Fortification of Expressed Breast Milk With Preterm Formula Powder vs. Human Milk Fortifier in Preterm (28-34 weeks' Gestation) Very Low Birth Weight Neonates: a Randomized non inferiority trial

STUDY PROTOCOL

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23 **Protocol summary in Institute format**

1. Full title of study:	**Fortification of Expressed Breast Milk with Preterm Formula Powder vs. Human Milk Fortifier in Preterm (28-34 weeks' Gestation) Very Low Birth Weight Neonates: a Randomized non inferiority trial**
1a. AIIMS Temporary Research Section Number for all Clinical Trials which are privately funded	Not applicable
2.1 **Name & signature of the candidate** 2.2 Department 2.3 Degree/course 2.4 Batch of admission to course 2.5 Month & year of submission of thesis 2.6 Email ID of the Candidate and Chief Guide	2.1 Dr. C Arunambika Signature_____ Department of Pediatrics MD January 2017 July 2019 arunambika.chinnappan@gmail.com jeevasankar@gmail.com
3. **Objectives of the study**	**Primary:** To compare the rate of weight gain from the time of enrolment until discharge from the hospital or 40 weeks' PMA, whichever was earlier, in preterm (28-34 weeks' gestation) VLBW neonates receiving expressed breast milk (EBM) fortified with preterm formula powder with those receiving EBM fortified with commercially available human milk fortifier (HMF). **Secondary:** Among preterm VLBW neonates receiving EBM fortified with preterm formula powder or HMF, to compare the 1. Proportion of babies growth restricted at term gestation (40 weeks PMA) 2. Rates of gain in length and head circumference from the time of enrolment until discharge from hospital and at 40 weeks PMA 3. Incidence of feed intolerance and other morbidities including late metabolic acidosis (LMA), necrotizing enterocolitis (NEC) stage 2 or more, anemia requiring blood transfusion(s), and osteopenia of prematurity

4. Why this study is required? Please provide brief justification.	1. Preterm VLBW neonates require higher energy, protein and mineral requirements, which are not met by unfortified breast milk alone. 2. Many newborn care units therefore fortify EBM with commercially available human milk fortifier (HMF). 3. But fortification with HMF is expensive and is also associated with high risk of complications like feed intolerance and late metabolic acidosis, which often warrant temporary or permanent withholding of fortification. 4. A simpler and more economical option is to use commercially available preterm formula powder, in place of HMF, to fortify EBM. An earlier study from our unit at AIIMS demonstrated no significant difference in the osmolality of milk fortified with HMF and preterm formula powder. 5. Fortification with preterm formula powder might be associated with lower risk of feed intolerance and similar (not lower) weight gain in preterm neonates. 6. No studies have, however, examined the rates of weight gain following fortification of EB with preterm formula powder.
6. Methodology	6.1. Number of patients: 124 6.2. Inclusion criteria 1. Preterm neonates born between 28 and 34 weeks of gestation 2. Birth weight less than 1500 g 3. Accepting oral feeds of at least 100ml/kg/day 4. Amount of EBM, as a proportion of total daily milk intake, 75% or greater at enrolment 6.3. Exclusion criteria 1. Major congenital anomalies 6.4. Study design: Randomized non inferiority trial 6.5. Dosages of drug: No drug is being used 6.6. Duration of treatment: Till the neonate reaches 40 weeks or 2 kg, whichever is later, provided the neonate is receiving EBM 6.7. Investigation specifically related to project: None; work-up including hematocrit, blood gas will be done in case of inadequate weight gain and serum calcium,

	phosphate, alkaline phosphatase (ALP) done if Osteopenia of Prematurity is suspected, as decided by the clinical team. 6.9 Permission to use copyrighted Questionnaire/ proforma: Not applicable 6.10 Brief methodology: Neonates born between 28 and 34 weeks of gestation, meeting the inclusion criteria will be approached for parental consent. After obtaining consent, the neonate will be enrolled and randomized to receive fortification of his/her mother's expressed breast milk (EBM) with preterm formula powder or human milk fortifier (HMF). Baseline information and anthropometric measurements will be recorded at enrolment. Enrolled neonates will be followed-up with weekly anthropometric measures. In case of inadequate weight gain, laboratory work-up including hematocrit, blood gas will be done and serum calcium, phosphorous, ALP will be done if OOP is suspected till the time of hospital stay. The incidence of various morbidities including feed intolerance and necrotizing enterocolitis (NEC) will be recorded prospectively until discharge from the hospital. Fortification will be continued till the neonate reaches 2 kg or reaches 40 weeks' post menstrual age (PMA), whichever is later (provided he/she is on EBM till that period). Fortification of milk will be done by the concerned staff nurse in the first few days after enrolment, following which the mothers will be trained to fortify EBM. At the time of discharge, mothers will be provided with supply of fortifiers for next two weeks. The supply will be replenished when the neonate turns up every 2 weeks for routine follow-up visits at high-risk clinic or for retinopathy of prematurity (ROP) screening. At 40 weeks, the final information regarding the preterm morbidities and anthropometric measures will be recorded.
7. Permission from Drug Controller General of India (DCGI)	1. Required ☐ 2. **Not required** ☑ 3. Received ☐ 4. Applied when ☐
8. Permission from DGFT, if required	1. Required ☐ 2. **Not required** ☑ 3. Received ☐ 4. Applied when ☐
9. a) Safety measures for proposed interventions b) Results of relevant laboratory tests	a) Safety measures are not required in both groups. b) Will be considered and treated as per standard when abnormal

c) Result of studies in human	c) Not applicable.
10. Plans to withdraw standard therapy in research	Yes ☐ No ☑
11. Plan for provision of coverage for medical risk	Not applicable.
12. How you will maintain Confidentiality of subject?	Information obtained by the study will not be disclosed to any unauthorized person
13. **Costs Involved (App. in Rs.)** 13.1 Investigations 13.2 Disposables 13.3 Implants 13.4 Drug / Contrast Media **Who will bear the costs of the requirements?** *(mark √)*	13.1. No investigations are done for the sole purpose of the study 13.2. Free; both HMF and preterm formula powder are available from the hospital supply. 13.3. Not required 13.4 Free 1. Patient 2. Project 3. √**Exempted** 4. Other Agencies (Name)_____
14. Participant Information Sheet *(mark √ if yes)*	√ Attached English version √ Attached Hindi version √ **Certified that Hindi version is a true translation of English version**
15. Participant Informed Consent Form *(mark √ if yes)*	√ Attached English version √ Attached Hindi version √ Certified that Hindi version is a true translation of English version
16. Whether any work on this project Has started or not?	X *(mark √ if yes, X if no)* *(Please enclose a separate certificate to this effect).*
17.Attached documents (If any)	17.1 Covering letter, through proper channel - Yes 17.2 Copy of the detailed protocol is mandatory - Yes 17.3 Undertaking that the study shall be done in accordance with ICMR and GCP guidelines - Yes 17.4 In case of multicentric study, IEC clearance of other centers must be provided – Not applicable

	17.5 Definite undertaking as to who will bear the expenditure of injury related to the project – Not a drug trial and no trial-related injury is expected 17.6 In case an insurance cover is intended, Insurance certificate must be provided (as per ICMR guidelines) - Not applicable 17.7: Permission as mentioned in 6.9 - Not applicable 17.8: Certificate/undertaking as mentioned in 16 - Yes 17.9: In case of Clinical trials, proof of registration of Clinical trial with ICMR needs to be submitted - Yes 17.10: Investigator should provide undertaking what they will do with the leftover sample tissue – No samples will be stored; only routine blood tests – as part of the clinical care – would be done. 17.11 Soft copy of all the documents in PDF in a two separate files (signed and unsigned) on a single CD - Yes 17.12 Others: Nil

INTRODUCTION

Preterm birth is a significant public health problem because of associated neonatal mortality, short and long term morbidities, and disability in later life. Prematurity is the leading cause of neonatal deaths and the second leading cause of all under-5 deaths.(1) With improvements in supportive care of preterm neonates and use of surfactant and antenatal steroids to women at risk of preterm labor, there is a substantial improvement of survival of preterm neonates in recent years.

With increased survival of preterm neonates, extra uterine growth restriction has become an important issue. The target for growth in these neonates will be to match intrauterine growth rates as close as possible(2). But it is almost impossible to achieve the intrauterine growth rates in most preterm neonates. The reasons are manifold: sickness level of preterm neonates in the first few weeks of life, inability to start enteral feeds, high energy requirements, and the inability to meet these requirements with breast milk alone.

Human milk is a key component of any strategy for enteral feeding in preterm neonates with beneficial effects in digestion and absorption of nutrients, neurodevelopmental effects, and decrease in incidence of complications like sepsis and NEC.(3,4) However, breast milk alone is not enough to meet the high energy, protein, minerals/vitamin requirements of preterm neonates. A simple comparison of the nutrient content of preterm milk and recommended dietary allowances (RDA) of preterm neonates – according to ESPGHAN 2010 – clearly shows that the nutrient needs of these neonates cannot be met even with full enteral feeding of breast milk. To circumvent this pertinent issue, most neonatal units fortify expressed breast milk (EBM) of preterm neonates with commercially available human milk fortifiers (HMF).(5)

According to a Cochrane meta-analysis of 14 trials , multi-nutrient fortification of breast milk increased growth rate – weight (mean difference [MD] 1.81 g/kg/day; 95% CI 1.23 to 2.40), length (MD 0.12 cm/wk; 95 CI 0.07 to 0.17), head circumference (MD 0.08cm/wk; 95% CI 0.04 to 0.12) with no effect on development and NEC (RR 1.57; 95% CI 0.76 to 3.23).(6)

The method of fortification of EBM in India is by using powdered HMF. The fortifiers used commonly in India are Lactodex (Raptakos Brett & Co., India), HIJAM (Endocura Pharma Ltd, India) and PreNAN (Nestle India Ltd).(7) On fortification with Lactodex HMF, assuming feed intake of 180 ml/kg/day, RDA of protein, vitamin A, vitamin D and iron are not met. In contrast,

with HIJAM, RDA of preterm neonates is met and no further supplements are needed. However, there is a paucity of data on safety with HIJAM.

At our NICU, over a 6-month period of observation from July to December 2016, the proportion of neonates developing feed intolerance following fortification with HIJAM was found to be 39.2% (29/74). Moreover, we also observed an unusually high incidence of late metabolic acidosis – about 10.8% – following fortification with HIJAM. Both these complications warrant withholding of fortification – temporarily or permanently – in these neonates thereby compromising their optimal growth and nutrition. The prevalence of feed intolerance in neonates receiving fortification with the other fortifier – Lactodex-HMF – was 26.7% (4/15). Though there are consensus that both feed intolerance and LMA is considerably less with the use of PreNAN, studies in this area are considerably limited.

The cost of fortification was also high, which for a 1 kg neonate will be around – INR 140-160/day with HIJAM (INR 20/sachet- 1g) , INR 35-46/day with Lactodex(INR 11.50/ sachet-2g), INR 198 – 200/day (INR 22/sachet – 1g) with PreNAN which would be a high burden for a low income family.

A simpler, more economical, and possibly more safe option to fortify EBM is to fortify it with preterm formula powder. Though preterm formula for fortification is routinely done in some low-income countries, research and literature in this area is still limited to recommend its use.(8,9)

We therefore plan to conduct a randomized trial to compare the efficacy and safety of fortification of EBM using preterm formula powder (Dexolac SPECIAL- DANONE, India) with fortification using HMF (PreNAN) in terms of short-term weight gain and incidence of feed intolerance and other morbidities like necrotizing enterocolitis (NEC), LMA, osteopenia of prematurity, etc.

REVIEW OF LITERATURE

Human milk, with its unique compositions, will be the ideal feed for term infants. Preterm infants, with less mature sucking reflex are usually given expressed breast milk. Breast milk is easily digested and absorbed. It promotes stool softness and helps in mineral absorption. Breast milk also has a major role in gut flora development. Available evidence suggests a reduction in incidence of necrotizing enterocolitis, late onset sepsis, retinopathy of prematurity with reduction in development of predictive factors of metabolic syndrome(According to a study of Bauer et al in 2011(10), the breast milk composition at various gestational ages are shown in Table 1. The average protein content is highest in extremely preterm human milk. The protein content tends to decrease across higher gestational age groups.

Table 1. Composition of human milk at different gestational ages.(10)

Composition	**Extremely Preterm (<28 weeks)**	**Very Preterm (28-31 weeks)**	**Moderately Preterm (32-33weeks)**	**Term**
Protein (g/dl)	2.3	2.1	1.9	1.6
Carbohydrates (g/dl)	7.6	7.5	7.5	6.2
Fat (g/dl)	4.4	4.4	4.8	4.1
Energy (kcal/dl)	77.8	77.6	76.7	67.7
Sodium (mmol/l)	10.6	10.6	10.4	11.2
Potassium (mmol/l)	14.0	13.1	12.1	11.5
Calcium (mmol/l)	6.2	6.5	7.4	5.4
Phosphate (mmol/l)	2.2	2.1	2.0	1.9

Preterm milk although has higher energy, protein, fat and minerals, as compared to term milk is still insufficient to meet the high requirements of growing preterm infants, when compared with the ESPGHAN recommendations of recommended dietary allowences, as can be seen in Table 2.

Table 2: Nutrient composition of unfortified breast milk, on fortification with Lactodex and HIJAM, and the RDA as per ESPGHAN 2010(5)**.**

COMPONENTS	HUMAN MILK [@] (180ML/KG/DAY)	EBM + LACTODEX[#] (180ml//kg/day)	EBM+ HIJAM[$] 180ml/kg/day	ESPGHAN (kg/day)
Energy	120.6	147.6	145.8	110-135
Protein, g	1.98	2.7	3.78	3.5-4.5
Fat, g	6.3	6.5	8.1	4.8-6.6
Vitamin A, IU	86.4	518	1202	1320-3300
Vitamin D, IU/day	14.4	151	734	800-1000
Calcium, mg	45.5	225	225	120-140
Phosphorous, mg	26.1	115	116	60-90
Iron, mg	0.16	0.09	2.7	2-3

[@] Human milk – Preterm mature milk. Deficient in calorie, carbohydrates, proteins, Vitamin A,D, Calcium, Phosphorous, Iron

[#] With Lactodex, Protein, Vitamin A,D, Calcium, Phosphorous, iron are deficient

[$] HMF – Meets RDA closely in all aspects

Target of growth in preterm infants would be to match the intrauterine growth curves as close as possible. However these infants are prone to extrauterine growth restriction, due to numerous factors. A study was done by Adriana et al, a retrospective analysis to assess the nutritional practices in NICU and their association with growth parameters of preterm infants. They included 305 preterm infants, stratified into 500-999gms and 1000-1499gms. According to them, insufficient management of parenteral nutrition like delay in starting parenteral nutrition and delaying aminoacids and lipids, restricting the maximum supply of nutrition and delay in hiking up the nutrients were important during the initial days. With regard to enteral nutrition, delay in starting minimal enteral nutrition, delay in hiking up feeds to attain a nutrition of 120kcal/kg/day, frequent interruptions in enteral feeding were important factors in growth of preterm neonates. The proportion of babies found to be SGA at term gestation was high around 82.5% in 500-999g group and 72.7% in 1000-1499g group.

According to Corpeleijin W.E. et al, having favourable postnatal growth after a period of growth restriction is associated with favourable neurodevelopmental outcome with insulin resistance and metabolic syndrome later in life. Also monitoring postnatal growth is complicated with current

growth charts reflecting nutritional practices rather than the reflection of preterm infants growth potential. Proportion of preterm infants that are severly growth retarded(<3rd centile) at admission to NICU is 1%, which increases to 55% at the time of discharge.

According to Su BH et al, the proportion of growth restriction in very low birth weight infants varies between 43% to 97% in various centers. The observed difference could be due to different reference growth charts and nonavailablity of a standard nutritional strategy.

Table 3: Studies on prevalence of growth restriction and analysis of possible causes.

AUTHOR/ YEAR	STUDY METHODOLOGY	OUTCOME MEASURES	RESULTS
DENG Ying et al China, 2016	Observational study N= 61 Time – 12 months	1.Growth parameters(z score) 2. Developmental parameter(DDST)	1.Proportion of underweight, growth retardation, emaciation, microcephaly, overweight, obesity were 15%, 16%, 11%, 13%, 20%, 10% respectively 2. 25% had abnormal developmental quotient. 3. SGA group had significantly higher incidence of growth retardation and abnormal DQ than AGA group.
Adriana et al	Observational study N=305 D1 = 500-999gm D2 = 1000-1499g	NICU nutritional practices and their influence of neonatal growth	Factors adversely affecting neonatal growth : Parenteral nutrition: Delay in starting parenteral nutrition, delay in achieving a calorie intake of 120 kCal/kg/day, delay in introducing aminoacids and lipids, failure to achieve maximum levels. Enteral nutrition : Delay in introducing MEN, delay in hiking up feeds, delay in achieving full feeds, lack of

			fortification, frequent interruptions. Proportion of growth restriction at term gestation : 82.5% in D1 group, 72.7% in D2 group.
Corpeleijin W.E. et al	1.Proportion of severe growth restriction(<3rd centile) at admission to NICU is 1%, while at discharge it is 55% 2. Monitoring growth in these babies is difficult due to lack of standard growth curves. 3. Favourable growth after a period of growth restriction is associated with favourable neurodevelopment although associated with insulin resistance and metabolic syndrome later in life.		
Su BH et al	1.Proportion of growth retardation in preterm infants at term gestation varies from 43 – 97% in various centers. 2.Observed difference in different centers can due to different growth curves used and the non availability of standard feeding techniques.		

A meta-analysis was done by Brown et al in 2016, to determine whether multinutrient fortified human breast milk (with human milk fortifiers) improves growth and development of the infant without increasing the adverse effects when compared to unfortified milk.(6) They included 14 trials in which 1071 infants had participated. The individual studies were small and had weak methodology. The analysis showed a low quality evidence that multi-nutrient fortification of breast milk increased growth rate – weight (mean difference [MD] 1.81 g/kg/day; 95% CI 1.23 to 2.40), length (MD 0.12 cm/wk; 95 CI 0.07 to 0.17), head circumference (MD 0.08cm/wk; 95% CI 0.04 to 0.12) with statistically insignificant effect on development (mental developmental index 2.2 more- 3.35 fewer to 7.75 more) and NEC (RR 1.57; 95% CI 0.76 to 3.23).

Kanya Mukhopadhyay et al did a similar RCT in Indian setting, involving 166 babies, randomized to receive unfortified breast milk and breast milk fortified with human milk fortifiers.(11) The results were analysed separately for SGA and AGA babies. The mean difference for weight gain was 3.2 g/kg/day, increase in length 0.18 cm/wk, head circumference 0.08cm/wk more in the fortified group, which was statistically significant. Serum levels of

sodium, calcium, phosphorous and ALP were comparable between the two groups. Similar incidence of sepsis, NEC, IVH, PDA was found, however there was a slightly increased in incidence of chronic lung disease in the fortified group(p=0.036). On subgroup analysis, the fortified SGA group(n=37) had statistically significant difference in gain of weight and increase in length as compared to the control group. In AGA group, there was no statistical significance in weight gain between the two groups, however the gain in length and head circumference was significant.

Table 4: Studies comparing Fortified and Unfortified milk in preterm VLBW babies.

AUTHOR/ YEAR	STUDY METHODOLOGY	OUTCOME MEASURES	RESULTS
Brown VE et al Cochrane library 2016	Systemic review Num of articles: 14 RCT Num of Infants: 1071	1.Anthropometric parameters 2. Developmental parameters 3. Complications	1. Fortification increases rate of growth: weight (MD 1.81g/kg/d;95% CI 1.23 to 2.40), length (MD 0.12cm/wk, 95% CI 0.07 to 0.17) and Head circumference (MD 0.08cm/wk, 95% CI 0.04-0.12) No difference in growth and developmental parameters beyond infancy. 2. Low quality evidence that fortification does not increase the risk of NEC.
Kanya Mukhopadhyay et al Indian Pediatrics 2007, India	Randomized Controlled trial HMF= 85 Unfortified controls= 81 Stratified for SGA and AGA	1.Anthropometric measures 2. Biochemical parameters 3. Length of hospital stay 4. Morbidities- PDA, LOS, CLD and IVH.	1. Fortified group had significantly better weight gain, increase in length and head circumference. 2. Sodium, calcium, phosphorous and ALP was comparable in both groups. 3. Incidence of LOS, PDA, IVH, and NEC were similar in both groups, but CLD was more in fortifier group.

There was a single study studying preterm formula powder as a fortifying option in feeding preterm VLBW infants. Probably this could be because majority of studies on fortifying breast milk was done on developed countries where human milk fortifiers are easy to obtain. In

developing, low income countries, where human milk fortifiers are difficult to obtain(12) in a community setting, preterm formula powder in fortifying breast milk is routinely used(6).

In this study done in Egypt by El Sakka et al, 50 babies (25 in each group), with <37 weeks babies, weighing <1500 gms, there was a statistically significant difference in the rate of weight gain – 3.0g/kg/day, length – 0.18cm/wk and head circumference 0.09cm/wk between the two groups.(8) Mean hemoglobin was significantly lower in the no fortification group(10.75±1.47 vs 11.94±2.32), when compared with the fortified group. However, there was statistically insignificant increase in feed intolerance (12% Vs 4%) and the duration of hospital stay in the fortification group.

Table 5: Study evaluating preterm formula as a fortifying option in preterm VLBW babies.

AUTHOR/ YEAR	STUDY METHODOLOGY	OUTCOME MEASURES	RESULTS
El Sakka et al Pediatric Rep 2016, Egypt	Prospective Case control study Preterm formula fortification, n=25 No fortification=25	1.Anthropometric measures 2. Biochemical parameters 3. Length of hospital stay. 4. Incidence of complications	1. Rate of gain of weight between 2 groups: 16.8 and 13.8g/kg/day, Length: 0.76 and 0.58 cm/ week, Head circumference 0.59 and 0.5 cm/week. 2. Hemoglobin was significantly higher in cases. 3. Duration of hospital stay was longer in the intervention group. 4. Incidence of feed intolerance and sepsis was higher in the fortification group. (statistically insignificant)

Various studies were done to compare the efficacy and safety of fortifying expressed breast milk when compared with using formula feeds in these infants. Wang et at, in 2012, studied 125 infants and found there was no statistically significant difference in gain of weight, length and head circumference. Serum levels of protein nutritional status like BUN, prealbumin and serum calcium was lower in the intervention group when phosphorous and ALP was comparable between the two groups. However, there was a statistically significant reduction in the incidence of sepsis in the fortified EBM group (16.1% Vs 31.7%).

A similar study was done by Wu Y et al in a similar setting, which also reinforced the above findings. They concluded that, there was no statistically significant difference in the gain of weight and head circumference, though there was a difference in the rate of gain of length. There was no difference in the incidence of extrauterine growth restriction or the age of rebounding to birth weight. They also found a significant reduction of feed intolerance and sepsis in the breast milk fed groups.

A study with similar objectives was done by Schaller et al with 108 babies and they differed from the previous two studies, in their conclusions. They found that the rate of gain of weight, length and head circumference in HMF group was slower than the formula fed group, however the gain in formula fed group surpassed the normal intrauterine nutrient assimilation rate in these infants. Also, the rates of late onset sepsis and necrotizing enterocolitis was higher in the formula fed group.

Table 6: Studies comparing benefits of EBM fortification with preterm formula feeding.

AUTHOR/ YEAR	STUDY METHODOLOGY	OUTCOME MEASURES	RESULTS
Wang et al Zhonghue Er Ke Ze Zhi 2012, China	Prospective controlled study HMF= 62 Formula feeds= 63	1. Velocity of gaining weight, length, head circumference 2. Time for regaining birth weight 3. Biochemical parameters 4. Complications	1. Length 0.7Vs 0.6cm/wk, HC 1.1 Vs 0.9, weight gain was similar in both groups. 2. Lower BUN, Prealbumin and calcium in HMF group with no difference in PO4 and ALP. 3. Sepsis was lower in HMF group (16.1% Vs 31.7%)
Wu Y et al Beijing Da Xue Xue Bao 2016, China	Randomized controlled study HMF= 62 Formula feeds=60	1.Velocity of gaining weight, length, head circumference 2.Time for regaining birth weight 3.Biochemical parameters 4. Complications	1. Gain of length more in HMF group, other parameters being similar 2. Age of rebounding to birth weight was longer in HMF group. No difference in the incidence of EUGR. 3. ALP was higher in HMF group.(363.98 Vs 299.73)

			4. Incidence of feed intolerance (6.5% Vs 18.3%) and sepsis (4.8% Vs 16.7%) lower in HMF group.
Schaller et al Pediatrics 1999,	Prospective controlled trial HMF= 62 Preterm formula feeds=46	1.Anthropometric measures 2. Complications	1. Rate of increase in weight, length, HC, Increase in skin fold thickness slower in HMF group. 2. Incidence of NEC and LOS lesser in HMF group. 3. The postnatal growth surpassed IU growth rate in Formula feed group.

Even though there is level 1 evidence to recommend enteral feeding with fortified breast milk for optimal growth in these infants, there are paucity of studies to recommend the single best fortifying option in terms of efficacy, safety and economical aspects. Fortifying with human milk fortifiers is found to have increased incidence of feed intolerance, late metabolic acidosis, necrotizing enterocolitis in addition to having high cost of fortification. Various studies have studied the complications of human milk fortifiers.

A retrospective cohort analysis was done by Cibulskis et al after a change of policy to use liguid human milk fortifier.(12) They studied 2 cohort using liquid HMF and powdered HMF, with a total of 100 neonates and found that incidence of metabolic acidosis was more common in liquid HMF than powdered HMF (54% vs 10%), with more discontinuation rates in liquid HMF group (62% vs 18%). There was no statistically significant difference in the incidence of feed intolerance between the two groups (27% vs 38%).

At our NICU, over a 6-month period of observation from July to December 2016, the proportion of neonates developing feed intolerance following fortification with HIJAM was found to be 39.2% (29/74). Moreover, we also observed an unusually high incidence of late metabolic acidosis – about 10.8% – following fortification with HIJAM. Both these complications warrant withholding of fortification – temporarily or permanently – in these neonates thereby compromising their optimal growth and nutrition. The prevalence of feed intolerance in neonates receiving fortification with the other fortifier – Lactodex-HMF – was 26.7% (4/15). The cost of

fortification was also high, which for a 1 kg neonate will be around – INR 140-160/day with HIJAM (INR 20/sachet- 1g) and INR 35-46/day with Lactodex(INR 11.50/ sachet- 2g), which would be a high burden for a low income family.

Hence, a consensus was reached in our unit – NICU AIIMS and HIJAM was replaced with PreNAN which reportedly had less complications.

Ganapathy et al studied the incidence of another rare complication – Necrotising enterocolitis and their economical implication with human milk fortifiers from a previously done RCT by Sullivan et al. They found the incidence of NEC (medical and surgical) to be 16% and 10% for Bovine based HMF as compared to 6% and 1% in case human milk based HMF is used. Additional costs incurred for medical and surgical NEC would be $74K and $198K respectively.

In a study done by R Agarwal et at in AIIMS, New Delhi, they found statistically significant increase in osmolality of breast milk on adding both human milk fortifier and low birth weight formula powder.(13) However there was no increase in osmolality on keeping it was six hours, thereby ruling out bacterial cause for increase in osmolality. The possible explanation could be that the maltodextrin present in these compounds was broken by amylase present in breast milk, thereby increasing osmolality. Since osmolality of feed is directly proportional to the incidence of feed intolerance, addition of these compounds could possibly increase the incidence of feed intolerance in infants.

However, a retrospective cohort study done by Moody GJ et al with 76 babies, they found although there is increase in the incidence of increased gastric residuals and episodes of vomiting with addition of human milk fortifiers, there is no difference in the number of feeds withheld or time to achieve full enteral feeding or the duration of hospital stay. They concluded, the addition of HMF does not adversely affect the outcome of the preterm neonate.

Table 7: Studies studying various complications of HMF.

AUTHOR/ YEAR	STUDY METHODOLOGY	OUTCOME MEASURES	RESULTS
1.CC Cibulskis et al J. of Perinatology 2015, USA	Retrospective cohort analysis N= 100 Liquid HMF=50 Powdered HMF=50	1. Incidence of metabolic acidosis 2. Incidence of feed intolerance 3. Incidence of NEC, LOS, Length of hospital stay.	1. Metabolic acidosis more common with Liquid HMF (54% Vs 10%) 2. Discontinuation rates were more common in Liquid HMF (62% Vs 18%) 3. No difference in feed intolerance between powdered and liquid HMF
2.Ganapathy et al Breastfeeding medicine 2012, California	Cost analysis of a prev RCT(Sullivan et al) n=207 Human milk HMF=138 Bovine HMF=69	1. Incidence of Medical and surgical NEC 2. Additional costs involved with surgical and medical NEC	1. Incidence of NEC(medical and surgical) 16% and 10% for Bovine based HMF and 6%, 1% for human milk HMF 2. Additional costs incurred for medical and surgical NEC- $74K and $198K resp
3.Moody GJ et al J Pediatric GE Nutr 2000	Retrospective Cohort study N=76	1. Milk intake, episodes of Abdominal distention, Gastric residual volume > 2ml/kg or >50% of prefeed, Bile stained/ blood stained GRV, emesis, regurgitation. 2. Number of episodes of Apnea and bradycardia; changes in abdominal examination 3. Number of hours feeds withheld.	1. Significant increase in number of episodes of GRV >2ml/kg and emesis after adding HMF. 2. No difference in number of feeds withheld or other clinical findings. 3. No delay in the time to achieve full feeds, complete oral feeds or hospital discharge. Thus, addition of HMF does not adversely affect outcome of preterm neonate.
4.R Agarwal et al Indian Pediatrics Jan 2004	Prospective Blinded study N= 48	1.Osmolality of breast milk with and without fortification. 2. Change in osmolality on storing for 20 min.	1. Fortifying with HMF and LBW formula resulted in significant rise in osmolality 2. No further rise was observed on storage upto 6 hours.

Keeping in view with the above high complication rate and high cost incurred with human milk fortifier, a simple, economical and more plausible option is to fortify breast milk with preterm formula powder as done in certain centers. However with preterm formula fortification, there is lack of adequate studies to recommend its use in a community setting. A single study was done

previously in Thailand by Khorana et al comparing the two – Human milk fortifier and preterm formula fortification.

According to the study by Khorana et al, which was a randomized controlled trial with a small sample size, n=33, they concluded there is no statistical difference in the growth parameters between the two groups.(9) The two groups had similar biochemical parameters and the incidence of complications were also similar in both the groups. However the cost incurred was 20 times higher in the human milk fortifier group. Thus their recommendation was to use preterm formula powder as a fortifying option in case human milk fortifiers are not available.

Table 8: Study comparing HMF and preterm formula powder as a fortifying option.

AUTHOR/ YEAR	STUDY METHODOLOGY	OUTCOME MEASURES	RESULTS
Khorana et al J Med Asso Thai 2014, Thailand	Randomized controlled trial N=33 HMF=18, Preterm formula fortification=15	1. Anthropometric measures 2. Biochemical parameters 3. Complications	1. No difference in growth parameters. 2. No difference in biochemical parameters in both groups. 3. No difference in the incidence of complications 4. Cost was 20 times higher.

Comparing the RDA of preterm infants and nutritional information of breast milk on adding human milk fortifier, in particular HIJAM, with that of preterm formula powder, DEXOLAC – SPECIAL, it can be seen that preterm formula fortification will be deficient in calcium, phosphorous, Iron, vitamin A, D. Thus, preterm formula fortification will additionally require supplementing deficient nutrients.

Table 9: Data comparing HIJAM fortification and fortification with preterm formula powder.

	RDA	HMF- HIJAM	Preterm formula
Energy(Kcal)	110-135	145.8	153
Protein	3.5-4.0	3.78	3.5
Fats	4.8-6.6	8.1	8.6
Carbohydrates	11.6-13.2	16.8	15.4
Calcium	120-140	225	104
Phosphate	0-90	116	56
Vit A	1330-3330	1202	788

Iron	2-3	2.7	0.9
Zinc	1.1-2.0	0.88	0.9
Vit D	800-1000	734	36

What is already known?

1. Preterm VLBW neonates require higher energy, protein and mineral requirements, which are not met by unfortified breast milk alone.
2. There is level I evidence to suggest the use of fortified EBM in preterm infants[5]
3. Traditionally HMF is used. Fortifiers used in INDIA – According to a 6 month observation in NICU, AIIMS during July – Dec, 2016
    a. Lactodex: Need of additional supplements. Protein requirement will not be met. Incidence of feed intolerance of 26.7%.
    b. HIJAM: High incidence of feed intolerance of 39.2% and late metabolic acidosis of 10.8%. High cost involved – INR 20/ sachet – 1 g.
    c. PreNAN : Needs additional Vit D supplementation. Reportedly has less complications, though limited studies. High cost involved – INR 22/ sachet – 1 g.
4. A simpler and economical option could be to use preterm formula powder in fortifying EBM.

GAPS IN KNOWLEDGE

1. Paucity of data from India on benefits and complications of fortification with various fortification options.
2. Lack of studies directly comparing HMF and preterm formula fortification.

RATIONALE OF CURRENT STUDY

1. Preterm VLBW neonates require higher energy, protein and mineral requirements, which are not met by unfortified breast milk alone.
2. Many newborn care units therefore fortify EBM with commercially available human milk fortifier (HMF).
3. But fortification with HMF is expensive and is also associated with high risk of complications like feed intolerance and late metabolic acidosis, which often warrant temporary or permanent withholding of fortification.
4. A simpler and more economical option is to use commercially available preterm formula powder, in place of HMF, to fortify EBM. An earlier study from our unit at AIIMS demonstrated no significant difference in the osmolality of milk fortified with HMF and preterm formula powder.
5. Fortification with preterm formula powder might be associated with lower risk of feed intolerance and similar (not lower) weight gain in preterm neonates.
6. No studies have, however, examined the rates of weight gain following fortification of EB with preterm formula powder.

Research methodology

Hypothesis

Among preterm (28 – 34 weeks) VLBW infants (P), fortification of expressed breast milk with preterm formula powder (I) results in weight gain from the time of fortification until discharge from hospital or 40 weeks PMA whichever is earlier(T), not lower than 2 g/kg/day (O), when compared with the expected weight gain of 13.5 gm/kg/day following fortification with commercially available human milk fortifier (HMF) (C).

Primary objective

To compare the rate of weight gain from the time of enrolment until discharge from hospital or 40 weeks PMA whichever is earlier, in preterm (28-34 weeks' gestation) VLBW neonates receiving expressed breast milk (EBM) fortified with preterm formula powder with those receiving EBM fortified with commercially available human milk fortifier (HMF).

Secondary:

Among preterm VLBW infants receiving EBM fortified with preterm formula powder or HMF, to compare the

1. Proportion of infants who are growth restricted at term gestation (40 weeks PMA)
2. Rates of gain in length and head circumference from the time of enrolment until discharge from hospital and at 40 weeks PMA
3. Incidence of feed intolerance and other morbidities including late metabolic acidosis (LMA), necrotizing enterocolitis (NEC) stage 2 or more, anemia requiring blood transfusion(s), and osteopenia of prematurity.

Outcomes

Table 10: Objectives and definitions of the study, the methods and time period of assessment.

S. No	Objective	Outcome measured	Definition	Method	Time Period
1.	To compare the rate of in-hospital weight gain.	Rate of weight gain from the time of fortification till discharge from hospital	Average of weekly weight gain during hospital stay. Weekly weight gain calculated as per g/kg/day taking mid weekly weight.	Standard weighing machine with accuracy of 1 gm	Till Discharge. 40 weeks PMA will be the end point if not discharged till then.
2.	To compare the proportion of infants who are growth restricted at term gestation (40 weeks PMA)	Proportion of infants found to be postnatal growth restricted.	Weight at 40 weeks PMA taken and plotted in Fenton growth chart.	Babies falling <10th centile at 40 weeks PMA using Fenton growth charts.	At 40wks PMA
3.	To compare the weekly gain in height and head circumference	Average of weekly gain in length	Baby laid in supine position, stabilized and head to foot length taken.	Infantometer	Till discharge and 40wks PMA
		Average of weekly gain in head circumference – OFC.	Widest possible circumference – Most prominent part of forehead to widest part of back of head.	Non-stretchable tape	
4.	To compare the incidence of morbidities	Late Metabolic Acidosis	Base deficit > 5mmol/L ; TCO2 <18	VBG	Till discharge and 40wks PMA
		Necrotizing Enterocolitits	Clinical symptomatology with X-ray/ USG showing Pneumatosis Intestinalis and/or Gas in bile duct.	Clinical symptoms ± X-ray/ USG signs	
		Osteopenia of prematurity	Phosphorous<4mg/ dL with ALP>800IU/L.	Serum Calcium, Phosphorous, ALP with X-ray wrist SOS	
		Anemia requiring blood transfusion	Refer table.12	PCV by capillary centrifuge.	
		Feed intolerance	Refer table.11	Clinical symptom assessment by treating team	Till discharge

Study design: Randomized non inferiority trial

Study setting: NICU, AIIMS, New Delhi

Study duration: 1 1/2 years, Oct 2017- June 2019.

Inclusion criteria:

1. Preterm neonates born between 28 and 34 weeks of gestation
2. Birth weight less than 1500 g
3. Accepting oral feeds of at least 100ml/kg/day
4. Amount of EBM, as a proportion of total daily milk intake, 75% or greater at enrolment

Exclusion criteria:

Major congenital anomalies

Sample size

Data and assumptions

The data on PreNAN is still not available. The mean weight gain of neonates receiving fortification with HIJAM in our unit was found to be 13.5 g/kg/day (SD 3.8). Assuming that fortification with preterm formula powder will result in weight gain not lower than 2 g/kg/day i.e, a minimum weight gain of 11.5 g/kg/day with the same SD, and an alpha error of 5% and power of 90%, we have to enroll 62 neonates in each group.

Screening and enrolment

All neonates delivered at 28 to 34 completed weeks of gestation and weighing less than 1500 g at birth will be eligible for enrolment in the study. Details of eligible neonates will be entered in the screening form. They will be followed-up daily till they reach enteral feeds of at least 100 ml/kg/day with >75% oral feeds being expressed breast milk (EBM). If found eligible, the parent(s) will be approached for consent for enrolling the neonate in the study. After obtaining written consent of either of the parents, the neonate will be enrolled in the study. At the time of getting consent, parents will be explained regarding the pros and cons of the study, follow up visit and the expected study duration.

Gestational age would be assessed from last menstrual period (LMP) or first trimester scan (T1 scan), if LMP not available. If both are not available or a discrepancy of 1 week or greater is noted between the two, Expanded New Ballard Score (ENBS) will be used. In case of invitro fertilization (IVF) conception, date of embryo transfer will be considered in place of LMP. All neonates will be screened clinically for major congenital malformations. A Level II ultrasound will also guide in ruling out internal organ malformations. Baseline information regarding baby's identity, baby's hospital course, mother's medical history and the socio- economic details will be collected at the time of enrollment. This process will be continued till the required sample size will be met.

Randomization

Neonates will be randomized into two groups after enrolment into the study. We shall use stratified block randomization, with appropriate for gestational age (AGA) and small for gestational age (SGA) neonates being the two different strata. Within each stratum, blocks of varying sizes will be used for the process of randomization. This will thus ensure an equal number of participants in each group.

Allocation concealment of the principal investigator will be ensured by using serially numbered, sealed, opaque envelopes kept in the NICU. The principal investigator shall open the next serially numbered envelope upon enrolment of the neonate and randomize to either of the two groups.

Blinding

Blinding of the intervention is not possible because of the practical difficulties in blinding: (1) different color, texture, and odor of the two fortifiers and (2) need to provide additional supplements (Ca/Po4) in formula powder group. The outcome variables have been made as objective as possible and protocols have been developed for management of complications to avoid being biased by the nature of fortification.

Intervention

Neonates in the intervention group will receive fortification by preterm formula powder- DEXOLAC SPECIAL, at 1 g per 25 mL of EBM. We shall prepare the sachets for each neonate with the exact amount of powder required for the given feed volume. The sachets will be prepared by the pharmacy. Amount of breast milk will be measured by using 20 or 50 mL

syringe and the appropriate amount of DEXOLAC SPECIAL powder will be added by mixing the contents of the sachet with EBM. Nutritional audit will be done after 1 week of reaching full feeds and additional supplements (vitamin D, calcium, phosphorous and iron) will be added accordingly. Parents will be provided with supply for 2 weeks of DEXOLAC SPECIAL at the time of discharge. Supply will be replenished, every 2 weeks, till the baby reaches 40 weeks/ 2 kg, whichever is later, provided the infant is still on EBM.

Neonates in the control group will be started with fortification by PreNAN/ other available fortifier, at 1 sachet (1g) to be added in 20 mL of EBM. Nutritional audit will be done after 1 week to ensure the baby is getting the adequate amount of nutrients. At the time of discharge, parents will be given supply for 2 weeks. Supply will be replenished every 2 weeks until the baby reaches 40 weeks/ 2 kg, whichever is later, provided the infant is on EBM.

Compliance of using fortification will be ensured by regular phone calls to parents and by enquiring them during the visits at the time of ROP screening.

Follow up of subjects

Growth assessment

At birth, the neonate's anthropometric parameters- weight, length and head circumference will be taken and the same will be repeated at the time of enrollment, i.e. at the start of fortification. Weight will be taken by a standard digital weighing machine with an accuracy of 1g with minimal clothing. Length will be taken by an infantometer and head circumference by a non-stretchable tape.

These measurements will be taken at weekly intervals during the period of hospital stay and the rate of growth will be calculated. Neonates found to have inadequate weight gain <10 g/kg/day with a feed volume of 180 ml/kg/day during the previous week will be evaluated for other possible associated causes with PCV and VBG. An organic cause if found will be treated. If no organic cause is found, feed volume of the baby will be increased upto 200 ml/kg/day. If the babies are found to have weight gain less than the cutoff 10 g/kg/day, will be crossed over to the other fortifier, which is PreNAN in case of intervention group and preterm formula powder i/c/o control group. Other fortifying options like cornstarch and MCT oil will be considered in individual cases as per consensus during ward rounds.

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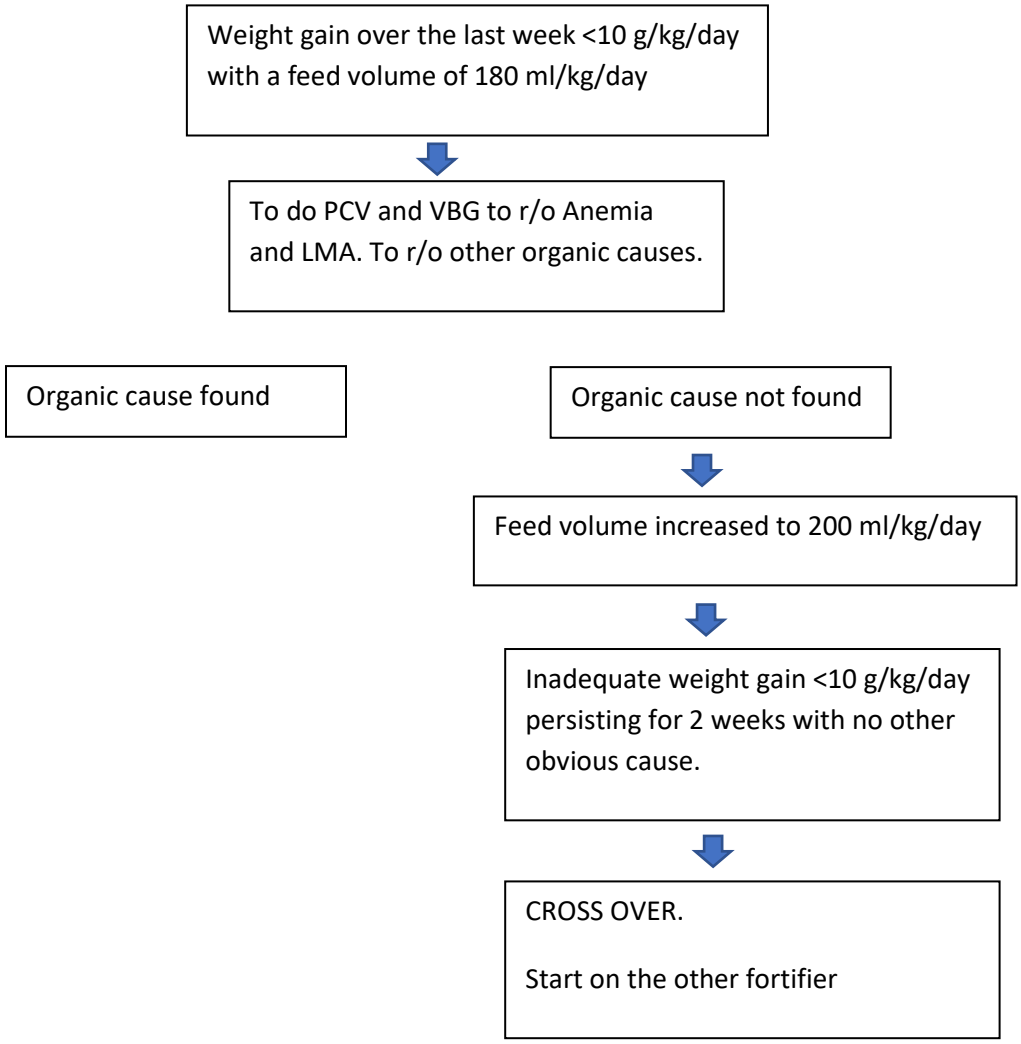

Weight gain over the last week <10 g/kg/day with a feed volume of 180 ml/kg/day
To do PCV and VBG to r/o Anemia and LMA. To r/o other organic causes.
Organic cause found
Organic cause not found
Feed volume increased to 200 ml/kg/day
Inadequate weight gain <10 g/kg/day persisting for 2 weeks with no other obvious cause.
CROSS OVER.
Start on the other fortifier

Assessment of feed intolerance

Enrolled neonates will be looked for features suggestive of feed intolerance like vomiting - number of episodes, quantity of vomitus, bile stained, fresh blood or altered blood; pre-feed aspirate volume and color; abdominal distention >2 cm from baseline.

Feed intolerance is diagnosed if the infant qualifies with 2 parameters or greater (14). If the pre feed aspirate is <25%, 25-50% and milky in nature or baby vomits >50% of feed volume and milky in nature, the action will be to stop fortification for 6 hours and reassess (see Table 11).

Table 11: Management of feed intolerance algorithm(14)

PARAMETER	FINDING	ACTION
Vomiting	>1 vomitus with yellow or green color/ fresh blood or altered blood*	Withhold feeding and evaluate for NEC/ sepsis
Pre-feed aspirate color	Bilious/ fresh blood/ altered blood	Withhold feeding and evaluate for NEC/ sepsis
Pre-feed aspirate volume	>50% of feed volume (to be checked after 3 feeds)#	Withhold feeding and evaluate for NEC/ sepsis
Abdominal girth	>2 cm increase over baseline in 24 hrs.	Withhold feeding.
*, # - If the pre feed aspirate is <25%, 25-50% and milky in nature or baby vomits >50% of feed volume and milky in nature, the action will be to stop fortification for 6 hours and reassess.		

Assessment of morbidities

Assessment of late metabolic acidosis (LMA)

Weekly venous blood gas will be done, as per unit protocol, for all enrolled neonates until the time of discharge. Late metabolic acidosis (LMA) will be diagnosed with a base deficit >5 mmol/L with TCo2 <18 after 3rd day of life. TCO2 will be calculated by summing HCO3 and amount of CO2 dissolved in plasma. Dissolved CO2 will be calculated by multiplying 0.03 and PCO2. Neonates meeting criteria for late metabolic acidosis but with adequate weight gain (minimum of 10 gm/kg/day) will only be observed. Those with LMA and inadequate weight gain will be treated with 7.5% sodium bicarbonate (dose: 0.6*Body weight*Base deficit) till 2 weeks of age or till 36 weeks PMA, whichever is later. Fortification will be continued in these neonates.

Assessment of osteopenia of prematurity(15)

Samples will be taken for calcium, phosphorous and ALP on suspicion of osteopenia of prematurity and at 40 weeks PMA. Osteopenia will be diagnosed when ALP> 800 IU/L along with

a serum phosphorous value of <4 mg/dL. Diagnosis will be confirmed by decreased bone mineralization observed on a radiograph.

Assessment of anemia requiring blood transfusion(15)

PCV will be done in case of inadequate weight gain <10 g/kg/day during the previous week or on suspicion of anemia by the treating clinical team. Venous blood will be taken in a capillary tube and centrifuged at 8000-12000 rpm for 5 min and measured. Need for blood transfusion will be assessed with the following guidelines:

Table 12: Table showing PCV cutoff for blood transfusion in neonates

S.No	Levels of respiratory support	Oxygen requirements	<28 days (PCV)	>28 days (PCV)
1.	Assisted ventilation	FiO2>0.3	<40	<30
		FiO2<0.3	<35	
2.	CPAP	Any FiO2	<30	<25
3.	Spontaneously breathing	Any age		
	a. Symptomatic anemia	FiO2≥0.35	<35	
		FiO2>0.21-0.34	<30	
	b. Oxygen therapy	FiO2>0.21	<25	
	c. Room air		<20	

Assessment of Necrotizing Enterocolitis (NEC) stage 2 or more(15)

Neonates will be observed by the treating clinical team daily for symptoms suggestive of NEC. After ruling out other causes, neonates with high suspicion of NEC will be done a USG and/or X-ray to look for pneumatosis intestinalis and/or portal venous gas. Neonates confirmed to have NEC will be treated according to the management guidelines.

Neonatal data collection at the time of discharge

At the time of discharge, neonates' anthropometric measurements will be recorded and details regarding the duration of hospital stay, type and mode of feed the baby is on, total duration of ventilator requirement and the total duration of TPN given will be recorded. Parents will be given 2 weeks supply of fortifiers and stressed on compliance, with the need of coming for follow up visits.

Post discharge follow up

Neonates will be followed up at 40 weeks PMA to assess the anthropometric status. Data regarding final diagnosis of morbidities like PDA, ROP, IVH, LOS and CLD will be recorded. Days of fortification given and the compliance will also be recorded. A final diagnosis regarding the presence or absence of EUGR will be assessed by using Fentons' growth charts.

Data collection and management

A case record form has been designed to record data pertinent to the study. The approval of faculty of neonatology division will be obtained prior to proceeding. All baseline variables and outcomes will be recorded in the Performa. Case record forms will be periodically checked and counter signed by the faculty. A database will be created in the Microsoft assess 2007 (Microsoft, Redmond, CA) for recording the variables.

Statistical analysis

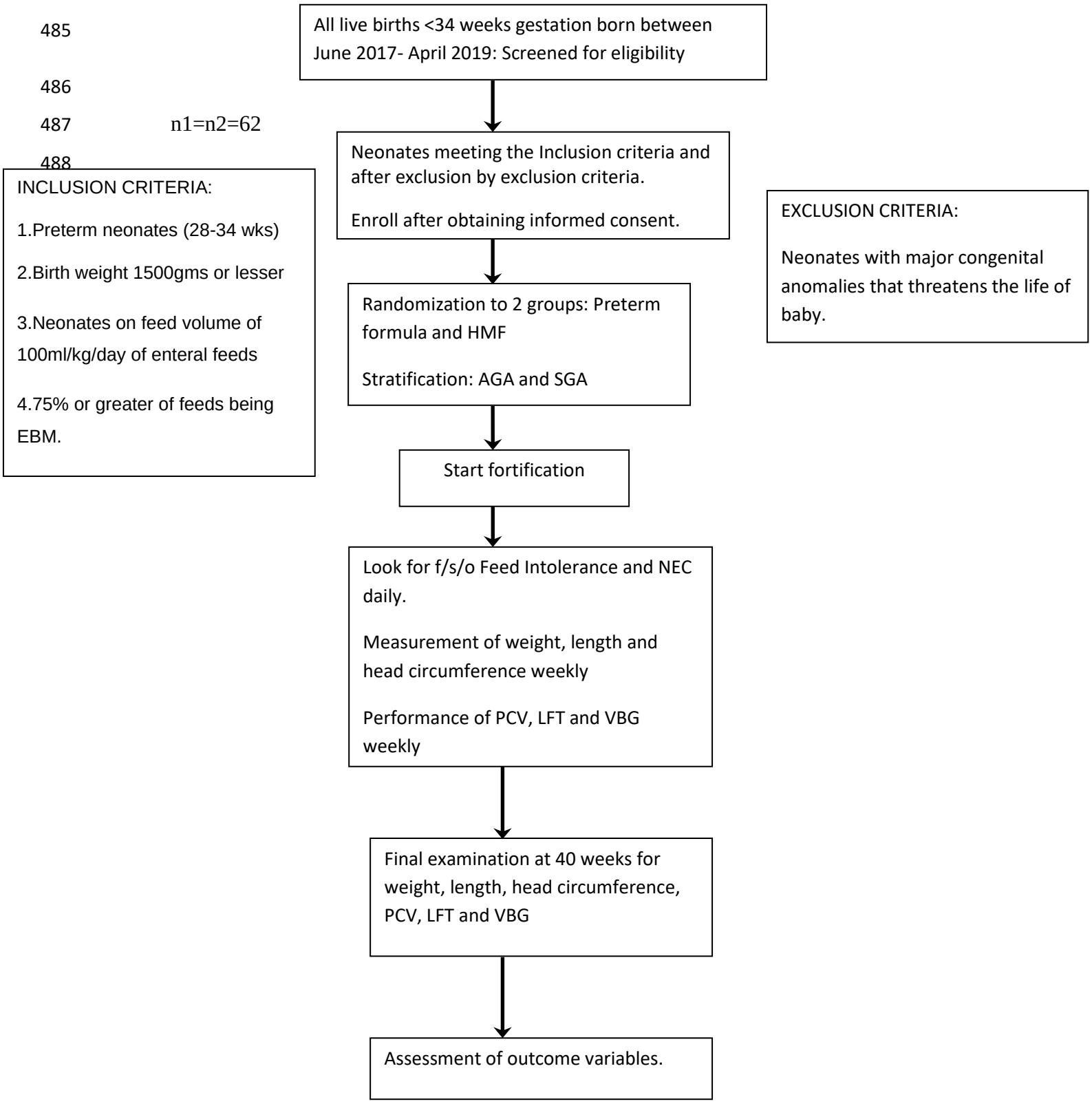
Data entry will be made in MS Assess 2007 (Microsoft Corp, Redmond, CA). Data analysis will be done by STATA/ SE 11.2 (Stata Corp, College station, Tx).

Data on feed intolerance, LMA, osteopenia of prematurity, NEC, anemia requiring blood transfusion, will be expressed as n (proportion). Test of significance will be Chi square test/ Fisher Exact test. Incidence of feed intolerance, LMA, OOP, NEC and anemia will be calculated by dividing the number of neonates who developed the condition by total number of neonates started on a particular fortifier.

Anthropometric parameters and biochemical parameters will be represented as mean ±SD and tested for significance by Student t test, assuming it to be normally distributed.

Being a non-inferiority trial, we intend to use both per protocol analysis as well as ITT for the key outcomes.

Study flow


All live births <34 weeks gestation born between June 2017- April 2019: Screened for eligibility
n1=n2=62
Neonates meeting the Inclusion criteria and after exclusion by exclusion criteria.
Enroll after obtaining informed consent.
INCLUSION CRITERIA:
1.Preterm neonates (28-34 wks)
2.Birth weight 1500gms or lesser
3.Neonates on feed volume of 100ml/kg/day of enteral feeds
4.75% or greater of feeds being EBM.
EXCLUSION CRITERIA:
Neonates with major congenital anomalies that threatens the life of baby.
Randomization to 2 groups: Preterm formula and HMF
Stratification: AGA and SGA
Start fortification
Look for f/s/o Feed Intolerance and NEC daily.
Measurement of weight, length and head circumference weekly
Performance of PCV, LFT and VBG weekly
Final examination at 40 weeks for weight, length, head circumference, PCV, LFT and VBG
Assessment of outcome variables.

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ANNEXURE 1:

PARENT/ LAR INFORMED CONSENT FORM (PICF)

Participant identification number for this trial: _____

Title of the project: FORTIFICATION OF EXPRESSED BREAST MILK WITH PRETERM FORMULA POWDER Vs HUMAN MILK FORTIFIER IN PRETERM (28-34WKS) VLBW NEONATES: A RANDOMISED NON INFERIORITY TRIAL

Name of the Principal Investigator: JR Dr **C Arunambika**. Mobile Num: **9791239124**

Name of Co- Investigator: Asst Professor Dr. **M Jeeva Sankar** Phone: **011-26546166**

The contents of the information sheet dated _____ that was provided have been carefully read by me/ explained in detail to me, in a language that I comprehend, and I have fully understood the contents. I confirm that I have had the opportunities to ask questions.

The nature and purpose of the study and its potential risks/ benefits and expected duration of the study, and other relevant details of the study have been explained to me in detail. I understand that my consent for my baby's participation is voluntary and that I can withdraw at any time in-between without giving any reasons, **without my baby's medical care or legal rights being affected**. I understand that the information collected from my baby, the samples taken and sections of any of medical notes may be looked at by responsible individuals from AIIMS and for **using it for publishing/ literature purposes**, without disclosing my neonates' identity.

Thus knowing all these, I give consent for my baby to take part in the study trial voluntarily **without any pressure by any means**.

Date: _____

Place: (Signature/ Left thumb impression)

Name of the parent/ LAR(Relation):

Son/ Daughter/ Spouse of:

Complete postal address:

This is to certify the above consent was obtained in my presence.

_____(Signature of Principal Investigator)

1. **Witness 1**	**2. Witness 2**
Signature:	**Signature:**
Name:	**Name:**
Address	**Address:**

ANNEXURE – 2

Parent/ LAR Information Sheet

i) **Title of the study.**

"Fortification of expressed breast milk with preterm formula powder vs. human milk fortifier (HMF) in preterm (28-34 weeks) very low birth weight neonates: A Randomized non inferiority trial"

Principal investigator: Dr. C Arunambika

Chief Guide: Dr. Jeeva Sankar M

ii) **Aims and methods of the research**

Heartiest congratulations to you and your family on birth of your baby. This is indeed a moment of great happiness for you. Still, as you know, your baby is born too soon from dates and is not fully mature.

Preterm neonates are neonates that are "Born too soon", i.e. before completion of 37 weeks. These neonates may have more health issues and may have longer hospital stay when compared to neonates born later. One important issue is the high incidence of extra uterine growth restriction in these neonates. It is because though preterm breast milk has composition that is different from that of term milk, it alone cannot meet the high demands of these neonates and when given alone can manifest with multiple deficiencies and growth failure.

Thus studies have shown advantages in short term growth parameters with the use of fortifiers with expressed breast milk. It is current standard of care in our hospital to fortify breast milk in neonates <34 weeks and <1500gms neonates until the baby reaches 2 kg or 40 weeks, whichever is later, provided the baby is getting expressed breast milk. A human milk fortifier known as PreNAN is used for routine fortification in our wards.

However there are concerns regarding high incidence of feed intolerance, late metabolic acidosis and high cost with HMF fortification. Another plausible idea could be to fortify EBM with preterm formula powder, which could be an acceptable alternative. Even though the studies in this area is limited, and additional supplements are needed to meet the neonates nutrient requirement, available studies have shown it to be equal in efficacy with possibly decreased incidence of feed tolerance with preterm formula fortification.

You are being requested to give consent for your baby to be included in the study of efficacy and complications comparing two fortifiers, that is being added to increase the nutrient content of breast milk, currently used- PreNAN and the proposed fortifier- preterm formula powder- DEXOLAC SPECIAL. This study will aim to assess the difference in gain of anthropometric parameters and the incidence of morbidities like feed intolerance, late metabolic acidosis, osteopenia of prematurity, anemia and NEC that are known to be caused by these fortifiers.

Thus this study is to compare both fortifiers with regard to anthropometric measures- rate of increase of weight, length, head circumference and the incidence of complications with respect to feed intolerance, late metabolic acidosis, osteopenia and necrotizing enterocolitis.

Your baby, being preterm, will be screened for the eligibility to enroll in the study using a screening form. If your baby satisfies the eligibility criteria, then you will be given a choice to join the study. Your baby will be enrolled only after your willful consent. After your consent, neonates will be allotted randomly to one of these two groups, which will not be under the control of investigator, who will be unaware of the nature of your fortification.

After enrollment, some baseline data and measurements will be collected regarding mother, baby and the baby will be started on fortification. Symptoms such as vomiting, abdominal distention will be monitored daily by the treating physician either by asking directly from the caretakers or from nurses monitoring chart. Weekly anthropometric measures- weight, length and head circumference along with the required blood sample around 1.5ml will be taken by the principal investigator. These procedures are routine in the care of neonates on breast milk fortification and since these are important for the study, it is being done by the principal investigator.

Even with refusal to give consent for participating in the study, the baby will be started on fortification with PreNAN and the investigations will be continued, with no compromised clinical care of the baby. Your neonates' participation will help us to get some idea regarding the complications and efficacy of the two fortifiers, thus in formulating a hypothesis that in future could guide the decision of feeding preterm neonates.

Any complications that may arise during the study will be evaluated carefully by the treating team and be treated as per the current standard treatment protocol.

iii) **Expected duration of the subject participation.**

Till the baby reaches term gestation/ 40 weeks PMA. Maximum of 12 weeks.

iv) **The cost burden/benefits to be expected from the research to the subject or to others.**

There will be no additional cost burden to you and your family due to this study. Neither will you be provided any financial benefit for being part of the study.

v) **Any risk to the subject due to participation in the study.**

This study will be done like routine clinical care of the baby. No additional blood samples or investigation or procedure will be done for the sole purpose of the study. There will no risks to the subject due to this study.

vi) **Provision of free treatment for research related injury and compensation of subjects for disability or death resulting from such injury.**

Not applicable

vii) **Maintenance of confidentiality of records.**

The confidentiality of the personal details would be maintained. Data related to the study will be used for analysis and will be shared with responsible individuals. The information could also be published in text of any format, without revealing the neonates identity.

viii) **Freedom of individual to participate and to withdraw from research at any time, without penalty or loss of benefits to which the subject would otherwise be entitled.**

Your baby's participation in this study is purely voluntary and you may choose to withdraw from the study at any time after agreeing to participate in the study, without having to give any reasons. Choosing not to participate at any time will not affect treatment services your baby may be requiring now or in future. You can ask questions about this project at any time. You may contact the investigator given below, if you have any questions or grievances about this research study, either directly, through mail or contact number.

Please feel free to ask about anything you do not understand. Please consider this information sheet and consent form carefully before you agree to participate in our study.

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