were recorded to enable accurate cross-study comparison.

The quality of the included RCTs was evaluated using the Cochrane Risk of Bias tool (29), which assesses key methodological domains such as randomization, allocation concealment, blinding, completeness of outcome data, and selective reporting. Most studies showed low risk of bias in random sequence generation, allocation concealment, outcome assessment, and data completeness. However, blinding of participants and personnel was often judged as high or unclear, largely due to the practical limitations of open-label or procedural feeding interventions. Finally, five studies were rated as having moderate quality, and one study demonstrated high methodological quality (14). A full summary of the assessments is provided in Table S3.

Ethical considerartion

Ethical approval was not required for this study because it is a systematic review of

published data and did not involve any new data collection from human participants or animals.

Data synthesis and statistical methods

While a meta-analysis was initially considered for NEC incidence, it was ultimately deemed methodologically inappropriate and clinically unsound due to the profound clinical and methodological heterogeneity across the evidence base. In accordance with Cochrane guidelines (Section 9.5.1) and the SWiM (Synthesis Without Meta-analysis) reporting framework, a single pooled estimate was avoided because the interventions differed fundamentally in their biological mechanisms (e.g., the local immunological action of buccal human colostrum vs. the systemic effects of enteral bovine colostrum supplementation). Furthermore, the high frequency of rare events (zero-event trials) for NEC in several cohorts meant that any forced pooling would be statistically precarious, highly sensitive to arbitrary continuity corrections, and prone to producing spurious (false-positive) results. Consequently, we prioritized a structured narrative synthesis and study-level effect estimates to ensure a rigorous, transparent, and clinically safe interpretation of the data, particularly given the high-risk nature of the VLBW population.

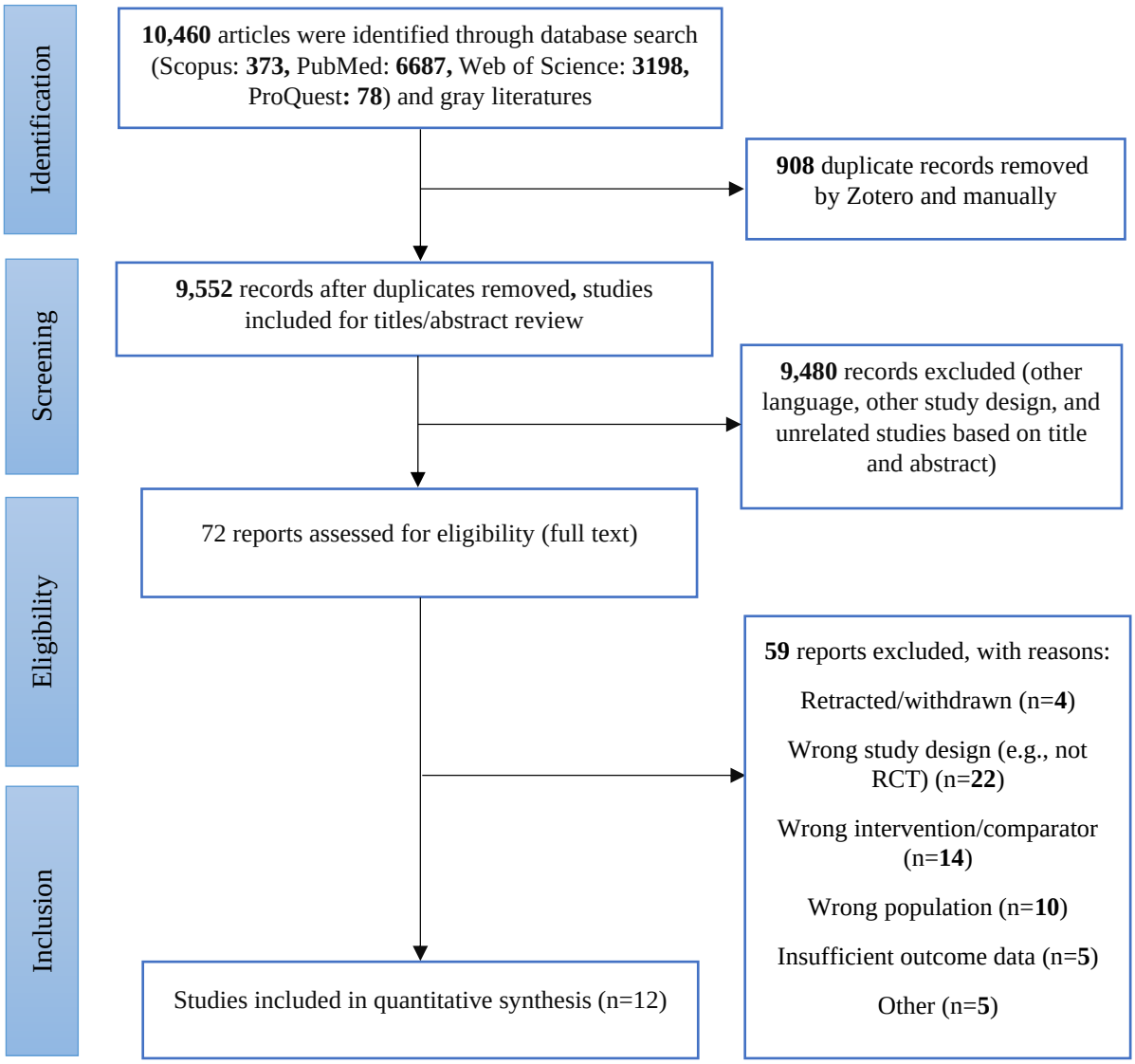

Figure. 1. PRISMA flowchart of the included eligible studies in the systematic review (30)

Results

General Characteristics of Included Studies

The included trials were conducted across a wide geographical range-Denmark, China, the United States, Egypt, Spain, Turkey, Iran, and Brazil- representing both high- and middle-income NICU settings and improving the external validity of the findings (Table 1). The studies spanned nearly a decade (2015–2024) and ranged from small single-center pilot RCTs involving 12 very low birth weight infants (19) to large multicenter protocols enrolling up to 622 extremely preterm infants (31). Most trials recruited between 70 and 350 neonates, typically <32 weeks' gestation or ≤1500 g birth weight. Follow-up periods generally covered the first 14–30 days of life, a critical window for assessing early feeding progression, immune responses, and clinical stability. Methodologically, the trials included open-label, single-blind, and double-blind RCTs as well as non-randomized comparative designs. Randomization procedures varied, incorporating block, stratified, web-based, or sealed-envelope allocation methods (32, 33), while large-scale studies employed DSMBs to strengthen safety oversight (31, 34). Despite heterogeneity in design, intervention duration, and administration routes, all studies shared a common goal: to evaluate safe, scalable colostrum-based strategies that enhance early immunity, feeding tolerance, and overall clinical outcomes in preterm infants.

Primary outcomes

Across the included RCTs, OBC supplementation was associated with several early benefits in preterm infants. It increased protein intake during the first week of life, even though most studies reported no significant difference in time to full enteral feeds (19, 32, 35, 36). OBC also promoted healthier early microbial patterns, with reductions in Moraxellaceae and Staphylococcaceae within 48–96 hours (14). Clinically, OBC was linked to a lower incidence of ROP (5% vs. 16%) without increasing NEC, sepsis, or feeding intolerance (35). Immunologic effects were consistently positive, with higher IgA, IgM, lactoferrin, and resistin levels reported in OBC-treated infants (14). In contrast, prolonged MEN did not shorten the time to full feeding but reduced the number of days with feeding intolerance, indicating improved gastrointestinal tolerance (9).

Secondary outcomes

Secondary outcomes offered additional insight into how colostrum-based interventions influence feeding, growth, immunity, and other clinical parameters in preterm infants.

Feeding progression and nutritional efficiency

Across multiple trials, OAC consistently supported earlier advancement of enteral or oral feeding. Several RCTs, including those by OuYang et al. (2021) (34), Martín-Álvarez et al. (2020) (37), Kelich et al. (2024), and Chen et al. (2022) (38), reported faster achievement of full feeds in the OAC groups. Newer studies, such as Behrooj et al. (2025) (39) confirmed these findings, although effects on discharge weight and hospital stay were minimal.

Growth and metabolic outcomes

Growth-related outcomes varied across studies. Some trials, such as those by Kelich et al. (2024) (33) and Martín-Álvarez et al. (2020) (37), found no significant differences in weight gain, while Farag et al. (2024) (35) observed notably greater weight gain, higher hemoglobin levels, and lower CRP among infants receiving colostrum. Metabolic parameters, including BUN, remained within safe limits, and transient changes such as elevated tyrosine levels (reported by Juhl et al.) (36) resolved without clinical consequences. Prolonged MEN was also linked to modest improvements in daily weight gain (14).

Respiratory morbidity and NEC incidence

NEC outcomes were generally favorable in colostrum-treated groups, with OuYang et al. (2021) reporting a significantly lower NEC rate (2.4% vs. 10.4%) (35). Other studies found similarly low NEC incidence across groups, limiting between-group differences (37, 38). MEN also showed potential protective effects, with NEC observed only in the early-advancement arm of one study (14).

Inflammatory and immune biomarkers

Colostrum consistently demonstrated immunomodulatory benefits. Studies reported reductions in pro-inflammatory cytokines (e.g., IL-6, IL-8, TNF-α) and increases in protective markers such as IL-10, IgA, IgM, and lactoferrin (37, 38, 40).

Microbiota and mechanistic outcomes

Microbiota-focused studies, particularly Sohn et al. (2016) (19), showed that OAC promotes early colonization with beneficial microbial taxa while reducing potentially pathogenic families. Mechanistically, these shifts support improved feeding tolerance and lower infection risk. Additional studies have suggested broader effects on gut microbial development, although complete results are still pending (31).

Length of hospital stay and mortality

Colostrum did not appear to prolong hospitalization, and in some cases, reduced the length of stay (39), as seen in the trial by Kelich et al. (2024) (33). Mortality data remain limited, with no clear differences reported (41).

Table 1. Summary of study characteristics of studies included in the systematic review

Study	Country	Design	Sample: GA/BW	Intervention/ Control	Key outcomes (primary)
(36)	Denmark and China	Pilot RCT	40 preterm infants; <32 weeks of gestation	Enteral bovine colostrum supplementation vs Mother's milk (MM) supplemented with donor milk (DM, Denmark) or infant formula (IF, China)	Higher enteral protein intake in the BC group (P < 0.05); Tendency for earlier achievement of full enteral feeding (China only)
(32)	South China	Multicentre RCT	350 preterm infants; <32 weeks of gestation	Enteral bovine colostrum supplementation vs MM + preterm formula (PF)	Time to full enteral feeding (TFF120: 120 mL/kg/day) - No significant effect on TFF120: aHR = 0.82 (95% CI: 0.64–1.06), P = 0.13
(19)	USA	Pilot RCT	12 preterm infants; ≤ 32 weeks	OBC/OMM: Mother's colostrum administered into the buccal pouch every 2 hours for 46 hours vs Standard care	Changes in oral microbiota composition
(35)	Egypt	RCT	211 preterm - BC group: 106 infants - non-BC group: 105 infants; ≤ 32 weeks	Enteral bovine colostrum supplementation vs Standard preterm formula without colostrum	Incidence of retinopathy of prematurity (ROP) - ROP incidence significantly lower in BC group: 5/100 vs. 16/100, p = 0.033
(40)	Spain	RCT	100 preterm neonates, Colostrum group: 48 infants, Control group: 52 infants; <32 weeks	OBC/OMM: 0.2 mL of mother's colostrum via the oropharyngeal route every 4 hours for 15 days vs No oropharyngeal colostrum	Serum levels of immunoglobulins (IgA, IgM, IgG1); lactoferrin and resistin levels; significant increases in IgA and IgM at days 15 and 30 in the intervention group compared to the control group.
(14)	Turkey	RCT	199 preterm Prolonged MEN group: 99 infants - Early advancement group: 100 infants; <32 weeks	MEN: MEN without increasing volume for the first 5 days vs Feed volumes increased by 20–25 ml/kg/day until reaching 150 ml/kg/day	Time to full enteral feeding (sustained for 72 h); Incidence of feeding intolerance; No significant difference in time to full enteral feed…
(39)	Iran	RCT	70 (final: 68); 28–32 weeks	Intervention: 0.4 ml colostrum every 3 hrs for 7 days + extraoral massage; Control: No colostrum	Incidence of late-onset sepsis (LOS) (clinical sepsis)

Study	Country	Design	Sample: GA/BW	Intervention/ Control	Key outcomes (primary)
(33)	Iran	RCT	80 (40 per group); 25–30 weeks	OBC/OMM: 0.1 cc breast milk via oropharyngeal method every 2 hours starting 48–72 h post-birth vs 0.1 cc sterile water (placebo)	Time to start enteral feeding
(38)	China	RCT	130 (final: 111); ≤ 32 weeks	OBC/OMM: 0.3 ml mother's milk via buccal swab before gavage feeding, every 3 h for 14 days vs 0.9% saline via swab, same protocol	Salivary sIgA levels on days 2, 7, 14
(37)	Spain	RCT	100 (final: 87); <32 weeks and/or <1500 g	OBC/OMM: 0.2 ml oropharyngeal mother's milk (OMM) every 4 h for 15 days vs No oropharyngeal milk (standard care)	Serum levels of IL-6, IL-8, IL-10, IL-1ra, TNF-α, IFN-γ at days 1, 3, 15, 30
(31)	USA	Double-blind RCT	622 planned (498 final analysis); < 1250 g (birth weight)	Intervention: 0.2 ml mother's milk every 2h for 48h, then every 3h until 32 weeks corrected gestational age (CGA); Control: 0.2 ml sterile water (placebo), same schedule	Incidence of late-onset sepsis (LOS)
(34)	China	Pilot RCT	252 (127+125); ≤ 32 weeks	OBC/OMM: 0.4 ml colostrum every 3h for 10 days via the oropharyngeal route vs 0.4 ml normal saline, same schedule	NEC (Bell stage 2/3), Late-Onset Sepsis (LOS)
(41)	Brazil	Non-randomized (historical control)	350 planned (175/group); ≤ 37 weeks and ≤ 1500 g (VLBW-PTNB)	OBC/OMM: 0.2 ml raw colostrum via oropharyngeal route every 3h (8x daily) for 7 days vs Historical control (same hospital, 3 years prior)	Attributable risk of death (mortality rate diff)

Abbreviations: MEN, minimal enteral nutrition (also termed MEF/trophic feeding); OBC/OMM, oropharyngeal/buccal administration of colostrum or mother's milk as reported by individual trials; GA, gestational age; BW, birth weight; RCT, randomized controlled trial; NR, not reported; NICU, neonatal intensive care unit.

Safety and adverse events

Across all reviewed trials, OBC and OAC/OMM demonstrated a consistently strong safety profile in preterm infants. No study reported an increase in NEC, sepsis, feeding intolerance, metabolic complications, or other serious adverse events attributable to these interventions. Large multicenter RCTs (32, 36) confirmed stable clinical and laboratory parameters, with BUN levels remaining within safe limits and no causal link identified for isolated PVL cases. Additional studies reported similar findings, including lower CRP levels and fewer suspected sepsis episodes in OBC-treated infants (35, 40), and even among very fragile VLBW infants, no adverse effects were observed (19). Trials incorporating real-time monitoring also recorded no apnea, bradycardia, desaturation, or other physiologic instability (33, 37-39). The most extensive data, involving more than 13,000 administered doses of OMM, showed no aspiration, apnea, bradycardia, or CMV transmission events (31, 34). Even studies without DSMB oversight reported no complications (41), further supporting the strong tolerability and safety of colostrum-based interventions.

Comparison of MEN and colostrum administration

As summarized in Table 2, this feeding strategy is associated with greater gastrointestinal maturation, higher daily weight gain, and a possible reduction in NEC. Colostrum, in contrast, is linked to earlier achievement of full enteral feeding, lower ROP rates, fewer suspected sepsis episodes, and more favorable inflammatory, immunologic, and microbial profiles. Both approaches demonstrated strong safety across trials, with only a minor, non-significant signal of increased PVL in one study.

Table 2. Summary of Key findings from independent RCTs assessing MEN and colostrum administration

Category	MEN	Colostrum administration
Main goal	Gastrointestinal priming to reduce NEC, improve weight gain	Enhance immunity, modulate microbiota, support growth, reduce inflammation
Weight gain	Higher daily weight gain (19g vs. 16g, $p < .001$) (Bozkurt et al., 2022)	Slightly higher or similar weight gain; improved Hb and reduced CRP (Farag, Moreno, etc.)
NEC incidence	No significant difference in NEC incidence between groups (0% vs. 5%, $p > 0.05$). Similarly, no significant differences were found in BPD, ROP, or mortality ($p = .06$)	No increase in NEC; safe profile across studies
Sepsis/infections	No significant difference in LOS or culture-proven sepsis	Fewer suspected sepsis cases ($p = .004$); lower CRP; no increase in LOS
Microbiota effects	Not reported	Significant shifts: ↓ *Staphylococcaceae*, ↑ *Planococcaceae* (Sohn et al., 2015)
ROP incidence	Not specifically addressed	Lower ROP incidence: 5% vs. 16% ($p = .033$) (Farag et al., 2024)
Safety / adverse events	No increase in adverse events	No increase in adverse events; slight PVL signal in one study (Yan et al., 2023)
Sample size/evidence	One large, well-powered trial (n = 199)	Mixed: large multicenter (n = 350) + smaller single-center RCTs
Clinical recommendation	Safe, improves weight, and may reduce NEC	Safe, enhances immunity, may reduce ROP and infections

Note: No single RCT included in this review directly compared MEN versus Colostrum. This table provides a parallel summary of findings from separate trials comparing each intervention to their respective control groups

Discussion

Prior reviews in neonatal care have typically focused on single interventions-such as probiotics, colostrum administration, or early feeding approaches like MEN-rather than directly comparing MEN with colostrum. Evidence syntheses on MEN (42, 43) consistently show reductions in NEC and feeding intolerance without adverse effects on growth, while meta-analyses of oropharyngeal colostrum (22, 25, 44) highlight its ability to lower sepsis risk and enhance immune and microbial outcomes.

This review expands the evidence base by providing the first comparative synthesis of MEN and colostrum administration using data from randomized controlled trials across 12 studies, including 7 newly included RCTs. While our qualitative synthesis suggests that MEN may be associated with improved daily weight gain, the evidence regarding its impact on NEC risk remains statistically non-significant across the included trial (14, 19, 31, 35, 40), preventing a definitive conclusion on its protective effect. On the other hand, colostrum administration consistently confers immunological and microbiota-related benefits, such as reduced systemic inflammation and elevated immunoglobulins. While these findings suggest a potential complementary role for these interventions, where MEN might optimize early gastrointestinal adaptation and colostrum supports mucosal immunity, these observations must be interpreted with caution due to the lack of statistically significant differences in primary clinical outcomes like NEC.

Methodological variation across studies-including differences in intervention duration, dosage, and administration route-limits the precision of direct comparisons and contributes to variability in effect estimates. Despite these constraints, the data provides a basis for further investigating early nutritional and immunomodulatory strategies. A key perspective of this review is that MEN and colostrum may function synergistically; however, to determine how best to integrate these approaches, larger and harmonized multicenter RCTs-specifically designed to reach statistical power for outcomes such as NEC-are urgently needed.

When interventions were examined separately, OBC administration was associated with a numerical, though statistically non-significant, trend toward lower NEC incidence. This pattern theoretically aligns with its proposed

immunomodulatory actions, such as strengthening mucosal immunity and fostering the growth of beneficial gut microbiota. In contrast, trials assessing MEN did not consistently demonstrate protection against NEC, though they remain important for supporting gastrointestinal maturation and improving feeding tolerance in preterm infants. Consistent with the reviewer's observation, it must be emphasized that based on the current data, there is insufficient evidence to confirm a significant clinical effect of either intervention on NEC risk. The lack of statistical significance may be explained by the low baseline NEC incidence in several trials, which limits statistical power, along with variations in intervention protocols-including differences in colostrum dose, feeding volume, and timing of initiation. Moreover, given the multifactorial nature of NEC, in which feeding strategies are only one component of a complex pathogenesis, the observed effects may be diluted (24, 43, 45, 46).

This review provides a comprehensive synthesis of findings from independent RCTs assessing MEN and colostrum, offering an integrated understanding of clinical, immunologic, and metabolic outcomes. However, it is important to note that as no single trial has performed a head-to-head comparison between these two interventions, our comparative insights are based on indirect evidence, which represents a methodological limitation. While both strategies appear safe, low-cost, and biologically plausible, several constraints exist. Specifically, the overall certainty of evidence was rated as 'low' per the GRADE framework due to methodological variability, high risk of bias in some trials, and small sample sizes with wide confidence intervals (imprecision). Consequently, consistent with the current data, there is insufficient evidence to confirm a significant clinical effect of these interventions on NEC incidence, and these findings must be interpreted with caution. Despite these limitations, the collective observations underscore the potential clinical value of MEN and colostrum as components of early neonatal care, though larger, harmonized, and adequately powered multicenter trials are urgently needed to provide definitive conclusions.

Conclusion

In conclusion, this systematic review establishes that both MEN and oropharyngeal colostrum are safe, feasible, and biologically plausible strategies for supporting early gastrointestinal function and immune maturation in preterm infants. Our synthesis confirms that while these interventions offer complementary benefits-specifically in enhancing early weight gain and supporting immunologic markers-there is currently insufficient clinical evidence to confirm a significant reduction in NEC incidence. The primary uncertainty remains whether these strategies can definitively alter major neonatal morbidities across diverse clinical settings. Therefore, rather than generic calls for more research, future studies must prioritize head-to-head comparative trials and standardized dosing protocols to determine the optimal timing and potential synergistic effects of these interventions, particularly in very preterm and high-risk infants. These findings support the continued clinical use of MEN and colostrum as low-cost, supportive components of neonatal care, while highlighting the urgent need for trials powered specifically for primary clinical outcomes like NEC

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Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

Maryam Zarafrooz: Conceptualization; Data curation; Formal analysis; Writing – original draft. Jamalodin Beigjani: Supervision; Project administration; Writing – review & editing.

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Appendix

Table S1. Search strategies of three international databases

Database	Keywords	Results	Date
PubMed	(("colostrum administration"[Title/Abstract] OR "minimal enteral feeding"[Title/Abstract]) AND ("necrotizing enterocolitis"[Title/Abstract] OR Dyspnea[Title/Abstract] OR Dyspnea[MeSH Terms] OR Pneumonia[MeSH Terms] OR Pneumonia[Title/Abstract] OR "Respiratory infection"[Title/Abstract] OR "Respiratory Tract Infections"[MeSH Terms]) AND ("Premature Birth"[MeSH Terms] OR "Premature infant"[Title/Abstract]))	6687	2/11/2025
Scopus	(TITLE-ABS-KEY("colostrum administration") OR TITLE-ABS-KEY("buccal colostrum") OR TITLE-ABS-KEY("minimal enteral feeding") OR TITLE-ABS-KEY("enteral nutrition") OR TITLE-ABS-KEY("enteral feeding")) AND (TITLE-ABS-KEY("necrotizing enterocolitis") OR TITLE-ABS-KEY(dyspnea) OR TITLE-ABS-KEY(pneumonia) OR TITLE-ABS-KEY("respiratory infection") OR TITLE-ABS-KEY("respiratory tract infections")) AND (TITLE-ABS-KEY("premature birth") OR TITLE-ABS-KEY("premature infant"))	373	
WOS	((TI=("colostrum administration") OR AB=("colostrum administration") OR TI=("buccal colostrum") OR AB=("buccal colostrum") OR TI=("minimal enteral feeding") OR AB=("minimal enteral feeding")) AND (TI=("necrotizing enterocolitis") OR AB=("necrotizing enterocolitis") OR TI=(dyspnea) OR AB=(dyspnea) OR TI=(pneumonia) OR AB=(pneumonia) OR TI=("respiratory infection") OR AB=("respiratory infection") OR TI=("respiratory tract infections") OR AB=("respiratory tract infections")) AND (TI=("preterm infant") OR AB=("preterm infant")))	3198	
ProQuest	("colostrum administration" OR "minimal enteral feeding") AND ("necrotizing enterocolitis" OR dyspnea OR pneumonia OR "respiratory infection" OR "respiratory tract infections") AND ("premature infant" OR "premature birth")	78	
Google scholar	("Buccal colostrum administration" OR "minimal enteral feeding") AND ("necrotizing enterocolitis" OR "respiratory infection" OR dyspnea OR pneumonia) AND ("preterm infants")	10 pages= 100	

Table S2. PRISMA checklist for search, screening, and study selection in the present systematic review

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Title page
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Structured abstract (250 words)
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Introduction, paragraphs 1–2
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Last paragraph of Introduction
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Methods → "Inclusion and Exclusion Criteria"
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Methods → "Search Strategy"
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Appendix 1 (full search strings)
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Methods → "Study Selection Process"
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Methods – "Data Extraction" section (Two reviewers extracted data independently using a standardized Excel form; no automation tools used; disagreements resolved by consensus)
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Methods – "Outcomes" section (NEC incidence; feeding progression; weight gain; inflammatory markers; immune biomarkers; microbiota changes; morbidity outcomes such as LOS, ROP, IVH; adverse events)
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Methods – "Data Extraction" (gestational age, birth weight, sample size, intervention type/dose/duration; assumptions documented when unclear)
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	For the narrative synthesis, results are presented using the effect measures reported in the original studies, such as Risk Ratios (RR), Odds Ratios (OR), or Mean Differences (MD) with their respective 95% Confidence Intervals (CIs), as shown in the summary tables.
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Studies were grouped for synthesis based on the type of intervention (MEN vs. OBC). A comparison of study characteristics (population, intervention

Section and Topic	Item #	Checklist item	Location where item is reported
			protocols, and outcomes) was performed to ensure clinical meaningfulness before including them in the narrative synthesis.
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Data were extracted directly from the included studies. In cases of missing summary statistics, we reported the available data as provided by the authors. No data conversions or imputations were performed as no quantitative pooling was conducted.
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Results of individual studies and the synthesis are visually displayed using comprehensive tables (Table 1: Study Characteristics) and a Risk of Bias summary figure. No forest plots were generated as a meta-analysis was not performed.
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Methods – "Data Synthesis" + Results – Table 2
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Not applicable. A formal meta-analysis was not conducted. Data were synthesized narratively due to high clinical and methodological heterogeneity.
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	Not applicable. Statistical quantification of heterogeneity (I^2, τ^2) was not performed as no pooling of data occurred. Heterogeneity was assessed qualitatively by comparing study designs, populations, and intervention protocols
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	Not applicable. Statistical quantification of heterogeneity (I^2, τ^2) was not performed as no pooling of data occurred. Heterogeneity was assessed qualitatively by comparing study designs, populations, and intervention protocols
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Not applicable. Reporting bias was assessed qualitatively by reviewing the completeness of outcomes across studies. Statistical tests for publication bias were not appropriate for this narrative synthesis
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	he certainty of evidence for each outcome was assessed qualitatively using the GRADE (Grading of Recommendations Assessment, Development and Evaluation) framework. Assessments were based on five domains: risk of bias, inconsistency (heterogeneity), indirectness, imprecision (sample size and confidence intervals), and publication bias. Detailed descriptions are provided in the 'Methods' and 'Discussion' sections
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Results – "Study Selection" + Figure 1 (PRISMA flow diagram)
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Results – "Study Selection" (reasons for exclusion reported; 61 full texts assessed, exclusions justified in text and PRISMA flow diagram)
Study characteristics	17	Cite each included study and present its characteristics.	Results → "Study Characteristics" + Table 2
Risk of bias in	18	Present assessments of risk of bias for each	Methods → "Risk of Bias Assessment" +

Section and Topic	Item #	Checklist item	Location where item is reported
studies		included study.	Supplementary Table 1
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Results – Individual Study Findings
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	A narrative synthesis was conducted for the included studies. Characteristics and risk of bias (assessed via ROBINS-I or Cochrane tool) for each contributing study are summarized in the 'Results' section
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Not applicable. A formal meta-analysis was not performed due to clinical heterogeneity. A structured narrative synthesis is provided instead (see Results section)
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	Not applicable. A formal meta-analysis was not performed due to clinical heterogeneity. A structured narrative synthesis is provided instead (see Results section)
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Not applicable. A formal meta-analysis was not performed due to clinical heterogeneity. A structured narrative synthesis is provided instead (see Results section)
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Not applicable. A formal meta-analysis was not performed due to clinical heterogeneity. A structured narrative synthesis is provided instead (see Results section)
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	The certainty of evidence for all outcomes was assessed qualitatively based on the GRADE framework (considering risk of bias, inconsistency, indirectness, imprecision, and publication bias). A summary of this assessment is provided in the 'Discussion' section under 'Strengths and Limitations' (pages 20 and 21)
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Discussion, paragraphs 1–2
	23b	Discuss any limitations of the evidence included in the review.	Discussion → "Geographical and Methodological Considerations"
	23c	Discuss any limitations of the review processes used.	Discussion → "Limitations"
	23d	Discuss implications of the results for practice, policy, and future research.	Discussion → Conclusion
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Methods → "Registration"
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Methods → "Registration"
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	Methods → "Registration"

Section and Topic	Item #	Checklist item	Location where item is reported
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Funding section
Competing interests	26	Declare any competing interests of review authors.	Competing Interests section
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Data Availability section

Table S3. Risk of bias assessment of included randomized controlled trials using the Cochrane risk of bias tool

Row	Study	Random sequence generation	Allocation concealment	Blinding of participants/personnel	Blinding of outcome assessment	Completeness of outcome data	Selective reporting	Other bias	Overall quality
1	(36)	Low risk	Low risk	Unclear risk (open-label)	Low risk (objective measures)	Low risk	Low risk	Low risk	Moderate
2	(32)	Low risk	Low risk	High risk (unblinded staff)	Low risk (blinded assessors)	Low risk	Low risk	Low risk	Moderate
3	(19)	Low risk	Low risk	High risk (impossible to blind)	Low risk	Low risk	Low risk	Low risk	Moderate
4	(35)	Low risk	Low risk	Unclear risk	Low risk (ophthalmologist blinded)	Low risk	Low risk	Low risk	Moderate
5	(40)	Low risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	Low risk	Moderate
6	(14)	Low risk	Low risk	Low risk (double-blind)	Low risk	Low risk	Low risk	Low risk	High
7	(39)	Low risk (block randomization + software)	Low risk (sealed opaque envelopes)	Low risk (double-blind: parents, evaluator, and analyst blinded)	Low risk (blinded neonatal specialist)	Low risk (68/70 completed)	Low risk (all outcomes reported)	Low risk (protocol registered in IRCT)	High
8	(33)	Low risk (block randomization with Sealed Envelope software)	Low risk (opaque covered syringes)	Low risk (all staff and parents blinded)	Unclear risk (not clearly described)	Low risk (no dropout reported)	Low risk (all outcomes reported)	Low risk (IRCT registered: IRCT20220509054798N1)	Moderate
9	(38)	Low risk (computer-generated random table, 1:1)	Unclear risk (not described)	Unclear risk (not explicitly stated for caregivers or parents)	Low risk (objective outcomes: ELISA for sIgA)	Low risk (9 dropouts reported with reasons)	Low risk (all planned outcomes reported)	Low risk (ethics approved, trial registered: ChiCTR2100046645)	Moderate
10	(37)	Unclear (no specific mention of random sequence method)	Unclear (allocation process not described)	High risk (no blinding of caregivers or participants mentioned)	Low risk (cytokine measurement likely blinded and objective)	Low risk (minor dropouts; reasons stated)	Low risk (no evidence of selective reporting)	Low risk (baseline characteristics balanced)	Moderate
11	(31)	Low risk (computer-generated block randomization stratified by birth weight)	Low risk (sealed, opaque envelopes used)	Low risk (double-blind; nurses and investigators blinded)	Low risk (outcomes are objective; blinding maintained)	Low risk (monitoring in place; dropout rate estimated and accounted for)	Low risk (protocol published; pre-registered trial)	Low risk (multi-center design; robust methodology)	High
12	(34)	Low risk (computer-generated randomization)	Low risk (opaque envelopes; managed by independent personnel)	High risk (intervention not blinded to nurses)	Low risk (blinded outcome analyst)	Low risk (8 dropouts with clear reasons; outcomes assessed for 252/260 infants)	Low risk (protocol and trial registration ChiCTR1900023697; all prespecified outcomes reported)	Moderate risk (early study termination; same statistician for interim analysis and final data)	Moderate

Row	Study	Random sequence generation	Allocation concealment	Blinding of participants/personnel	Blinding of outcome assessment	Completeness of outcome data	Selective reporting	Other bias	Overall quality
13	(41)	High risk (non-randomized, historical control used)	High risk (group allocation not concealed)	High risk (open-label; caregivers aware of group allocation)	Moderate risk (outcomes extracted from records; potential detection bias)	Moderate risk (historical control data may be incomplete; losses <20% addressed in protocol)	Low risk (detailed protocol; registered trial: ReBEC RBR-2cyp7c; ethics approval documented)	High risk (non-randomized design; protocol still under implementatio; external validity limited)	High