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Original Article

Fortification of Expressed Breast Milk with Preterm Formula Powder Versus Human Milk Fortifier in Preterm (28-34 Weeks) Very Low Birth Weight Neonates: Longitudinal Comparative Study

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ABSTRACT

Background: Extrauterine growth restriction (EUGR) remains a persistent challenge among preterm neonates despite improved survival. Human milk, though optimal, fails to meet nutritional requirements of Very Low Birth Weight (VLBW) babies, necessitating fortification. Human milk fortifiers (HMF) improve growth but are expensive and may cause feed intolerance. Fortification with preterm formula powder (PTF) may serve as an economical and effective alternative in low-resource settings.
Methods: T`his longitudinal comparative study enrolled 142 preterm (28–32 weeks) VLBW (<1500gm) neonates. Sixty-seven received PTF fortification and 75 received HMF; data were analyzed for 66 and 68 neonates respectively at discharge, with 105 babies completing follow-up until term equivalent age (TEA). Growth parameters, feed tolerance and morbidities were compared between groups.
Results: Baseline neonatal and maternal characteristics were similar between groups. Weight gain velocity until discharge was significantly higher in HMF group (14.6 ± 1.2 vs. 13.9 ± 1.3 g/kg/day; p=0.001). Linear, head growth and comorbidities were comparable. No cases of NEC were observed. Feed intolerance episodes were slightly higher in the PTF group but not statistically significant. At TEA, mean weight and head circumference were marginally higher in the HMF group, though not significant. Both groups had high rates of EUGR (≈86%) and severe EUGR (≈82%).
Conclusion: Preterm formula powder fortification demonstrated comparable safety and efficacy to HMF, with acceptable tolerance, better compliance and lower cost, representing a feasible alternative for fortification in resource constrained NICUs without increasing adverse outcomes.

Keywords*: Preterm infants, VLBW, Human milk fortifier, Preterm formula, Fortification, Extrauterine growth restriction.*

INTRODUCTION

Preterm birth contributes significantly to neonatal morbidity, mortality, and long-term neurodevelopmental impairment. Although advances in neonatal intensive care improved survival, extrauterine growth restriction (EUGR) remains a persistent concern. Achieving intrauterine-equivalent growth in very low birth weight (VLBW) neonates is difficult due to limited nutrient reserves, feeding intolerance, delayed establishment of enteral nutrition, and the inadequacy of breast milk alone to meet increased nutritional requirements.

Human milk is universally recognized as the optimal feed for preterm infants, providing immunological protection, improved digestion, neurodevelopmental benefits, and a reduced risk of sepsis and necrotizing enterocolitis (NEC). However, its macronutrient and micronutrient content falls short of the energy and protein needs of VLBW infants.

Fortification of expressed breast milk (EBM) with commercially available human milk fortifiers (HMF) enhances weight, length, and head growth without increasing NEC risk[1].

Despite these benefits, the use of HMF is often limited in low-resource settings. Feed intolerance may require temporary or permanent withholding of fortification, compromising nutrition. In addition, the high cost of commercial HMF (INR 250–400/day for a 1-kg neonate) places a substantial burden on families of lower socioeconomic status.

Alternatively, fortification using preterm formula powder (PTF) offers a low-cost option, providing balanced macronutrients with acceptable tolerance. Small randomized trials have shown comparable growth outcomes between PTF and human milk fortifier (HMF), without significant differences in morbidities, though evidence remains limited and requires further validation, especially in resource-limited settings[2]. In this context, we conducted a longitudinal comparative study in a tertiary-care NICU in coastal Andhra Pradesh, India, to evaluate the safety and efficacy of PTF versus HMF fortification in preterm VLBW neonates, focusing on growth outcomes (weight gain, length, head circumference), feed tolerance, major morbidities, and the incidence of growth restriction at term equivalent age.

METHODOLOGY

Study Design and Setting

This was a longitudinal comparative study conducted in the Level III NICU of Rangaraya Medical College – Government General Hospital, Kakinada, Andhra Pradesh, between November 2022 and December 2023. The hospital is a tertiary referral center catering to high-risk pregnancies and preterm deliveries across five districts.
Participants

Inclusion criteria:

1. Preterm neonates born at ≤34 weeks gestation.
2. Birth weight <1500 g.
3. On oral feeds ≥100 mL/kg/day.
4. Expressed breast milk (EBM) constituted >75% of total daily intake at enrolment.

Exclusion criteria:

1. Major congenital anomalies.
2. Death or referral before initiation of fortification.

Gestational age was determined using last menstrual period or first trimester scan. If unavailable, or if discrepancy >1 week was noted, Expanded New Ballard Score was applied.

Sample Size

Based on prior studies reporting mean weight gain of 15–16 g/kg/day with HMF, a 6% effect size, 90% confidence, and 80% power, 45 neonates per group were required. To account for attrition, a target enrolment of 100 was set (50 per group).

Enrolment and Allocation

Eligible neonates meeting inclusion criteria were enrolled after parental consent. Assignment to PTF or HMF groups was done alternately. In multiple gestations, infants were allotted alternately to minimize bias. However, due to cost constraints, several families opted directly for the PTF arm.

Intervention

- **PTF group:** EBM was fortified with *Dexolac Special Care* (Danone Nutraceuticals, India) at 1 g per 25 mL. Sachets with premeasured powder were prepared by the clinical team.
- **HMF group:** EBM was fortified with *Lactodex HMF* (Raptakos Brett & Co, India) at 1 g sachet per 25 mL.

Fortification was initiated and supervised by trained nurses initially; mothers were later trained to continue preparation. Nutritional audits were performed weekly, and supplementation adjusted as required. At discharge, parents were advised to continue fortification until infants reached 2 kg or 40 weeks postmenstrual age (PMA).

Data Collection

Baseline maternal, demographic, and neonatal details were recorded at enrolment. Anthropometry (weight, length, head circumference) was measured at birth, enrolment, discharge, and 40 weeks PMA using standard techniques (digital scale, infantometer, and non-stretchable tape). Growth velocity was calculated as average weekly gain.

Morbidities including late-onset sepsis, NEC (≥Stage II), bronchopulmonary dysplasia, intraventricular hemorrhage (Grade III–IV), PDA, neonatal cholestasis, anemia requiring transfusion, retinopathy of prematurity, and osteopenia of prematurity were documented during hospitalization and follow-up.

Outcome Measures
Primary outcomes
- Weight gain velocity (g/kg/day) during hospitalization.
- Velocity of length and head circumference gain.

Secondary outcomes
- Feed intolerance episodes.
- Incidence of NEC, late-onset sepsis, BPD, PDA, ROP, IVH (Grade III–IV), anemia requiring transfusion, neonatal cholestasis, osteopenia.
- Mortality during hospital stay.
- Proportion with extrauterine growth restriction (EUGR; <10th centile by Fenton chart) at 40 weeks PMA.

Definitions
- **Feed intolerance:** diagnosed if ≥2 of the following were present: vomiting >1 episode (bilious/bloody), gastric aspirates >50% of previous feed (after 3 feeds), or abdominal distension >2 cm over baseline in 24 h.
- **Anemia requiring transfusion:** based on National Neonatology Forum India (2020) thresholds for Hb/Hct, adjusted for respiratory support[8].
- **Metabolic bone disease:** ALP >800 IU/L with serum phosphorus <4 mg/dL.

Compliance and Follow-Up
Parents bore the cost of fortifiers. Compliance was reinforced by counseling and verified at high-risk follow-up and ROP screening clinics. At 40 ± 1 week PMA, anthropometry and morbidity outcomes were reassessed.

Statistical Analysis
Data were analyzed using SPSS version 25.0. Continuous variables were summarized as mean ± SD and compared using Student's *t*-test. Categorical variables were expressed as proportions and analyzed using Chi-square or Fisher's exact test. $p < 0.05$ was considered statistically significant.

RESULTS
Baseline neonatal characteristics were comparable between the two groups (Table 1). The mean gestational age was 31.7 ± 1.6 weeks in the PTF group and 31.5 ± 1.4 weeks in the HMF group. Weight gain velocity from enrolment to discharge was significantly higher in the HMF group compared to the PTF group (14.6 ± 1.2 vs 13.9 ± 1.3 g/kg/day; p = 0.001), while length and head circumference gains were similar (Table 2). The incidence of feed intolerance, late-onset sepsis, metabolic bone disease, anemia requiring transfusion, and major neonatal morbidities did not differ significantly between groups (Table 3). Anthropometric measurements at term-equivalent age were comparable in both groups (Table 4). Comparative cost analysis demonstrated substantially lower fortification costs with preterm formula powder compared to human milk fortifier(Table5).

Data is summarized in Figure 1, Tables 1-5.

Figure 1: Study Flow Diagram

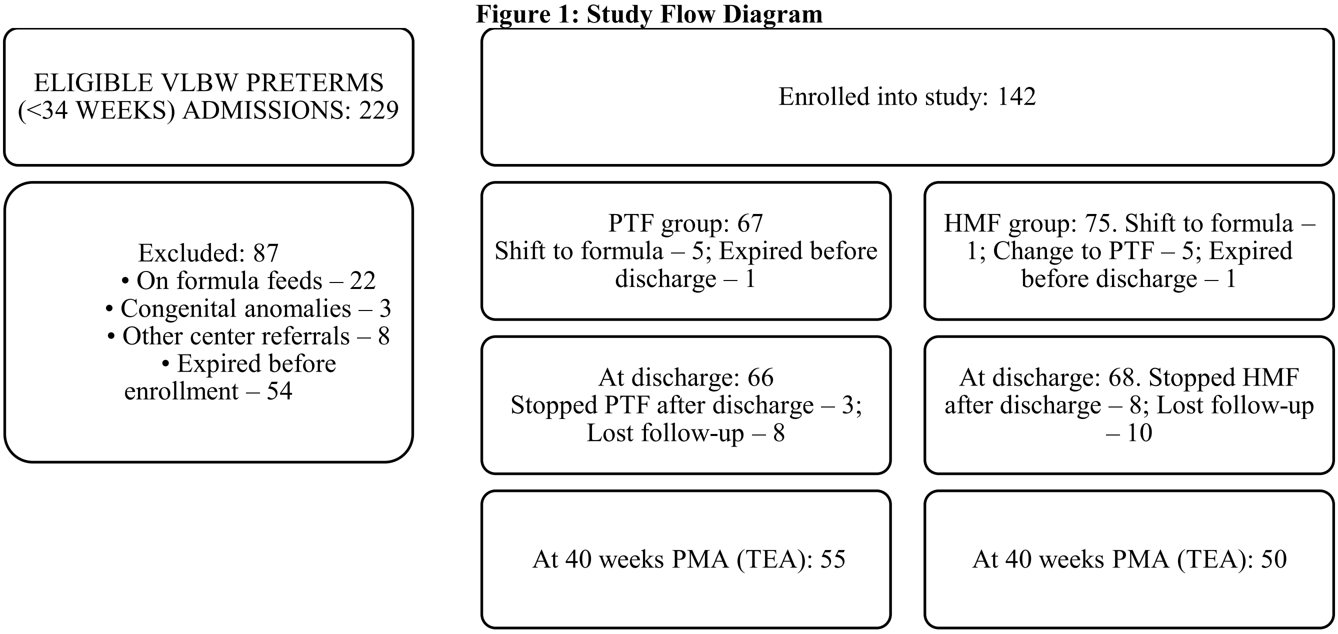

Table 1: Baseline characteristics of study population

Neonatal characteristics Variable, n(%)	Preterm formula fortification (n- 67)	Human Milk Fortifier (n- 75)	p value
Gestation, Mean (SD), wk	31.7 (1.6)	31.5 (1.4)	0.6
Birth weight, Mean (SD), gm	1263 (153)	1293 (132)	0.2
Intrauterine growth			0.87
SGA	26 (39.5)	26 (34.5)	
AGA	40 (59)	48 (64)	
Male gender	43 (64)	33 (44)	0.016
Day of enrolment, Median (IQR)	9 (7,11)	10 (7,12)	0.2
Mean feed volume at enrolment Mean (SD), (ml/kg/day)	138 (19)	140 (17)	0.4
Mean weight at enrolment, Mean (SD), gm	1236 (132)	1206 (103)	0.14
Duration of stay(day), Median (IQR)	26 (19,32)	27 (22,31)	0.2
Gestational age at discharge, mean ± SD	35.2 ± 1.4	35.5 ± 1.3	0.18
Mean weight at discharge (gms), ± SD	1574.1 ± 115	1583.4 ± 89	0.60
Mean length at discharge, ± SD	45.23 ± 2.5	44.87 ± 2.4	0.3
Mean OFC at discharge, ± SD	31.56 ± 1.4	31.18 ± 1.1	0.08

Table 2: Primary outcome of the study group

Primary outcome	**PTF** **n– 66**	**HMF** **n- 68**	**p value**
Weight gain from enrolment to discharge g/kg/day, Mean ± SD	13.9 ± 1.3	14.6 ± 1.2	**0.001**
Length gain from birth to discharge Cm/week, Mean ± SD	1.2 ± 0.2	1.2 ± 0.08	0.1
OFC gain from birth to discharge Cm/week, Mean ± SD	0.78 ± 0.08	0.79 ± 0.08	0.5

Table 3: Secondary outcomes and morbidities

Secondary outcomes	**PTF,** **n – 66**	**HMF,** **n – 68**	**p value**
Feed intolerance requiring withholding fortification for 24 hours	11 (17)	8 (12)	0.42
Withheld fortification for 6 hours	32 (49)	17 (25)	**0.005**
Necrotising Enterocolitis	-	-	
Late onset sepsis	8 (12)	6 (9)	0.5
Metabolic Bone Disease	2(3)	2(2.9)	1.0
Neonatal cholestasis	1 (1.5)	0	0.5
Anemia (Requiring transfusion)	21(32)	18(27)	0.49
PDA requiring surgery	1(1.5)	1(1.5)	1.0
ROP requiring Anti VEGF	7(11)	6(9)	0.7
ROP requiring LASER	1(1.5)	1(1.5)	1.0
Evolving BPD	5(7.6)	5 (7.4)	1.0
Severe IVH (Grade III, IV)	1(1.5)	1(1.5)	1.0
Severe EUGR (<3rd centile)			
ALL	44 (80%)	42 (84%)	0.59
SGA	19 (100%)	20 (100%)	
AGA	25 (69%)	22 (73.3%)	

Table 4: Anthropometry at TEA

Anthropometry at Term Equivalent Age	**PTF, n – 55**	**HMF, n – 50**	**P value**
Weight at TEA (grams)	2154 ± 27	2208± 262	0.31
Length at TEA (cm)	48.9± 1.7	48.84±1.6	0.83
OFC AT TEA (cm)	33.75±1.0	33.42±0.7	0.06

Table 5: Comparative cost of fortification

Study	**PTF**	**HMF**	
V Gupta et al[14]	Rs 190	Rs 2000	10 times higher with HMF fortification

Khorana et al[4]	19 times reduction in the cost with PTF fortification as compared to HMF fortification		
Kanya M et al[9]	Cost was significantly higher in HMF group		
Chinnappan A et al[3]	Rs. 8	Rs. 280	35 times higher in HMF group
Pedaveeti M et al[7]	Rs. 1,39,750 ±57,112	Rs. 1,89,771 ±63,502	Costs during stay are 1.35 times higher in HMF
Andrea W et al[5]	21 cents	28 cents	Absolute difference of 7 cents
Present study	INR 12 / day	INR 240 /day	~20 times higher cost in HMF group

DISCUSSION

In the present study involving 134 neonates (66 PTF, 68 HMF), both fortification methods achieved satisfactory postnatal growth without major adverse effects. The mean weight gain velocity was slightly higher in the HMF group (14.6 ± 1.2 g/kg/day) than the PTF group (13.9 ± 1.3 g/kg/day, p = 0.001), but the difference, although statistically significant, was clinically small. Linear and head circumference growths were comparable between groups, demonstrating that PTF fortification effectively supports somatic growth.

These findings are consistent with several international and Indian studies. **Chinnappan et al.**[3] (AIIMS, New Delhi, 2021) conducted a randomized non-inferiority trial on 123 VLBW preterm infants and found no significant difference in mean weight gain between PTF and HMF fortification (15.7 vs 16.3 g/kg/day; mean difference –0.5 g/kg/day, 95% CI –1.9 to 0.7). They concluded that PTF fortification was non-inferior to HMF for growth outcomes and morbidity profile. Similarly, **Khorana et al.** [4] (Thailand, 2014) observed nearly identical weight gain rates between the two groups (18.9 vs 18.1 g/kg/day) and comparable biochemical indices, supporting the nutritional adequacy of PTF fortification. **Andrea Willeitner et al.** [5] (Oklahoma, Canada, 2018) also reported equivalent weight gain (18.3 vs 16.9 g/kg/day, p = 0.38) and no difference in feed intolerance or sepsis, reinforcing that preterm formula fortification can achieve growth similar to commercial HMF.

The linear and head growth results in our study (1.2 cm/week and 0.78–0.79 cm/week) parallel observations from these studies. **Mukhopadhyay et al.**[6] (PGIMER, Chandigarh) compared various fortification methods in a small cohort and reported significant improvements in weight and length gain across all fortified groups compared to unfortified human milk, though without major inter-fortifier differences.

Feed intolerance and necrotising enterocolitis (NEC)

Feed intolerance remains a common clinical concern while fortifying human milk. In our study, about 17% of PTF and 12% of HMF infants experienced feed intolerance requiring temporary withholding of fortification for 24 hours; however, none developed NEC. These rates align with published data indicating comparable gastrointestinal tolerance between the two fortifiers. **Chinnappan et al.**[3] reported fewer feed-intolerance episodes in the PTF group (1.4 vs 6.8 per 1000 patient days), while **Pedaveeti M et al.**[7] (2022, Karnataka) found a higher but still acceptable rate of feed intolerance in HMF (25.7%) compared to PTF (7.7%, p = 0.02). Conversely, **Khorana et al.** [4] and **Andrea**[5] **et al.** observed no significant difference, with NEC incidence being rare in both groups.

The 2020 Cochrane review on human milk fortification (14 RCTs, n = 1110) also concluded that fortification does not significantly alter the risk of feed intolerance or NEC (RR 1.05 and 1.37, respectively). These findings affirm that fortification—even with bovine-based components—remains safe if introduced at appropriate feed volumes and monitored closely.

A 2020 Cochrane review comparing early (20–40 mL/kg/day) versus late fortification (≥ 100 mL/kg/day) found little or no effect on linear growth, head growth, NEC, or time to regain birth weight (low certainty of evidence). Our study, similar to those by **Chinnappan**[3], **Andrea**[5], and **Khorana**[4], also introduced fortification around 120–140 mL/kg/day. This suggests that timing of fortification may be less critical than ensuring sustained adequate nutrient intake once tolerance is established.

The small difference in weight gain could stem from compositional variations—HMF typically delivers ~2 g protein and 80 kcal/100 mL versus 1.7 g protein and 78 kcal in PTF fortification. **R. Agarwal et al.**[10] demonstrated that osmolality of fortified milk with either HMF or PTF remains below 450 mOsm/kg, the safety threshold recommended by the American Academy of Pediatrics, suggesting that PTF does not increase gastrointestinal risk.

Secondary morbidities

The secondary outcomes—late-onset sepsis, metabolic bone disease, anemia, cholestasis, patent ductus arteriosus (PDA), bronchopulmonary dysplasia (BPD), retinopathy of prematurity (ROP), and intraventricular hemorrhage (IVH)—were comparable between groups. No differences were noted in sepsis incidence (12% vs 9%) or transfusion-requiring anemia (32% vs 27%). Similar equivalence in morbidity outcomes was reported by **Chinnappan et al.**[3], **Khorana et al**[4]**.**, and

Pedaveeti M et al.[7], indicating that nutrient source does not significantly influence systemic complications in preterm infants. The absence of severe NEC or major adverse events reinforces the safety of PTF fortification.

Anthropometric outcomes at term-equivalent age (TEA)

At 40 weeks PMA, both groups achieved comparable anthropometric measurements (mean weight ≈ 2.1 kg, length ≈ 48.8 cm, OFC ≈ 33.5 cm). However, around 85% of infants in both groups remained below the 10th centile (EUGR) on Fenton charts. This mirrors global observations, such as those of **Horemuzova et al.**[11] and **Pedaveeti M et al**[7]**.**, who found persistent postnatal growth restriction despite optimal fortification strategies. The high EUGR rates highlight the multifactorial etiology of poor growth, including delayed TPN initiation, limited maternal nutrition, and underlying intrauterine growth restriction (IUGR).

Early amino acid and lipid supplementation has been linked to reduced EUGR and improved neurodevelopment (Silvia Iacobelli et al., 2018; A.S. Pages et al., 2014). However, such interventions are often limited by cost and logistics in Indian government hospitals. Nevertheless, several studies, including **Roze et al.**[12] (EPIPAGE cohort), demonstrate that breast-milk-fed preterms—even with slower initial growth—show better cognitive outcomes, underscoring that human-milk-based nutrition should remain the foundation of NICU feeding practices.

Subgroup analysis

Subgroup analysis by gestational age and birth-weight categories revealed comparable growth across groups, except for marginally higher weight gain in HMF among 28–30 and 32–34 week AGA infants. SGA infants demonstrated slightly better weight gain with PTF, though not significant. Previous studies also note minimal interaction between intrauterine growth status and fortifier type. Bauer et al.13 showed similar postnatal energy expenditure and proportional tissue accretion between AGA and SGA preterm infants once adequate nutrition was established. Hence, the choice of fortifier does not appear to influence growth differently across growth-restriction strata.

Cost analysis and practical implications

Cost was a major determinant of fortifier choice in this study. The daily cost of fortification per infant was approximately ₹ 12 for PTF versus ₹ 240 for HMF—a 20-fold difference. Similar findings were reported by Chinnappan et al.3 (₹ 8 vs ₹ 280), Khorana et al.4 (19-fold cost reduction), V. Gupta et al.14 (₹ 190 vs ₹ 2000), and Pedaveeti M et al.7 (1.35-fold higher overall hospital cost in HMF group) . Andrea et al.5 observed a smaller but notable difference (21 vs 28 cents per feed). Collectively, these data emphasize that PTF fortification can reduce neonatal feeding costs substantially without compromising safety or efficacy. In resource-limited NICUs, such savings could enhance accessibility to adequate nutrition for a greater number of preterm infants.

Clinical relevance and public-health significance

When synthesizing evidence across studies, the pattern remains consistent: PTF fortification achieves growth and morbidity outcomes comparable to HMF. Although a few studies, including ours, noted a marginally higher weight gain velocity in HMF groups, the differences are unlikely to be clinically meaningful. The equivalence in linear and head growth indicates that PTF provides sufficient macronutrient support for tissue accretion and neurodevelopment.

Our findings, consistent with multiple randomized trials and systematic reviews, confirm that fortification of EBM with PTF is a safe, effective, and economical alternative to commercial HMF for preterm VLBW infants. This has major implications for neonatal care in low- and middle-income countries, where HMF availability and affordability remain barriers. By adopting PTF fortification, NICUs can ensure continuity of human-milk-based feeding without significant financial burden to families. Implementation of standardized fortification protocols and parental education regarding continuation at home could further enhance growth outcomes.

The persistent challenge of EUGR despite fortification calls for comprehensive nutritional strategies—early initiation of enteral feeding, optimal protein intake, timely introduction of TPN when feasible, and follow-up growth monitoring. Policy support to include affordable fortification options in national neonatal nutrition guidelines is warranted.

Strengths and limitations

The strengths of this study include its design in a resource-limited NICU and the relatively large sample size. Direct comparison of two fortification strategies under uniform clinical conditions adds to its validity. Limitations include lack of long-term neurodevelopmental follow-up, unavailability of precise biochemical markers, and absence of individualized nutrient analysis. Economic constraints precluded TPN and may have affected early postnatal growth. Despite these, the study adds meaningful local data to the growing body of evidence supporting cost-effective neonatal nutrition.

CONCLUSION

Fortification of expressed breast milk with preterm formula powder demonstrated comparable efficacy and safety to human milk fortifier in preterm VLBW infants, with substantial cost savings. PTF may be adopted as a practical alternative in resource-limited neonatal units. Future research should evaluate long-term neurodevelopmental outcomes and explore individualized fortification to optimize protein and energy intake in preterm VLBW infants.

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