Effect of Hindmilk on Growth Velocity of Very Preterm Infants
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Project Summary:
Extra-uterine growth restriction (EUGR) is one of the most common findings among very preterm infants at discharge from neonatal intensive care units. EUGR is associated with major morbidities such as bronchopulmonary dysplasia (BPD), retinopathy of prematurity (ROP) and impaired neurodevelopment. EUGR is caused by slow postnatal growth and largely results from energy and protein deficits. These deficits occur despite the current fortifications of human milk. Hindmilk, the milk at the end of a breast pumping session, has higher fat and energy content compared to the composite milk. Feeding hindmilk can be a natural and innovative way to provide additional calories for very preterm infant. Hindmilk is rich in some fatty acids that are currently under investigation to prevent BPD and ROP and improve neurodevelopment. The aim of this study is to assess growth benefits of feeding hindmilk and to explore whether it can improve fatty acids profile in very preterm infants.

1. **What you want to do.**
   Hypothesis: In very preterm infants (born less than 32 weeks gestation) with poor postnatal growth velocity (<15 g/kg/day), feeding hindmilk would improve average weight gain by at least 4 g/kg per day.
   Research question: Does feeding hindmilk improve weight gain in very preterm infants with poor growth velocity?
   Primary outcome: Difference in average weight gain (in g/kg per day) between the week after feeding hindmilk and the week before.
   Average weight gain is calculated as: (end weight – start weight in grams) / start weight then divided by number of days (7 days in our study as we use average weight gain over 7 days).
   American Academy of Pediatrics continues to recommend that postnatal growth of preterm infants should approximate the intrauterine growth of fetuses of the same gestational age (15-20 g/kg per day).[1] Therefore, poor postnatal growth velocity is defined when average weight gain is less than 15 g/kg per day).
   Secondary objectives/outcomes:
   1. Effect of feeding hindmilk on:
      a. Anthropometrics (weight, length and head circumference Z scores) at 36 weeks postmenstrual age (PMA) and at discharge.
      b. Body mass index (BMI) at 36 weeks PMA and at discharge.
   2. Incidence of EUGR at 36 weeks PMA and at discharge.
   3. Incidence of BPD, ROP and late-onset sepsis.
   4. Correlation between human milk content (fat, protein and energy) and weight gain.
   5. Effect of hindmilk on erythrocyte membranes fatty acids profile.
   6. Changes in mother's milk volumes after feeding hindmilk.

   Note: secondary outcomes 1 to 3 will be compared between infants received hindmilk for at least 2 weeks and the rates of these outcomes among NICUs. These rates are published publically by the Canadian Neonatal Network.

2. **Why this is a reasonable thing to do**
   The nutritional management of very preterm infants aims to result in growth patterns that approximate the intra-uterine fetal growth patterns.[1,2] However, poor growth during NICU stay is common and results in a high rate of EUGR, one of the most common findings in very preterm infants at discharge.[3-5] A study from Calgary has shown that 65% of very preterm infants are growth restricted at 37 weeks PMA.[4] EUGR and the associated caloric and protein deficits not only cause slower growth velocity, but also are associated with major morbidities, including BPD, ROP and impaired neurodevelopment.[2,6] Moreover, epidemiologic studies have described additional long-term health consequences of growth restriction, such as an increased risk of cardiovascular (hypertension), renal and metabolic morbidities, all explained under the concept of developmental origins of disease.[6-8]
   Human milk provides the ideal nutrition for term infants. It is also recommended for preterm infants, but does not alone provide optimal nutrition. The growth and neurodevelopmental needs of very preterm infants are best met by appropriate fortification of human milk. Nonetheless, current fortification regimen (4 packets added to 100 ml of human milk) has been unsuccessful in preventing EUGR in almost two thirds of very preterm infants.[4] Fortification with formula on top of the currently used human milk fortifiers has become a common practice to improve growth velocity. This practice increases milk osmolality which is a risk factor for feeding intolerance and necrotizing enterocolitis (NEC).[9] Powder formulas have also been associated with increased rate of sepsis.[10] Alternative sterile liquid formulations displace large amount of mother's own milk.

Foremilk and hindmilk for very preterm infants:

There is a large variation in the protein and energy contents of human milk between foremilk, the first milk that comes out of the mother's breast, and hindmilk, the milk that comes out at the end of the breast pumping session.[11] Foremilk is low in calories and fat compared to hindmilk. Fat content is known to be the major source for energy in the breast milk. Hindmilk is considered a natural way of providing additional calories to preterm infants. Furthermore, the degree of prematurity is inversely related to the ratio between foremilk: hindmilk volumes. This may partially explain the higher rate of postnatal growth failure in the more immature infants.[12] Although, it is difficult to precisely identify the time point in in which production of hindmilk starts, current literature define hindmilk as milk collected after the first 3 minutes of pumping.[13,14]

Nutrient composition of hindmilk in preterm infants:

In addition to the higher fat (1.2-fold) and energy (1.1-fold), concentrations of retinol α-tocopherol (vitamin A) and γ-tocopherol (vitamin E) are approximately a 1.2-fold higher in hindmilk compared to composite milk.[13] With the higher fat concentrations, hindmilk offers a great opportunity to naturally increase intakes of long chain poly-unsaturated fatty acids (LC-PUFAs). LC-PUFAs such as docosahexaenoic acid (DHA) and arachidonic acid (AA) are considered essential for maturation of the developing brain, retina and other organs in preterm infants. Several randomized controlled trials have been recently completed to explore effects of LC-PUFAs supplementation on visual acuity, BPD and neurodevelopmental outcomes of very preterm infants.[15,16]

Very preterm infants often have low plasma concentrations of vitamin A.[17] A recent Cochrane review indicated a small reduction; although significant, in the risk of BPD with repeat intramuscular doses of vitamin A.[18] The need for frequent muscular injections has limited the use of this method to prevent BPD. Vitamin E supplementation reduces risk of ROP.[19] Parenteral vitamin E (intravenous and intramuscular) has been associated with increased risk on intraventricular hemorrhage (IVH) and sepsis nevertheless enteral vitamin E supplementation has been shown to be safe in 10 randomized controlled trials.[19] Furthermore, the calculated daily dose that a very preterm infant will get from all enteral sources including hindmilk is significantly lower than that associated with increased risk of IVH and sepsis (30 IU/kg per day).[19] Nitrogen concentrations are almost similar between hind and composite milk (1.01-fold).[13]

Availability of hindmilk in NICU:

Preterm infants require small milk volumes. Therefore, their mothers are usually able to pump more milk than infants' need. It is beneficial to the infant if the small volumes they consume are higher in fat and calories rather than watered down with the foremilk volume. On average mothers of very preterm infants produce 545 mL per 24 hours (inter-quartile range: 224, 1817).[12] Most very preterm infants on enteral feeding require less than 225 ml of human milk when there weight is less than 1500 g. Previous studies showed that 62-82% of mothers of very low birth weight (VLBW) infants produce enough milk volume to enable fractionation and hindmilk feeding after 3 weeks from delivery.[12,20]

Use of hindmilk in Calgary:

Use of hindmilk (cream project) started in September 2016 as part of a quality improvement project to improve weight gain in very preterm infants. Eligibility criteria for including very preterm infants in the cream project are:

1) Infants is on full enteral feeds for more than 2 weeks
2) Poor weight gain (<15 g/kg per day) despite optimization of energy and protein intakes
3) Mothers have enough milk supply (>150% of daily needs)

Mothers are approached by NICU lactation consultants (LCs) and the benefits of higher fat milk is discussed. Mothers separate their foremilk (and freeze it to feed at a later time) from hindmilk. Mothers use the following instructions to separate their milk: "1) have extra breast milk storage containers ready, 2) begin pumping breasts with the breast pump, 3) about three minutes after the milk starts, turn the pump off. This is usually equal to one-third of the usual pumped amount, 3) label this container with "foremilk" which is frozen to be used later, 4) Change containers and continue pumping until two minutes after the milk flow stops, 5) label these bottles "hindmilk"." Hindmilk collection sheet is kept at bedside. The sheet is completed weekly by lactation consultant, participant's mother, bed side nurse and dietitian.

To date, a total of 34 mother-infant pairs were included in the cream project. Preliminary analysis for these infants revealed an increase in weight gain by 4.2 g/kg per day in the following week of using hindmilk. This analysis does not capture any change to the nutrition plan after the hindmilk use. There were 8 (23.5%) infants with limited benefit (less than 3 g/kg per day increase from the week before feeding hindmilk). Human milk analysis would

have helped identify fat and energy contents of the hindmilk of these mothers.

Hindmilk and erythrocyte membrane fatty acids profile:
The fatty acids (FAs) profile of the erythrocyte membrane measures the percentage of FAs in the red blood cells (RBCs) from a dried blood spot. Measurement of the RBC FAs provides less biological variability than the measurement in plasma because of a slower turnover rate.[21] Dietary supplementations of LC-PUFAs for 2 weeks have been shown to significantly alter RBC FAs profile.[22] Advantage of the RBC FAs profile is the small amount of blood to perform the test. FAs profile includes measurement for DHA, AA, and Eicosapentaenoic acid (EPA). Because hindmilk has 20% more fat than the composite milk, it is sensible to propose that the former FAs would be considerably higher in preterm infants fed hindmilk. Current literature suggest that supplementations with DHA using tuna oil to lactating mothers of very preterm infants[23], and not directly to these infants[16], may reduce the risk of BPD and hay fever particularly in boys. As we are measuring the fat contents of the composite and hindmilk, we will be able to explore effects on FA status particularly the levels of LC-PUFAs. This will set the basis for a larger study to address potential effects of hindmilk on brain, retina, and neurodevelopment.

In conclusion, EUGR is common in very preterm infants and is associated with short- and long-term morbidities. EUGR is largely due to nutritional deficits which occur despite standard fortification. This is in part due to large variation in human milk contents between mothers. Because very preterm babies need small volumes it is important to maximize their benefits from the small amount they get. To date, there have been very few studies conducted to evaluate benefits of hindmilk use in preterm infants. These studies were mostly small in size, done in countries where fortification is not used or done in more mature infants.[14,24,25]

3. Why this is important

This study represents an innovation in optimized nutrient delivery that may have a significant impact on morbidity of very preterm infants. This study would, (i) explore growth benefits of feeding hindmilk to very preterm infants, (ii) provide better understanding of the role of hindmilk in achieving favorable FAs profile (higher DHA and EPA proportions), (iii) will set the basis for a larger study to evaluate effects of hindmilk on BPD, ROP and neurodevelopment and (iv) may lead to a practice change to utilize hindmilk earlier to prevent slow growth velocity and EUGR. In addition, we need to systematically evaluate our current practices of hindmilk use with a prospective and detailed nutrition assessment.

4. How you are going to do it

4.1 Methodology:

Study design: This will be a prospective cohort study in very preterm infants admitted to NICU at Foothills Medical Centre.

Inclusion Criteria:

- Very preterm infants (<32 weeks at birth)
- On full enteral feeds for more than 2 weeks (full feed is defined at the time of reaching 120 ml/kg/day with no parenteral nutrition used)
- Poor weight gain (<15 g/kg per day) despite optimization of energy and protein intakes by RDs (calories intake: 125-135 Kcal/kg per day and protein: 4-4.5 g/kg per day)
- Mothers have enough milk supply (>150% of infant's daily needs)

Exclusion Criteria:

- Congenital anomalies
- Small for gestational age infants (< 10th percentile) at birth

4.2 Study protocol:

RDs (JS and HB) and LC (CM) will screen and identify eligible mother-infant pairs. Once mother's consent obtained, LC will hand out the "hindmilk information sheet" and teach the mother how to separate her milk. A sample (10 ml) of composite pumped fresh milk will be collected and placed in the fridge. These samples will be sent for analysis at NorthenStar Milk Bank in Calgary within 24 hours of collection to minimize any changes to contents.[26] Samples for Erythrocyte membrane FA profile will be collected on dried blood spot (30-100 µL). These samples will be collected within 72 hours of the consent. It will be coordinated with blood tests ordered by the clinical team within that period. Dried blood spots are stable in room temperature for 28 days however we will store them in the -80°C freezer in the neonatology research room at FMC within 72 hours of collection. Another milk sample, hindmilk this time, will be sent for testing within 24-48 hour of starting hindmilk. This is to ensure that assessment of milk contents is performed in the same mother's

lactation stage. The second dried blood spot will be drawn after 2-4 weeks of starting hindmilk. The 2-4 weeks period is to allow timing with other blood work-ups. In General, blood glucose is done every 2-3 days in babies with poor growth and growth laboratory testing is normally done every 2-3 weeks.

Data on fluid volumes, feeds, macronutrients intakes and any change in nutrition plan will be collected from electronic dietitian's notes. RDs will ensure proper and detailed documentation of nutritional information. Average weight gain (in gram/kg per day) is calculated as mentioned before. Average weight gain will be compared initially between the week before and the week after starting hindmilk. The day that hindmilk started will be used to identify the start point however it will not be used in either the pre- or the post-hindmilk calculation. Clinical team will be encouraged to not order other changes to the nutrition plan for the first week after starting hindmilk. Daily weight, weekly length and head circumference will be collected from the electronic charts in SCM. Furthermore, weight, length and head circumference at completed gestational week will be used to calculate Z scores using Fenton Z scores calculator.[27]

Data on maternal and neonatal characteristics will be collected from their electronic and physical charts. Composite milk, foremilk and hindmilk volumes will be collected from the collection sheet that has been already used in our NICU.

Secondary outcomes EUGR, BPD and ROP will be compared between infants who received hindmilk and the general rates published publically by the Canadian Neonatal Network each year.

4.3 Sample size and feasibility:

Our local data indicate that very preterm infants with extra-uterine growth restriction have an average weight gain of 13 g/kg per day (standard deviation (SD)= 7). A previous small study of 15 preterm infants showed a 7 g (SD= 4.4 g) increase in weight gain when hindmilk was fed.[14] However, our preliminary data indicated a 4 g/kg/day increase in weight gain with hindmilk. The 4 g/kg per day increase is considered clinically significant given it is enough to restore good weight gain (>15g/kg per day) and potentially reduce incidence of EUGR in very preterm infants. Thus, we calculated the sample size based on this difference. In order to permit detection of 4 g/kg per day with 90% power and 0.05 alpha error, a sample of 33 VLBW infants is needed using a calculation of repeated measures in STATA software (STATA 11.0, Texas, USA). Because the cream project included 34 infants in 14 months, we expect to recruit all subjects for the prospective study in 18 months.

4.4 Statistical analysis:

Descriptive statistics using mean, median, SD and interquartile range (IQR) will be used to describe the study population. Paired *t* test will be used to compare average weight gain before and after hindmilk use. Chi-square test will be used to compare EUGR rates and other categorical outcomes. Linear regression analysis will be used to assess the relationships between weight gain and hindmilk and to adjust for potential confounding factors including gestational age and postnatal age. For all tests, a probability of P value < 0.05 will be considered statistically significant.

4.5 Definitions:

EUGR will be defined as weight less than 10th percentile on Fenton growth charts. EUGR will be determined at 36 weeks and at discharge. BPD will be defined according to Child Health and Human Development as the requirement for positive pressure support (CPAP or high flow nasal cannula $\geq$ 1 liter per minute (LPM)), or oxygen dependency at 36 corrected gestational age.[28] ROP will be defined according to the international classification1 or requiring treatment.[29]

5. Why you/your group should do it

Dr. Alshaikh has an expertise and training in neonatal nutrition. He is skilled in study design and statistical analysis and has a Master degree in Epidemiology. He also worked on a previous study that involved measurement of fatty acids profile during his research nutrition fellowship at the Children's Hospital of Philadelphia. Our group has been working on the cream project for the last 14 months. Both JillMarie Spence and Hope Boychuk are registered dietitians and have been working in the NICU at FMC for a while. Christel Major is our NICU lactation consultant. Our group also has a neonatal-perinatal fellow and a clinical assistant. Both are very interested in developing expertise in neonatal nutrition. The section of neonatology also has a research assistant and a statistician. Both are familiar with studies conducted in our NICU. Analysis of milk samples will be performed in the NorthernStar milk bank in Calgary. Jannette Festival is the milk bank director. She will help with the analysis of human milk samples.

Potential limitations:

Hindmilk might not be available all the time or sufficient to meet babies needs during the study. Infants will be considered on hindmilk if more than or equal to 80% of their weekly milk volume are given as hindmilk. Most

mothers in the cream project were able to feed their infants hindmilk until 36 weeks PMA.
Some infants may receive blood transfusions during the study period. Erythrocyte FA profiles of these infants are likely to reflect the donor red blood cells. Infants who are on hindmilk and received any blood transfusion between the collection dates of the dried blood samples will be excluded from the analysis for erythrocyte FAs profiles. The number of infants requiring blood transfusion has become very limited in our NICU after the recent implementation of natural cord clumping.

6. Dissemination and translation of results and plans for next steps in study of topic:

Findings from this study will generate insight into the effects of hindmilk on growth velocity of very preterm infants. It will also improve our knowledge about the hindmilk content and growth of very preterm infants. The results of this study will be presented first on provincial level given that the neonatology groups in Edmonton and Lethbridge are interest in the hindmilk project. In addition, the results will be presented in national and international conferences and will be published in a peer reviewed scientific journal. Also, the study will strengthen the collaboration between the neonatal nutrition group and the NorthenStar milk bank for future studies.
The measurement of the erythrocyte membrane FAs profile will provide comprehensive information in regarding changes of FAs in very preterm infants after using hindmilk. If the results of the study indicate a significant increase in LC-PUFAs, this will be used to get funding for a large study to address the effects of hindmilk on BPD or neurodevelopment as a primary outcome.

7. Budget

Analysis of milk samples ($10/sample x 33 subjects x 2 time points)	660.00
Shipment for milk samples ($20/sample x 33 subjects x 2 time points)	1320.00
Cost of human milk analyzer standardization	335.00
Erythrocyte FA profile ($179/sample x 33 subjects x 2 time points)	11,814.00
Shipment for dried blood spots (FA profile) (once every month x 18 month)	360.00
Research assistant ($35/hour x 2 hours/week x 78 weeks)	5460.00
Conference presentation and travel	1000.00
Syringes, gloves, papers	50.00
Total	**$20,999.00**

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