ARTICLE

The effects of early vs late human or bovine milk fortification on growth and safety parameters in low birth-weight preterm neonates: A Systematic Review and Meta-analysis

Clayton John B. Chua, Roffel D. Felisilda, Christine M. Guzman

BACKGROUND: Premature infants benefit from human milk fortifiers (HMF) for optimal growth, nutrition, neural development and reducing complications. However, the ideal timing for HMF introduction is still debated due to preterm infant's immature digestive system and inconclusive study results, often marred by methodological flaws.

OBJECTIVE: : To compare the effects of early human milk fortification (EHMF) vs late human milk fortification (LHMF) on low birthweight (LBW) preterm neonates.

MATERIALS AND METHODS: A systematic search for randomized controlled trials comparing EHMF vs LHMF on LBW preterm neonates was conducted, covering databases such as Cochrane CENTRAL, Pubmed, Google Scholar and clinical trials registries iCRTP, ISRCTN and HERDIN from January 2000 to July 2024. Statistical analysis was performed using Review Manager version 5.3, with mean differences for continuous outcomes and risk ratio (RR) for dichotomous outcomes. A sensitivity analysis using the leave-one-out method and meta-regression was conducted to address potential confounding factors and publication biases were assessed through funnel plots.

RESULTS: The EHMF group showed potential benefits in reducing the incidence of retinopathy of prematurity (ROP) and neonatal sepsis, achieving a faster time to regain birth weight. However, there may be an increased risk of bronchopulmonary dysplasia and the effects of necrotizing enterocolitis and feeding intolerance (FI) may be unclear. Interestingly, hospital outcomes (length and time to reach full feeds) and adverse effects (extra-uterine growth restriction) no significant difference between groups were noted.

CONCLUSION: EHMF could impact growth parameters and reduce adverse outcomes, although most effects were not significant. The wide confidence intervals around some effect sizes suggest a need for more robust studies to resolve these uncertainties.

KEYWORDS: *preterm, milk fortification, enrichment, early vs late, RCT*

Correspondence: Clinical Trial and Research Division
Clinical Research Department
Philippine Children' s Medical Center Quezon Ave., Quezon City
Philippines
Phone: (02) 8588 9900 local 1308
Email: ctrd@pcmc.gov.ph

The authors affirm that this manuscript is original, has been read and approved by all contributing authors, that each author meets the established criteria for authorship, and that the content represents a truthful and accurate account of the work conducted.

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Introduction

In 2020, an estimated 13.4 million babies were born prematurely, and around 900,000 children died in 2019 due to complications of preterm birth.[1] The neonatal mortality rate in the Philippines stands at 14.23 per 1000 live births with a LBW of 13%.[2,3] Over the past few decades, there has been a rising trend in preterm survival rates, and the key to management is ensuring adequate nutrition to enhance both short-term and long-term outcomes.[4]

Neonates born prematurely miss some or even all the crucial period during the third trimester, characterized by nutrient accretion and growth. However, there is great potential for improvement in the nutritional care of preterm infants. Breast milk alone does not provide enough nutrients for preterm infants; it lacks the recommended daily amount of iron, zinc, calcium, phosphorous and vitamins A and D.[5,6.] Preterm infants are at high risk for mineral deficiency, which can lead to increased risk for osteopenia of prematurity since calcium and phosphorous deposition occurs during the last trimester of pregnancy.[7,8] Additionally, their gastrointestinal tract immaturity, including reduced gastrointestinal motility and enzyme activity, along with corticosteroid therapy, further hinders their growth.[9,10] Due to these factors, most preterm infants fail to show adequate growth during the first few weeks of life, and they do not achieve expected growth parameters by term gestation.[11,12,13]

Total parenteral nutrition (TPN) is beneficial for providing early aggressive nutrition and improving long-term outcomes for preterm infants. However, it can also lead to some complications such as transient metabolic acidosis, hyperlipidemia, azotemia, parenteral nutrition-associated liver disease (PNALD) and central line associated bloodstream infection (CLASBI)[14] if administered for a prolonged period. Therefore, it is essential to prioritize early enteral feeding and to initiate full enteral feeding as soon as the condition allows. A day of depriving infants of enteral nutrition can increase the risk of extrauterine growth restriction by 8%.[15]

In addressing the impact on growth parameters and adverse clinical outcomes in LBW preterm neonates, it is essential to consider how does EHMF compare to LMHF.

The study aims to provide vital insights for clinicians and neonatologists on the safety and effectiveness of EHMF in preterm neonates. This comprehensive systemic review and meta-analysis seek to establish a strong foundation for practice guidelines and drive progress in future research, with a specific focus on short-term and long-term outcomes on preterm infants. Although recent

clinical trials highlights the benefits of EHMF, they have not shown significant differences in complication rates, particularly necrotizing enterocolitis (NEC), between milk fortification groups. As a result, there is an urgent need for a consensus on the optimal timing for initiating HMF, as practices for its onset vary widely worldwide. LHMF, often starting at TFR 100ml/kg/day due to traditional or fear-driven approaches, can lead to complications despite careful parenteral and enteral feeding strategies. It is imperative to balance these approaches and potential risk factors, such as feeding intolerance (FI), sepsis, NEC and mortality.[16] This delicate balance demands the utmost attention and consideration, as the decisions made can profoundly impact neonatal care.

Defining EHMF and LHMF yields varied results. Some studies suggest EHMF as fortification starting from the 1st feeding to 40mg/kg/day and LHMF as 75 to 120ml/kg/day. EHMF is already gaining wide acceptance in several NICUs worldwide, as evidenced by the multiple feeding protocols published in clinical reports.[59,60,61] This promising trend suggests potential benefits for the future of neonatal care.

Recent systemic review and meta-analysis studies from 2020 and 2021 with 2 and 3 studies respectively, indicate a trend of non-significant effects on growth parameters, hospital course and clinical outcomes associated with EHMF.[34,35] These studies emphasize the urgent need for future research and standardized approach on feeding protocols and initiation of HMF. Current variability in feeding practices could result in delayed nutrition and extended hospital stays,[34,35,62] emphasizing the need for prompt action to tackle these challenges.

Despite recent RCT and meta-analysis showing the benefits of EHMF and no significant differences in the rates of complications especially NEC, there is still a wide range of practice variations in the introduction of HMF. In the Philippines and our institution, the predominant approach is still the traditional and fear-driven method, which involves starting fortification late at 100ml/kg/day.

Study Objectives

General

To compare the effects of EHMF vs LHMF on growth parameters and safety in LBW preterm infants.

Specific Objectives

To compare the effects of early vs late fortification of breastmilk in LBW preterm neonate, with possible benefits in the following:

- Weight in g/kg/day

- Length in cm/week
- HC in cm/week
- Days to full feeds
- Days of TPN
- Days of Hospital stay
- Days to regain BW

To compare the effects of EHMF vs LHMF on adverse clinical outcome rates in LBW preterm neonates

- NEC
- Neonatal sepsis
- Bronchopulmonary Dysplasia (BPD)
- Retinopathy of Prematurity (ROP)
- FI
- EUGR
- Death

Materials and Methods

This systemic review and meta-analysis followed the Preferred Reporting Items for Systemic review and Meta -analysis (PRISMA) guidelines and was conducted between April to September 2024.

Inclusion Criteria

Population: Preterm infants low birth weight neonates

Intervention: Human milk feeding (MoM, DBM, PBM) with EHMF (either human or bovine derived) starting when feeding is at $\leq$ 40ml/kg/day

Control: Human milk feeding (MoM, DBM, PBM) with LHMF (either human or bovine derived) starting when feeding is at $\geq$ 75ml/kg/day

Outcome: Primary: comparison of growth parameters (weight in g/kg/day, length and HC in cm/week) and incidence of NEC. **Secondary:** comparison on the ff: adverse effects (Neonatal sepsis, BPD, ROP, FI, Extra-uterine growth restriction (EUGR), Death) and hospital outcomes (days to full feeds, days of TPN, days of hospital stay, days to regain BW)

Method: Published RCT or Clinical Trial written in English language with publication dates 2000-2024.

Exclusion Criteria

- **Population:** Preterm with multiple congenital anomalies or chromosomal abnormalities
- **Intervention and Control:** Mother could not provide own milk and refusal to use DBM, PBM and use of formula milk
- **Methods:** Studies other than RCT, non-English language publications, unavailability of full text article and unpublished data.

Search Strategy

We conducted a comprehensive search inclusive of a wide range of sources, including Cochrane CENTRAL, MEDLINE via Pubmed, Google Scholar, English Databases and published clinical trials through the iCRTP, ISRCTN and HERDIN from 2000-2024.

The search was guided by a combination of medical subject headings (MeSH) term and free text used were preterm OR premature, AND low birth weight AND milk fortifier OR milk fortification, AND enrichment, AND early AND delayed OR late fortification AND randomized controlled trials OR RCT OR controlled clinical trial OR clinical trial. Studies not meeting the inclusion criteria were excluded and the remaining studies were retrieved and reviewed.

Eligibility Screening

The primary investigator and co-investigator performed an independent and thorough review of abstracts and full-text articles for eligible studies generated by the search strategy. In the presence of discrepancies between the two reviewers, disagreements were resolved by consulting an independent reviewer.

Risk of Bias in individual studies

The Cochrane Collaboration GRADE (2011) was used to categorize the ROB and level of evidence of the included studies into three levels: Low, Unclear or Moderate and High risks. The following trial characteristics were appraised: Random sequence generation, allocation concealment, blinding of participants, personnel and outcome assessment, incomplete data outcome, selective reporting and others. The answers to all domains of bias based on the algorithm generate a proposed judgment on the ROB. A low-risk bias indicates low-risk assessment in all domains; unclear risk means unclear risk assessment for all domains; and high-risk denotes a high-risk assessment in one or more key domains.

Data Collection

The primary researcher and the co-investigator extracted the data of eligible studies and placed in a data collection form and tabulated where population, intervention, comparator, outcome and methodology were included. For any missing data of interest, the investigators contacted the study authors to provide the data, which was then computed based on appropriate statistical tools.

Statistical analysis

Mean differences were calculated for continuous outcomes. Risk ratio (RR) with 95% CI were calculated for dichotomous data. Heterogeneity was assessed using the Chi-squared test for heterogeneity and the I^2 statistics. The effect size (MD or RR) was calculated for each study and then pooled using a fixed-effect model to obtain an overall

effect size. 95% CI were calculated to estimate the precision of the pooled effect size. Statistical analysis was performed using Review Manager version 5.3.

Sensitivity analysis was done using the leave-one-out method to evaluate how results would change if studies were excluded one at time from the analysis. Meta-regression analysis was performed to evaluate the effect of any confounding factor for outcomes.

RESULTS

Figure 1 is a summarized flowchart of the study selection process. 1004 potentially relevant articles were retrieved using the search strategy - excluded articles at each stage with reasons indicated. The remaining six full-text articles were reviewed and included in the systemic review and meta-analysis.

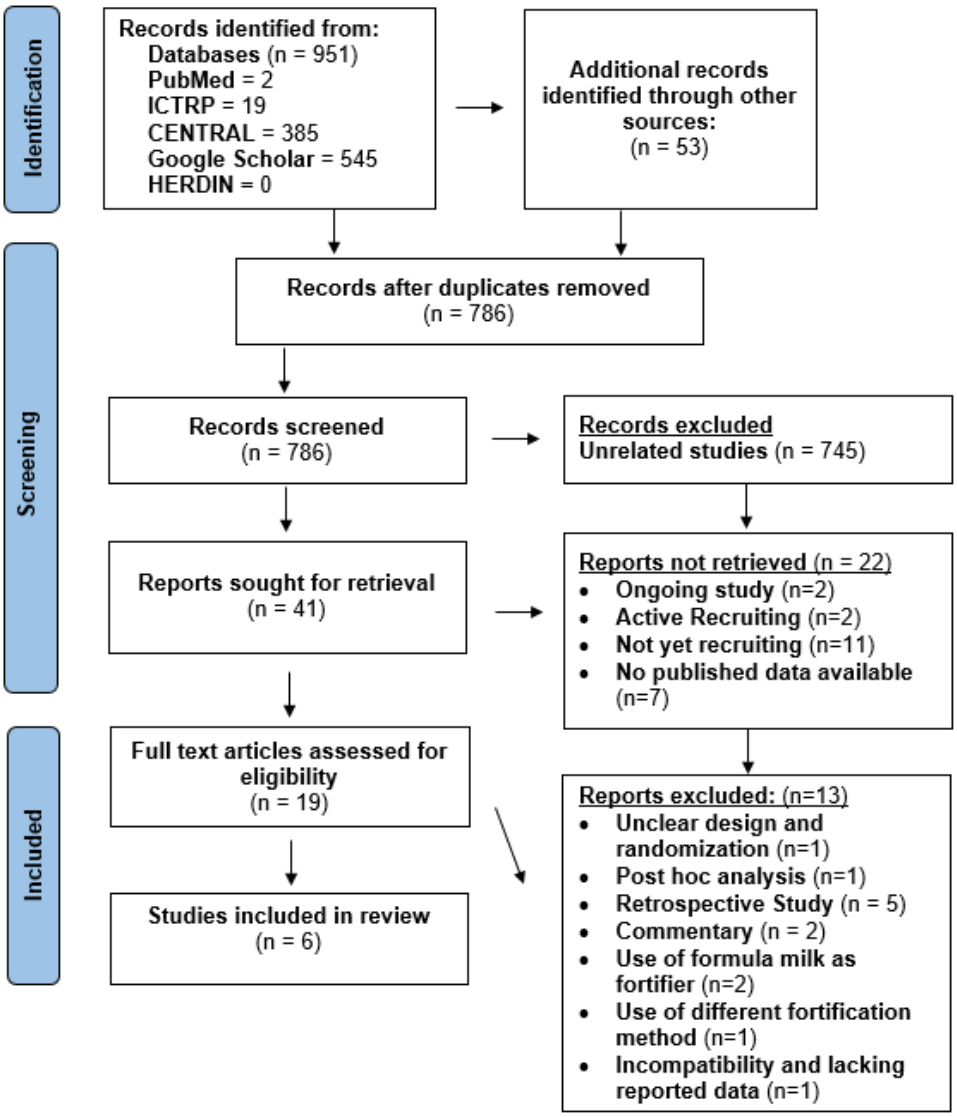

Figure 1. *PRISMA Flow Diagram of Study Selection*

Study Characteristics of Individual Studies

All six studies,[63-68] were prospective RTCs comparing the effects of EHMF vs. LHMF in preterm neonates. A total of 599 participants were involved, with 50.4% allocated to the early fortification group and 49.6% to the late fortification group.

Two studies[63-64] were conducted in a multi-center setting, one in several centers in the USA and the other in USA and Austria, while the remaining studies were single-center studies.[65-68] Two studies were conducted in the USA; one from Iran and one in India. Detailed study characteristics for each investigation are provided in **Table 1.**

Interventions

Study interventions varied in term of fortifiers used. Four studies[64-68] used various CMBF such as Aptamil FMS,[69] Enfamil Liquid HMF,[70] Similac HMF[71] and Lactodex[72], while two studies[63,66] used HMBF like Prolact+ H^2MF.[73]

The studies also differed in the form of fortifiers used, with 67% using liquid-based HMF[63,64,66,67] and 33% using powder-based HMF.[65,67] Additionally, the studies varied in the methods for commencing fortification, showing diversity in research approaches. Despite this, feeding increments were comparable across all studies.

Early fortification was started at different feeding levels, with 20.5% of cases starting during the first feedings,[65,67] 37.7% at 20-25ml/kg/day,[64,66] 18.3% at 30ml/kg/day[68] and 23.5% at 40ml/kg/day.[63]

Late fortification was initiated at 12.1% of cases at 75ml/kg/day,[65] 27.3% at 80ml/kg/day,[67,68] at 39.4% at 100ml/kg/day[63,64] and 21.2% at over 120ml/kg/day.[68]

Table 1: *Characteristics of the Studies Included*

Author	Sullivan	Taheri	Shah	Salas	Wynter	Gupta
Location	USA Austria	Iran	USA	USA	USA	India
Year	2010	2016	2016	2023	2024	2024
Methods						
Design	RCT	RCT	RCT	RCT	RCT	RCT
Blinding	Unblinded	Double Blind	Unclear blinding	Single Blind	Double Blind	Unblinded
Study Site	Multi-center	Single cen-ter	Multi-center	Single center	Single center	Single center
Sample Size	207	80	99	150	52	120

Inclusion	BW 500-1250g 27-29 weeks AOG Intention to receive MoM Ability to adhere to feeding protocol on the basis of use of MoM Initiation of PN within 48 hours of birth Initiation of feeding within 21 days after birth	BW < 2000g 28-34 weeks AOG	BW < 1500g	≤ 28 weeks AOG	BW 1000-1500g Admission to NICU within 24 hours of birth Maternal intent to supply BM and informed consent for the use of donor human milk	≤ 32 weeks AOG
Exclusion	Major Congenital Anomalies Likelihood of transfer to a non-study institution during the study period	Major congenital anomalies Transferred to another hospital Did not attend neonatal clinic for ff up visits Fed with formula milk	Major congenital anomalies or chromosomal abnormality Died or expected to die w/ in 72 hrs Mother could not provide own milk and refuse to use donor breast milk	Major congenital anomalies Terminal illness in whom decision to withhold or limit life support have been made	Major congenital anomalies BW < 3rd percentile for GA and sex on Fenton curve Enteral feeds not initiated within 24hours of birth	Major congenital anomalies Hemodynamically unstable neonates Surgical problems
Intervention						
Type of Milk used	Pasteurized Human Milk	Breast Milk	Breast Milk	Breast Milk Donor milk	Breast Milk	Breast Milk Pasteurized DBM
Fortifier used	**Prolact+ H²MF;** Prolacta Bioscience, Monrovia, California **(Donor HMBF)**	**Aptamil FMS powder** Nutricia GmbH, Erlangen, Bavaria, Germany **(Bovine)**	**Enfamil**; Mead Johnson, LLC, Evansville, IN **(Bovine)**	**Prolact,** Prolacta Bioscience, Inc, CA **(Donor HMBF)** **Enfamil Liquid HMF, High protein** Mead Johnson Nutrition, Evansville, IN **(Bovine)**	**Enfamil Liquid HMF, High protein** Mead Johnson Nutrition, Evansville, IN **(Bovine)**	**Lactodex HMF** Raptakos, Brett & Co, Ltd. **(Bovine)**

Form	Liquid	Powder	Liquid	Liquid	Liquid	Powder
Early Fortification (EHMF)	TFR 40ml/kg/day	1st feeds	TFR 20ml/kg/day	TFR 20-25ml/kg/day	1st feeding	TFR 30ml/kg/day
Late Fortification (LHMF)	TFR 100ml/kg/day	TFR 75ml/kg/D	TFR 100ml/kg/day	TFR >120ml/kg/day	TFR 80ml/kg/day	TFR 80ml/kg/day
Outcome						
Primary	Duration of TPN	Difference in growth indices weight, Length, HC	Number of days to reach full feeds >140ml/kg/D	FFM for age, z score at 36 weeks PMA or discharge Body composition measurements at 36 weeks PMA or hospital discharge converted to z score values	Weight velocity in g/kg/day at 28th DOL HC and length at 28th DOL	Days to regain BW Days to reach full feeds FI BCS proven sepsis
Secondary	Daily Weight Weekly recumbent length and HC Ventilator Days O2 Days ROP BPD LONS NEC FI	NEC Neonatal Sepsis FI	Daily weight NEC FI Length, HC and Weight velocity 4 wks after birth and 36 wks PMA Days till BW is regained TPN Days Days of hospital stay Ventilator days Protein and Caloric intake for the 1st 4 weeks of life Milk Intolerance LONS BPD PDA Severe IVH (Grade 3-4) PVL ROP	Weight gain velocity in g/kg/d from birth to 36 weeks PMA Declines in weight for age z score from birth to 36 weeks Length gain velocity (cm/wk) from birth to 36 weeks PMA measured with length boards Declines in length for age z score from birth to 36 weeks HC gain velocity in cm/wk measured with a flexible tape measure Declines in HC for age z score from birth to 36 weeks SIP NEC Death	NEC Metabolic acidosis ROP LONS NPO occurrences	Weight gain velocity in g/kg/d Length gain in cm/wk HC gain (cm/wk) Duration of hospital stay in days

Risk of Bias in Individual Studies

We assessed the methodological quality of six studies.[63-68] **(Figure 2a and 2b).** Overall, the study level ROB was moderate. Despite low ROB in some areas, some uncertainties and potential sources of bias persists. Most studies adequately conducted random sequence generation and allocation concealment, suggesting a low risk of selection bias. However, unclear allocation concealment in some studies and the lack of blinding raises concerns about performance bias. Unclear detection bias in most studies [63,65-68] poses challenges in assessing reliability in their findings. Low risk for attrition bias due to short follow up periods and nature of study populations. Low risk for reporting bias on all studies as they offer comprehensive results including both positive and negative findings. Unclear risk concerning other potential sources of bias in all studies[63-68] due to insufficient information to make a comprehensive assessment beyond the mentioned domains.

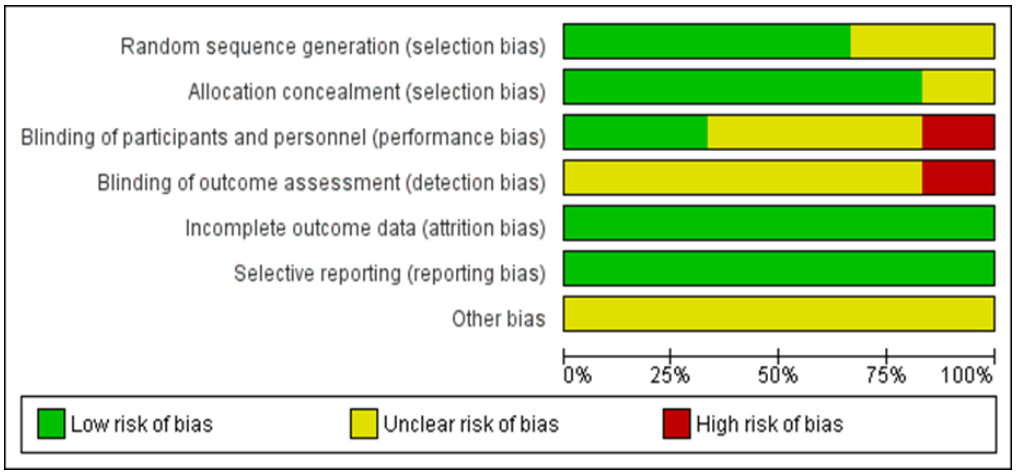

Figure 2a: *Risk of Bias Assessment Graph of included studies using the Cochrane GRADE Tool*

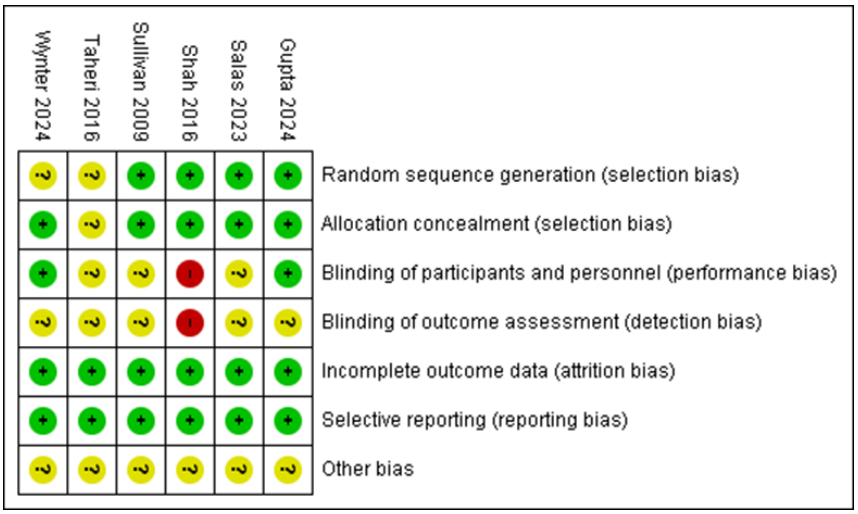

Figure 2b: *Risk of Bias Assessment Summary of included studies using the Cochrane Grade tool*

RESULTS

1. Change in growth parameters

1.1 Weight (g/kg/day)

Data for analysis of this outcome were available from three trials,[63,64,68] representing the average difference in weight gain between EHMF and LHMF groups. The combined mean difference between groups is 0.43 g/kg/day (95% CI of -0.36 to 1.21 g/kg/day), indicating more weight gain for the EHMF group, but was not statistically significant between groups. No significant heterogeneity between studies noted. ($Chi^2 = 1.51$, df = 2, p = 0.47, $I^2 = 0\%$)

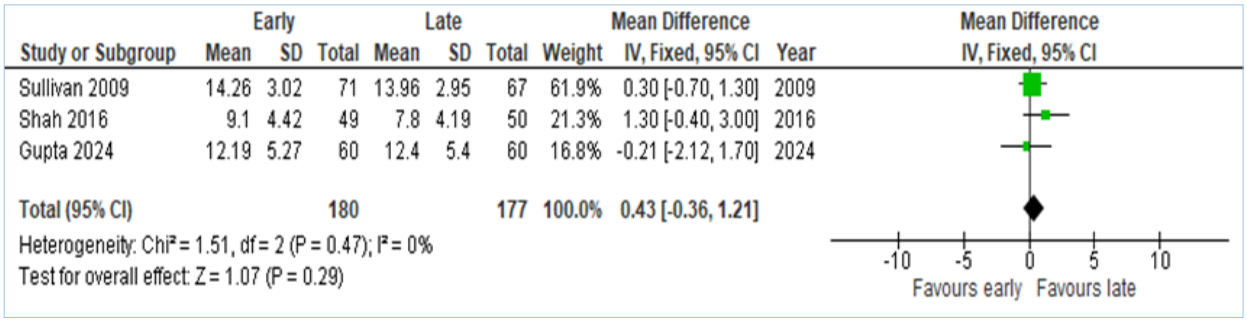

Figure 3. *Forest plot of comparison: EHMF vs LHMF, outcome: Weight gain in g/kg/day*

1.2 Length (cm/week)

Data for analysis of this outcome were available from three trials,[63,64,68] representing the average difference in length gain between EHMF vs LHMF groups. The combined mean difference between groups is 0.02 cm/week (95% CI of -0.05 to 0.09 cm/week), indicating no statistically significant difference in length gain between groups. The high precision of pooled effect indicates no difference between groups. No significant heterogeneity between studies noted. ($Chi^2 = 2.27$, df = 2, p = 0.32, $I^2 = 12\%$)

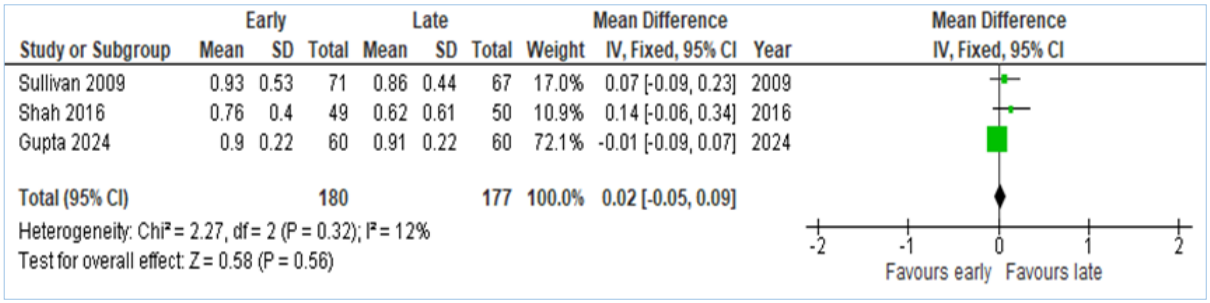

Figure 4. *Forest plot of comparison: EHMF vs LHMF, outcome: Length gain in cm/week*

1.3. Head Circumference (cm/week)

Data for analysis of this outcome were available from three trials,[63,64,68] representing the average difference in head circumference gain between EHMF and LHMF groups. The combined mean difference between groups is 0.005 cm/week (95% CI of -0.001 to 0.011 cm/week), indicating no statistically significant difference in HC gain between the EHMF and LHMF groups. The high precision of the pooled effect indicates no difference between groups. No significant heterogeneity between studies noted. ($Chi^2 = 1.38$, df = 2, p = 0.50, $I^2 = 0\%$)

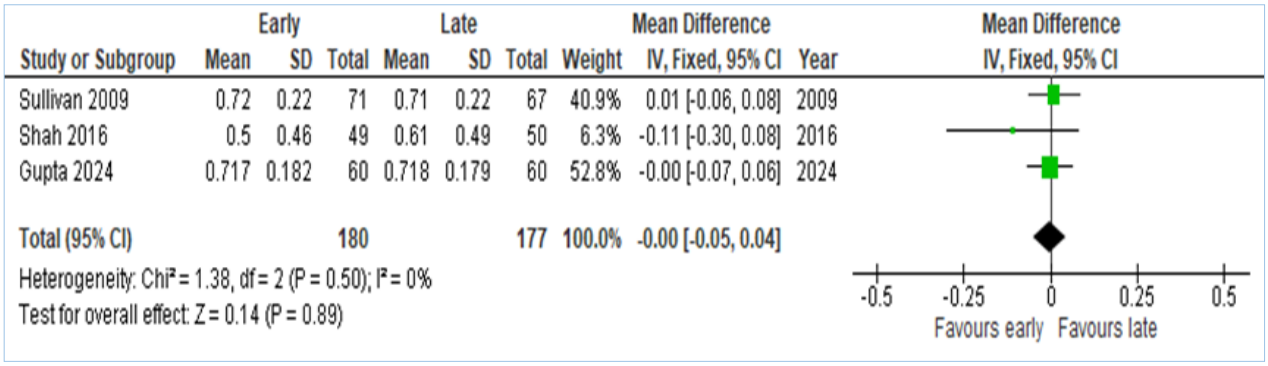

Figure 5. *Forest plot of comparison: EHMF vs LHMF, outcome: Head circumference gain in cm/week*

2. Days to reach full feeds

Data for analysis of this outcome were available from four trials.[63,64,67,68] The overall effect size between EHMF and LHMF groups (Mean Difference = 0.16, 95% CI: -0.23, 0.54) indicates no statistically significant difference in mean days to full feeds between groups.

The high precision of the pooled effect indicates no difference between groups. Neither group consistently reached full feeds significantly faster or slower than the other. No significant heterogeneity between studies noted. ($Chi^2 = 1.74$, df = 3, p = 0.63, $I^2 = 0\%$)

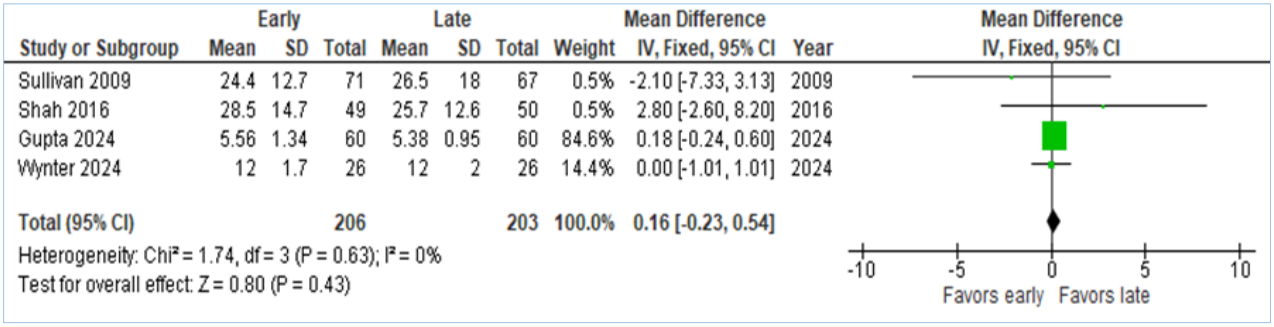

Figure 6. *Forest plot of comparison: EHMF vs LHMF, Outcome: Days to reach full feeds*

3. Days of TPN

Data for analysis of this outcome were available from three trials.[63,64,67] The overall effect size between EHMF and LHMF groups (Mean Difference = -0.03, 95% CI: -1.92, 1.87) indicates no statistically significant difference in mean days of TPN between groups.

No significant heterogeneity between studies noted. ($Chi^2 = 0.77$, df = 2, p = 0.68, $I^2 = 0\%$)

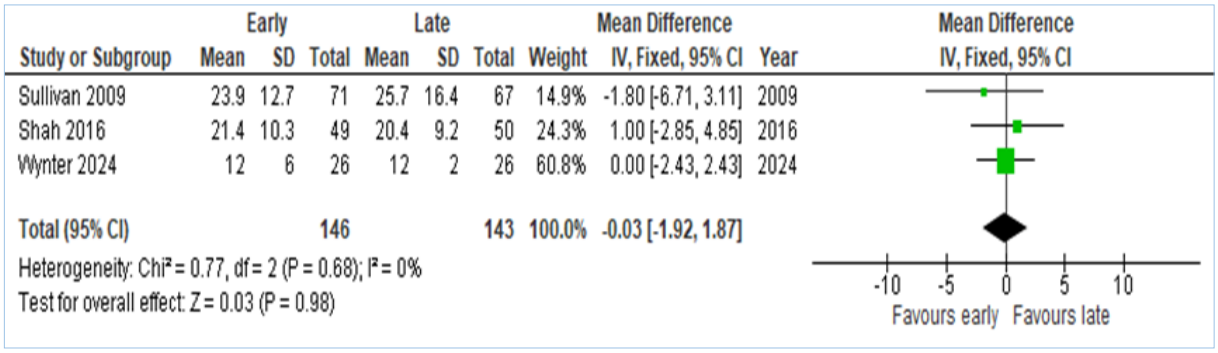

Figure 7. *Forest plot of comparison: EHMF vs LHMF, Outcome: Days on TPN*

4. Days of Hospital Stay

Data for analysis of this outcome were available from four trials.[64,64,67,68] The individual studies show varying mean differences in days of hospital stay, with some favoring the EHMF and others favoring the LHMF group. However, there needs to be a consistent pattern across studies. The overall effect size between groups (Mean Difference = 0.36, 95% CI: -3.68, 4.39) indicates no statistically significant difference in mean days of TPN between the EHMF and LHMF groups. No significant heterogeneity between studies noted. ($Chi^2 = 0.40$, df = 3, p = 0.94, $I^2 = 0\%$)

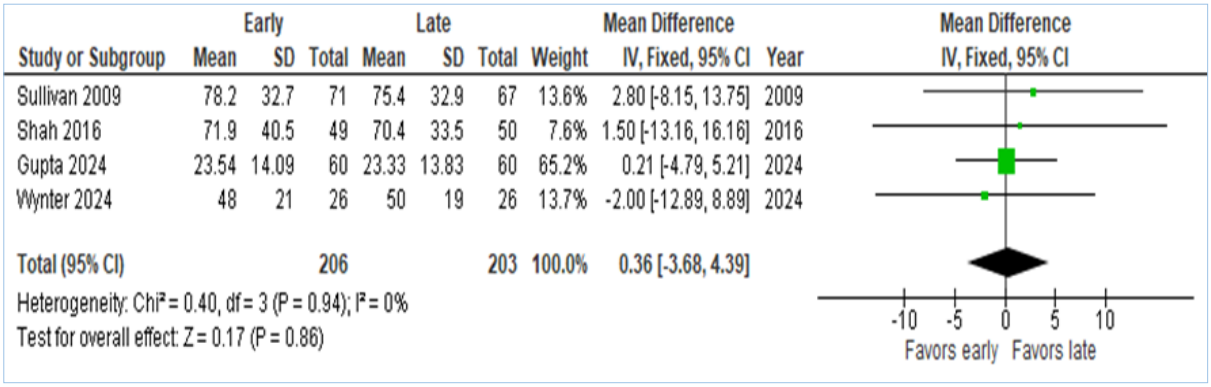

Figure 8. *Forest plot of comparison: EHMF vs LHMF, Outcome: Days of Hospital Stay*

5. Days to Regain BW

Data for analysis of this outcome were available from three trials with 180 newborns.[63,64,68] It is evident in the forest plot that the EHMF group has faster time to regain BW, (Mean Difference = -0.68, 95% CI: -1.50, 0.13) but, the overall effect size between groups indicates no statistically significant difference in mean days to regain BW between the EHMF and LHMF groups. No significant heterogeneity between studies noted. (Chi^2 = 2.48, df = 2, p = 0.29, I^2 = 19%)

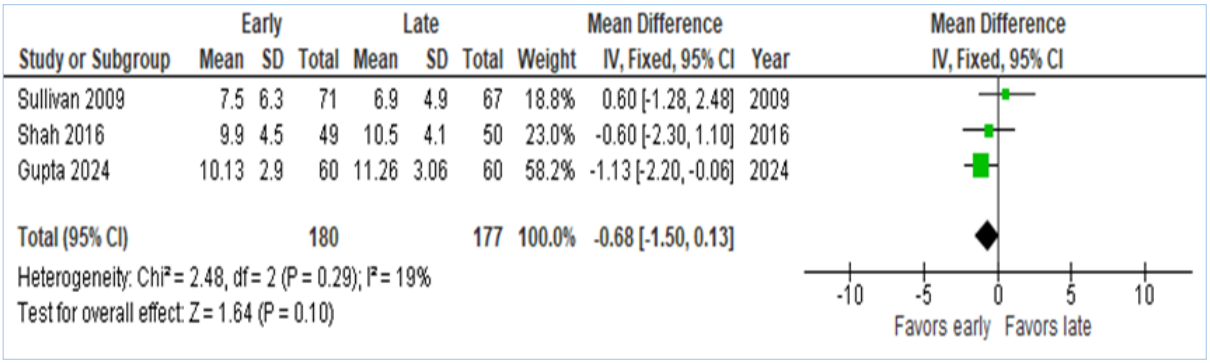

Figure 9. *Forest plot of comparison: EHMF vs LHMF, Outcome: Days to regain BW*

Adverse events

1. NEC

Data for analysis of this outcome were available from six trials.[63-68,] The estimated risk ratio 1.35 (95% CI 0.85 to 2.14) indicates a slightly higher risk for the EHMF group but was no statistical significance in NEC incidence between EHMF and LHMF groups. No evidence of heterogenicity across studies noted. (Chi^2 = 2.61, df = 5, p = 0.76, I^2 = 0%)

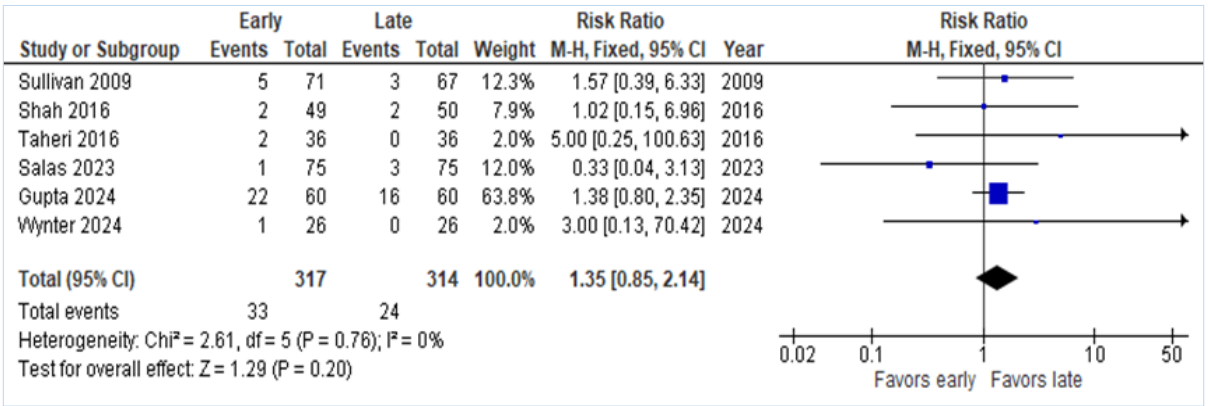

Figure 10. *Forest plot of comparison: EHMF vs LHMF, Outcome: NEC*

2. Neonatal sepsis

Data for analysis of this outcome were available from five trials.[63-65,67,68] The pooled effect for neonatal sepsis suggests a potentiation benefit of EHMF, however the estimated risk ratio 0.81 (95% CI 0.51 to 1.30) indicates no significant difference in the incidence of neonatal sepsis between EHMF and LHMF groups. No evidence of heterogenicity across studies noted. ($Chi^2 = 1.57$, df = 3, p = 0.67, $I^2 = 0\%$)

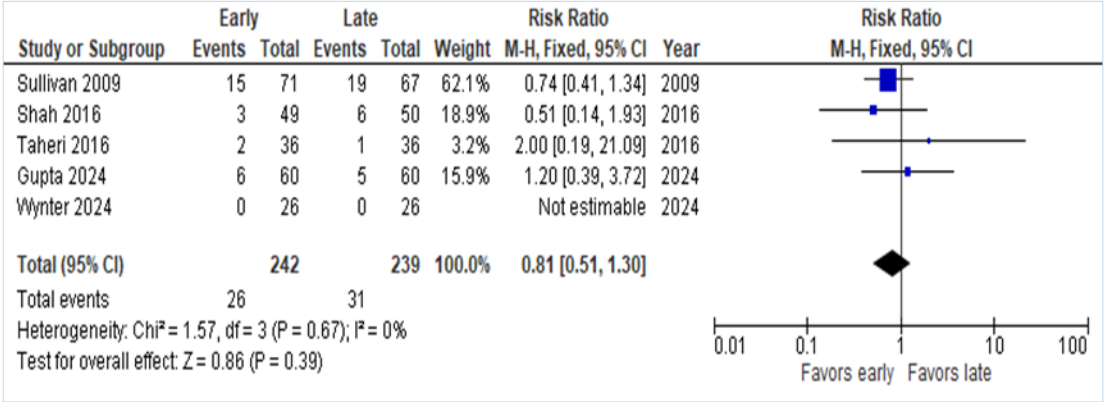

Figure 11. *Forest plot of comparison: EHMF vs LHMF, Outcome: Neonatal sepsis*

3. BPD

Data for analysis of this outcome were available from three trials.[63,64,67] Though the forest plot would suggest a possible increased risk for BPD with the EHMF group, the estimated risk ratio of 1.19 (95% CI 0.82 to 1.74) indicates no significant difference in the incidence of BPD between EHMF and LHMF groups. No evidence of heterogenicity across studies noted ($Chi^2 = 0.33$, df = 2, p = 0.85, $I^2 = 0\%$)

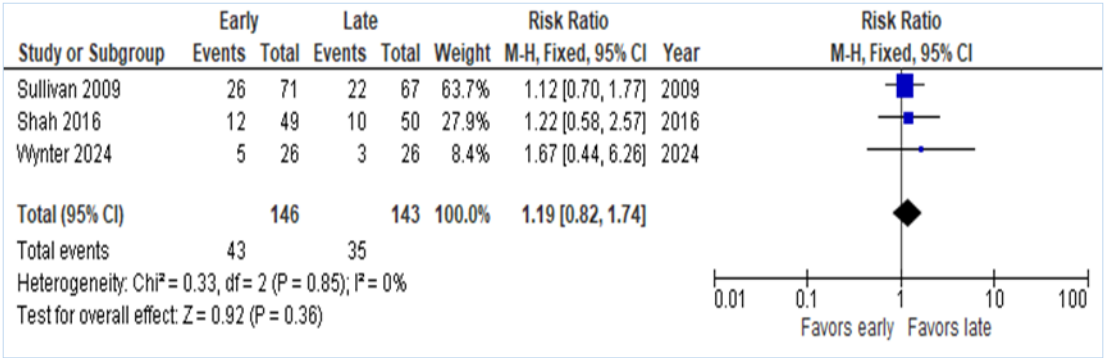

Figure 12. *Forest plot of comparison: EHMF vs LHMF, Outcome: BPD*

4. ROP

Data for analysis of this outcome were available from three trials.[63,64,67] The pooled effect shows a potential decrease in the incidence of ROP with the EHMF group, but the estimated risk ratio of 0.79 (95% CI 0.53 to 1.17) indicates no significant difference in the incidence of ROP between EHMF and LHMF groups. No evidence of heterogenicity across studies noted (Chi² = 0.17, df = 1, p = 0.68, I² = 0%)

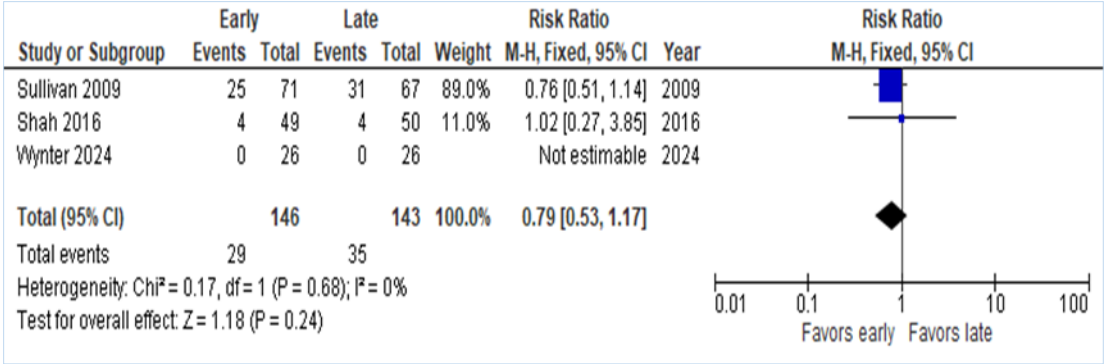

Figure 13. *Forest plot of comparison: EHMF vs LHMF, Outcome: ROP*

5. FI

Data for analysis of feeding tolerance were available from six trials.[63-68] The forest plot indicates a potential increase in FI in the LHMF group, however, the estimated risk ratio of 1.11 (95% CI 0.87 to 1.43) indicates no significant difference in the incidence of FI between EHMF and LHMF groups. No evidence of heterogenicity across studies noted. (Chi² = 1.5, df = 4, p = 0.83, I² = 0%)

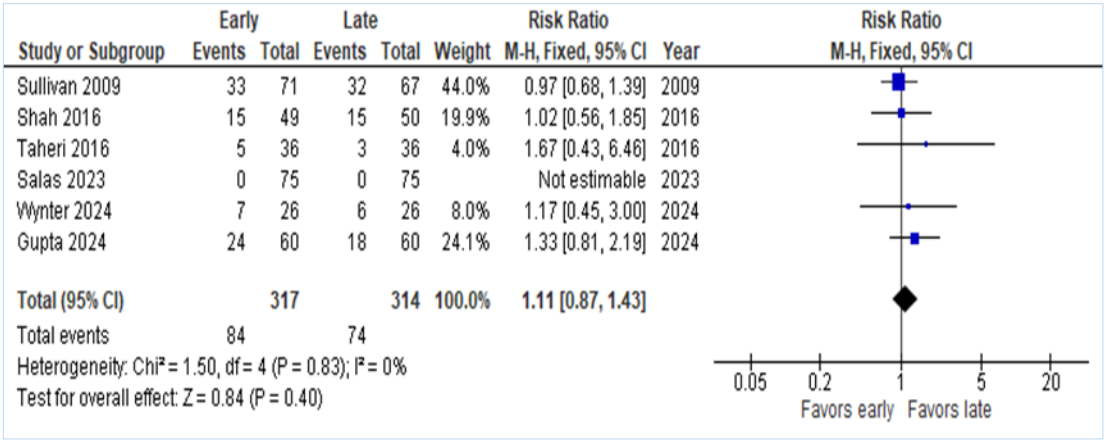

Figure 14. *Forest plot of comparison: EHMF vs LHMF, Outcome: FI*

6. EUGR

Data for analysis of this outcome were available from three trials.[63,64,66] The forest plot indicates a slightly lower risk in the incidence of EHMF group, however, the estimated risk ratio of 0.93 (95% CI 0.75 to 1.15) indicates no significant difference in the incidence of EUGR between EHMF and LHMF groups. The high precision of the pooled effect indicates no difference between groups. No evidence of heterogenicity across studies noted. ($Chi^2 = 2.14$, df = 2, p = 0.34, $I^2 = 7\%$)

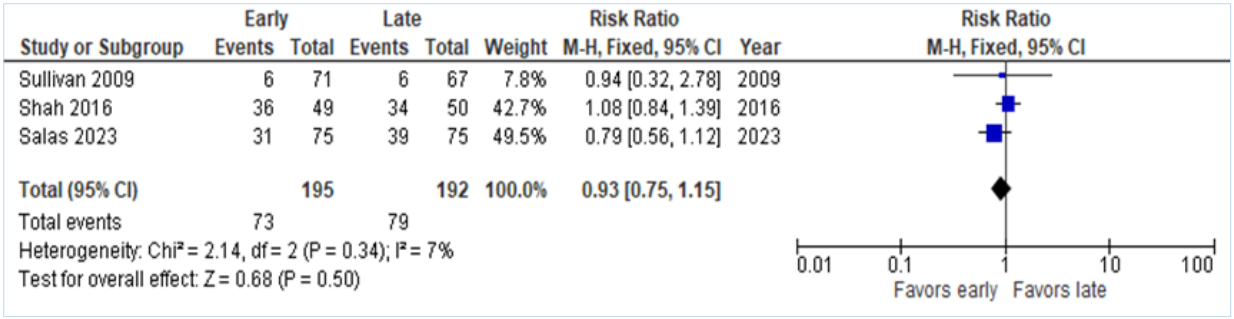

Figure 15. *Forest plot of comparison: EHMF vs LHMF, Outcome: EUGR*

7. Death

Data for analysis of this outcome were available from four trials.[63,64,66,68] The forest plot indicates an increased risk of death against the LMHF group, however, the estimated risk ratio of was 1.30 (95% CI 0.58 to 2.89) indicates no significant difference in the incidence of death between EHMF and LHMF groups. No evidence of heterogenicity across studies noted. ($Chi^2 = 1.20$, df = 3, p = 0.98, $I^2 = 0\%$)

Figure 16. *Forest plot of comparison: EHMF vs LHMF, Outcome: Death*

DISCUSSION

Summary of results

No significant differences were found between groups regarding growth parameters, average TPN days or mean length of hospital stay, aligning with previous meta-analyses.[34,35,62] A comprehensive systemic review and meta-analysis identified six studies[63-68] assessing 599 low and very low birth weight (500-2000g) preterm infants. All the data was combined into one comparison, specifically looking at the effects of EHMF (≤ 40 ml/kg/day) and LHMF (≥ 75 ml/kg/day), revealing that only one study[63] utilized donor HMBF. While the others[-68] employed CMBF, all at 24 kcal/oz. Feeding commenced as early as the first day of life and as late as the 96th hour. The studies conducted in various economic settings also differed in their approaches in feeding increments and timing of nutritional fortification, showing a range of strategies available for managing nutrition in LBW preterm infants.

The study found that EHMF is generally safe and well tolerated in preterm infants. The EHMF group revealed a potential reduction in the incidence of ROP and neonatal sepsis, with faster BW recovery. Conversely, there might be an increased risk of BPD and the effects on NEC and FI incidences are unclear. The study also hinted at a potentially higher mortality risk for the EHMF group, though all results were not statistically significant.

Interestingly, due to the high precision of the pooled effects on growth parameters (length cm/week and HC cm/week), hospital outcomes (day to reach full feeds) and adverse effects (EUGR), indicates that even if there were larger sample sizes, there would probably be no difference noted for these outcomes.

Our study had notable strength, including a meticulous methods involving an exhaustive search, precise data extraction, use of local database, thorough appraisal and in-depth analysis, the low heterogenicity rate between studies and the inclusion of three additional studies for analysis, have significantly minimized biases. Our comprehensive literature search leaves little room for the possibility of missing other relevant completed studies. The adequately powered analysis made this study identify meaningful differences between groups, even if the differences are relatively small, thus providing strong evidence for the robustness and reliability of the findings. Additionally, we have uncovered several potential future data sources.

The study had notable limitations, including the exclusion of non-English and unpublished studies, a small and limited number of trials, an imbalanced study ratio

favoring the CMBF group, challenges in using some RCTs due to communication issues with authors, the inclusion of some underpowered trials and an insufficient number of RCTs to adequately assess publication or reporting bias through funnel plot symmetry. These factors contributed to the low quality of evidence for all outcomes, which were further downgraded due to the lack of blinding and small sample sizes.

Agreements and disagreements with other studies and reviews

Recent systemic reviews and meta-analyses[34,35,59] compared EHMF vs. LHMF in preterm infants, evaluating outcomes like growth, hospital stay, adverse effects and long-term development. A 2021 study[62] which combined data from three RCTs[63-65] defined EHMF at ≤ 40 and LHMF at ≥75ml/kg/day. Like our study, it did not find significant evidence for EHMF in growth parameters, length of hospital stays or incidence of NEC, FI and sepsis. However, it lacked data on other outcomes like hospital course (days to full feeds, days on TPN, days to regain BW) and other adverse outcomes (ROP, BPD, EUGR). A 2020 Cochrane review[35] involving two RCTs[63,64], used different volume thresholds from ours at ≤ 100ml/kg/day and ≥ 100ml/kg/day; four studies[65-68] included in our studies were not yet included in the Cochrane review. Like our study, this review also concluded that EHMF may have some or no effect in improving growth or reducing hospital stay, calling for larger RCTs. Unique to this review was the inclusion of neurodevelopmental outcomes after 12 months corrected age. Another 2020 review[34] including the same three RCTs,[63-65] without specific volume thresholds but with a unique data conversion method,[79] which could have affected the interpretation of results that EHMF was associated with a significant increase in days of hospital stay, which, in turn, led to an inferred increased risk of FI and NEC. However, this was contradictory to the results for FI with pooled data noting no significant difference (RR 1.27; 95% CI 0.72, 2.24) and for NEC with a pooled estimate of RR (1.68; 95% CI 0.6,4.70), which was not significant. This highlights the importance to adhering to clear and supportive informative statements to accurately report findings in reviews, maintaining the research's integrity. In contrast, our study provided an appropriate methodology. The reviews collectively indicate insufficient evidence to conclusively recommend one over the other, highlighting the need for further research to guide clinical nutrition strategies for preterm infants effectively.

Two similar systemic reviews[74,75] have compared EHMF vs LHMF in preterm infants, highlighting their safety, tolerability and potential benefits. The first review[75] analyzed two RCTs[63,64] focusing on

short-term growth parameters, FI, length of hospital stay and PMA on discharge, also noting NEC and sepsis. The second review[76] examining three RCTs[63-65] and two retrospective studies,[77,78] concluded that EHMF is safe and well-tolerated and potentially beneficial for preterm infants, found no significant impact on growth outcomes and hospital stay duration.

Like our study, both reviews highlight the need for additional RCTs to further explore these findings. However, it is critical to note that the absence of GRADE analysis and meta-analysis in both reviews, suggesting a gap in assessment of the quality of evidence and the strength of recommendations. Given this context, the conclusion is that while EHMF shows promise for use in preterm infants, particularly in terms of safety and tolerance, definitive claims regarding its benefits over LHMF cannot be made without further high-quality research. Like the conclusions with the meta-analysis above, again underscore the importance of conducting more robust studies, to provide clearer guidance on the use of fortifiers in clinical practice.

Five retrospective studies[76,77,79-81] compared EHMF and LHMF in preterm infants. A 2022 study[79] (n= 90, BW < 1,500g) found that EHMF (≤ 100ml/kg/day) reduced EUGR before discharge (p = 0.03), while LHMF (≥ 100ml/kg/day) shortened the time to regain BW. (p = 0.03). Like our study, TPN duration and time to regain BW were not statistically significant in this study. However, this study noted a significant reduction of EUGR (p = 0.03) before discharge, favoring the EHMF group and a significant reduction in days to regain BW (p = 0.03), favoring the LHMF group, in contrast to our study, which suggested no significant differences between groups. A 2020 study[80] (n = 215, 31 to 36 weeks, BW < 2500g). EHMF (80ml/kg/day) vs LHMF (160ml/kg/day). This study reported EHMF group has a reduced time to regain BW (p < 0.0001), shorter length of stay (p = 0.03) and a lower incidence of FI (p = 0.007), in contrast to our study, which suggested a potential increased incidence of FI, no difference in length of stay and faster days to regain BW, favoring the EHMF group. What was unique to this study was the association of EHMF with reduced maximum weight loss as % BW (p = 0.03), decrease in weight z score at 10 days (p =0.01) and multiple improvements of jaundice outcomes: serum bilirubin levels (p < 0.001), phototherapy (p < 0.0001) and % conjugated serum bilirubin/total serum bilirubin were similar between groups. In a 2019[81] (n = 394, BW 500-1250g), conducted across multiple centers. EHMF (≤ 60ml/kg/day) and LHMF (≥ 60ml/kg/day). This study shares many similarities with our own, which revealed that EHMF led to better growth outcomes and a reduction in CLD without increasing NEC

risk, contrasting our study's observations of a non-significant risk of CLD in the EHMF group; this study noted that CLD was higher in the LHMF group ($p = 0.008$). Additionally, growth velocity improvements with EHMF were significant in this study. No difference in other clinical outcomes like NEC was noted, aligning with our results. A 2016 study,[77] ($n = 271$, BW $\leq$ 1300g). EHMF (25ml) and LHMF (100ml/kg/day). While adverse clinical outcomes (NEC and EUGR) remained consistent between groups, the EHMF group showed a significant improvement in BW and HC at discharge ($p < 0.01$), a finding that was contrary to our results, which showed no significant difference between groups. Lastly, a 2012 study[76] ($n = 95$, < 31 weeks gestational age). EHMF (initiated at first feeds) and LHMF (50 -80ml/kg/day). In line with our study, results showed no significant difference in weight gain and FI between groups. Notably, this study also looked at bone mineral status and found that the EHMF group had a lower incidence of elevated alkaline phosphatase levels.

Across the multiple retrospective studies comparing EHMF and LHMF in preterm infants, results indicates that EHMF can lead to improved outcomes such as reduced EUGR, faster recover of BW and improved growth metrics including HC at discharge. EHMF has also been associated with shorter hospital stays, lower incidence of FI and better bone mineral status. However, findings also suggested variability in outcomes such as the incidence of CLD and NEC, with some studies noting no difference between EHMF and LHMF in these areas. Overall, EHMF appears to offer several benefits in the care and development of preterm infants, although outcomes can vary depending on specific infant characteristics and study designs.

One RCT involving three arms compared different fortification commencement times. In a 2022 study,[82] conducted in Hajar Hospital in Shahrekord, Iran, ($n = 90$, 28-32 weeks and BW < 2,000g). The study consisted of three fortifications = 90: Group A at 30cc/kg/day, Group B at 70cc/kg/day and Group C at 100ml/kg/day. The study's objectives was to investigate and compare the effects of early and late breast milk fortification on growth parameters in preterm infants.

Despite being excluded from final analysis due to data incompatibility and lack of results for adverse outcomes (NEC, sepsis and nutritional intolerance). Like our study, found no significant difference in growth parameters (length and HC) and clinical outcomes across groups. Interestingly, Group B $p < 0.05$), showed significantly better weight gains than Groups A and C.

One multi-center survey study involving four arms compared no HMF, early, middle and late fortification groups. In a 2022

study,[83] conducted in China, 985 preterm infants with BW < 1,800g were involved. The study had three fortification groups comprising early started at ≤ 80ml/kg/day, middle at 80-100ml/kg/day and late at ≥ 100ml/kg/day. Like our study, no significant differences in the incidence of FI, NEC and late-onset sepsis. However, our study identified some key differences, such as the middle fortification group considered LHMF in ours, exhibited the fastest growth velocity, the least dramatic decrease in z scores of weights and length and the lowest incidence of EUGR. The no HMF group exhibited the slowest growth velocity, the most significant decline in z scores and the highest incidence of EUGR (61.6%). This study also noted no significant difference in metabolic bone diseases in all groups ($p > 0.05$).

Conclusion

This study found that the timing HMF introduction, whether early or late, does not significantly impact preterm infants' growth parameters, length of hospital course or adverse clinical outcomes. This study aligns with a recent 2021 meta-analysis on the topic,[59] reinforcing the understanding that addition of HMF to breast milk is a beneficial practice regardless of the specific timing of its introduction. Moreover, it offers flexibility in clinical practice, allowing for a varied feeding fortification timings to suit the unique needs of preterm infants, making it a promising approach in neonatal care. Further research with larger sample sizes and stronger study designs is needed to provide more definitive evidence on the optimal timing of fortification.

Our comprehensive systemic review and meta-analysis, which confirmed and strengthened the findings of most previous studies. This has significant implications for future research and clinical practice. Our findings support the conclusion that there is no difference in growth parameters, hospital outcomes and adverse clinical outcomes between EHMF and LHMF groups. This is an important finding with the potential to guide future research and practice, leading to more informed and effective clinical management of preterm infants.

It is imperative to highlight that the low certainty of evidence for most outcomes, indicating the urgent need for further research to confirm these findings. The low quality of evidence for most outcomes was due to a lack of blinding and small sample sizes. However, this also presents an exciting opportunity for future trials to provide data on outcomes like post-discharge growth and long-term outcomes, especially neurodevelopmental outcomes.

Future research with multi-centered RCTs with larger sample sizes, uniform

definition, structured feeding and fortification approach and various fortifiers (HMBF and CMBF) could lead to significant advancements in the field. We identified several studies useful for future research. (**Appendix H)**

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