Freeze Dried Human Milk: Liquid Gold to Powdered Uncertainty

Heidi E. Karpen, M.D.
Professor of Pediatrics
Associate Director Neonatal-Perinatal Medicine Fellowship Training Program
Division of Neonatology
Emory University/Children's Healthcare of Atlanta

1

Disclosures

Dr. Karpen has the following to disclose:

- Prolacta Bioscience, Research grant recipient
- Progeny Health, Medical Advisory Board

2

Objectives

- Briefly review the biology of human milk and its benefits for the "At-Risk" infant
- Discuss difficulties/complications of providing adequate nutrition especially in preterm/critically ill infants
- Compare and contrast the differences between pasteurization/preservation methods for donor human milk
- Describe the process of freeze drying human milk and the effects on its nutritional and bioactive components
- Discuss the limitations in current knowledge on the risks/benefits of freeze dried milk in the outpatient and inpatient settings

3

Benefits of Human Milk

- **Decreased risk of:**
  - Respiratory tract infections and otitis media
  - Sudden infant death syndrome
  - GI infections such as Rotavirus
  - IBD and Celiac disease
  - Asthma, atopic dermatitis and eczema
  - Obesity and Type I and II diabetes
- **Positive effect on:**
  - Neurodevelopmental outcomes
  - Severity of Retinopathy of Prematurity (ROP)

American Academy of Pediatrics Section on Breastfeeding. Breastfeeding and the use of human milk. Pediatrics. 2012; 129(3): e827-e841

4

Breast Milk Constituents

- **Lactose:** carbohydrate source in human milk
  - Ideal choice for infants who are preterm or who are "at-risk"
- **Proteins:** HM- whey proteins; limited bovine protein exposure
- **Fats:** MCTs and LC-PUFAs
- **Vitamins/Minerals:** Ca, Phos, Mag, iron, Vitamins (B complexes, C, A)
- **Prebiotics:** Milk Oligosaccharides
- **Probiotics:** HM comes ready made!

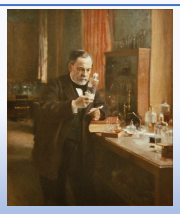
5

Human milk factors that protect the infant and promote gut maturation/immunity

- **Growth factors** (EGF, GH, glutamine), **Anti-inflammatory molecules** (IL-10, TGF-β2)
- **Lactoferrin** – A multifunctional protein that facilitates iron absorption and inhibits bacterial growth; present in quantities 100x greater than found in bovine milk.
- **Lysozyme** – An antibacterial protein that kills gram positive and gram negative bacteria; present in quantities 3000x greater than found in bovine milk.
- **Secretory IgA** – An antibody custom to pathogens in the maternal environment; present in quantities 4000x greater than found in bovine milk.
- **Human Milk Oligosaccharides (HMOs)** – Indigestible, short chain sugars that serve many functions in the intestinal tract, including pathogen binding and promotion of microbiome development; third most abundant factor in human milk compared to trace amounts found in bovine milk.
- **Preterm milk contains high levels of protective factors**

6

Methods of HM Preservation- Pasteurization

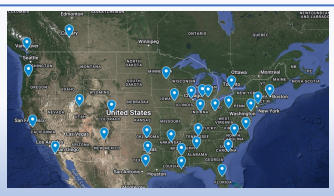


http://en.wikipedia.org/wiki/File:Tableau_Louis_Pasteur.jpg

- Holder pasteurization is the most common and highly effective processing technique used globally by milk banks to eliminate bacteria and viruses.
- A low-heat method of processing: milk is warmed to 62.5º C, held for 30 minutes, rapidly cooled, and then frozen until use.
- Some loss of protective factors found in MOM, but most bioactive components preserved.

7

HMBANA Milk Banks



https://www.hmbana.org

- Non-profit association, established 1985
- 29 HMBANA milk banks in US and 3 in Canada
- Largest supplier: over 11 million ounces distributed in 2024!
- Donor moms are screened for medication use, travel and medical history, and for infectious illness
- Milk is pooled, Holder pasteurized, and sampled for bacterial contamination then frozen and shipped

8

Coming soon… Georgia's HMBANA Milk Bank!!

MED MILK PROJECT
Building Georgia's Milk Bank

https://medmilk.org/

9

Prolacta

https://www.prolacta.com

- For-profit company FDA regulated milk processing facility, tissue grade handling
- Extensive screening, including DNA fingerprinting
- VAT Holder pasteurization which eliminates bacteria and viruses
- PDHM is guaranteed for caloric density and protein content
- Only produced of a HMBF product for addition to MOM or DM
- Paid donations (or mothers may elect to donate their payments to the NICU Parent Network)

10

Methods of HM Preservation: Retort Processing/Sterilization

Ni-Q: For-profit company

- Paid donations
- Produces a sterilized human milk product with a shelf life of 12 months at RT and does not need to be refrigerated or frozen until opened
- Guarantees 20kcal/oz and 1.1g Protein/100mL

- Sterilized by heating to 121ºC for 5 min, with added pressure of 15 pounds per square inch above atmospheric pressure
- Provides mothers a safe way to supplement rather than "milk sharing"
- Milk can be ordered by hospitals and by families for post discharge donor milk supply.
- Extensive screening, including DNA fingerprinting

11

Sterilization vs. Pasteurization

Study Attributes	Meredith-Dennis (2017)[7]	Lima (2017)[8]
Types of Milk	Holder; Vat-Processing; shelf stable	Raw; Holder; shelf stable
Sample size	N = 3 per milk type	N = 12 per milk type
Study Design	Cross-sectional (each milk type was from different donors)	Cross-over (each milk type was from a combined milk pool from 60 donors)
Lactoferrin	*Higher in Holder vs shelf stable	Not measured
Immunoglobulin A	*Higher in Holder vs shelf stable	Holder retained 87%; shelf stable retained 11%
Lysozyme	*No difference between Holder vs. shelf stable	Holder retained 54%; shelf stable retained 0%
Bacteria levels	Not measured	B. Cereus detected in 3 Holder samples; no bacteria detected in shelf stable.
Other	Holder was higher in HMO, protein, and other factors, likely due to the fact that Holder milk was from preterm donors.	Not measured
Conclusions	Difference in processing and pooling of milk may contribute to differences in nutrient and bioactive composition and warrant further research.	Significant loss of bioactive proteins in shelf stable milk compared to Holder. Holder requires post pasteurization testing for B. Cereus.

*This study was a cross-sectional study, so difference in composition may be attributed to different donors and different stages of lactation; therefore, the scale of differences was not assessed due to lack of control.

Meredith-Dennis, L. et al. (2017) . J Human Lact, Lima, R. et al. (2017) Curr Dev Nutr

12

> J Pediatr Gastroenterol Nutr. 2022 May 1;74(5):668-673.
doi: 10.1097/MPG.0000000000003401.

Nutritional and Safety Concerns of Infant Feeding Trends

Dina M DiMaggio [1], Nan Du [2], Anthony F Porto [3]

Affiliations + expand

PMID: 35149650 DOI: 10.1097/MPG.0000000000003401

- The purpose of this study was to establish the prevalence of contemporary infant feeding practices such as *informal human milk sharing, imported European infant formula, toddler formula and homemade formula* and gain insight into the parental reasoning for their choices.
- An anonymous, cross-sectional, voluntary electronic survey
- Of 2315 respondents, 18% were following at least one contemporary feeding practice
- Thirty six percent of parents using donor breast milk obtained from unregulated sources, 14% of the respondents were using European infant formula, 5% were using toddler formula for their infants, and 2% were making homemade infant formula.

13

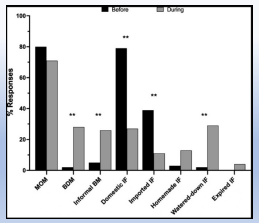
> BMC Pediatr. 2023 Jun 24;23(1):320. doi: 10.1186/s12887-023-04132-9.

Infant feeding practices and parental perceptions during the 2022 United States infant formula shortage crisis

Karina Cernioglo [1 2], Jennifer T Smilowitz [3 4]

Affiliations + expand

PMID: 37355589 PMCID: PMC10290398 DOI: 10.1186/s12887-023-04132-9



- In May of 2022, parents in the U.S. experienced a critical infant formula shortage due to supply chain issues and the recall of several infant formula products over contamination concerns.
- The number of individuals that used at least one unsafe infant feeding practice increased from 8% before the infant formula shortage to 48.5% during the shortage ($p < 0.001$).
- Compared to before the formula crisis, parents reported an increase in use of:
  - banked donor milk use from 2 to 28% ($p < 0.005$)
  - use of human milk from informal sharing from 5 to 26% ($p < 0.005$)
  - use of watered-down infant formula from 2 to 29% ($p < 0.005$).

14

Freeze Drying- Lyophilization

- **Freezing:** Breast milk is frozen at a very low temperature (below -40°F).
- **Vacuum:** Then pressure within the freeze-drying chamber is reduced, creating a vacuum.
- **Sublimation (Drying):** Heat is applied to the frozen breast milk, causing the ice to turn directly into water vapor (sublimation) without melting.
- **Packaging:** The resulting powder is then vacuum-sealed in a food-grade bag to protect it from moisture, oxygen, and UV light
- Freeze-drying results in a powdered product that needs to be reconstituted prior to consumption
- Appeal is that it allows for a longer shelf life, easier transport, and a lower cost of *storage*

15

Pros and Cons of Freeze Dried Breast Milk

- **Extended Shelf Life:** Freeze-dried breast milk can be stored for up to three years at room temperature, whereas fresh milk has a limited shelf life.
- **Nutrient Retention:** The freeze-drying process is purported to be gentler on the milk and helps retain the nutritional value of breast milk, including proteins, fats, and carbohydrates.
- **Convenience:** Freeze-dried breast milk powder is easy to use; simply add warm water to make a bottle or sprinkle it on solid foods.
- **Versatility:** It can be used for bottle feeding, supplementing nursing, or fortifying baby food.
- **Peace of Mind:** It provides a convenient and long-term storage solution for breast milk, reducing the risk of waste and freezer space/malfunction issues.
- **High Lipase Milk:** freeze drying decreases lipase activity possibly making taste more pleasant for these infants
- **Cost:** Freeze-drying services can be more expensive than traditional storage methods.
- **Potential Risks:** While generally safe, some concerns have been raised about the potential for contamination during handling and the lack of research on long-term effects.
- **Bacterial contamination:** FD does not kill bacteria or viruses in the milk and other bacterial contaminants may be introduced during production or useage at home. None of the current companies pasteurize the milk before freeze drying
- **Changes in milk:** FD an result in alterations in macronutrients, micronutrients or vitamins. Loss of antimicrobials and bioactive components.
- **Reconstitution errors:** Errors water to powder ratios when reconstituting feedings can lead to adverse outcomes like water intoxication, dehydration and dangerously low or high sodium levels.
- **Accessibility:** Not all regions or cities have access to freeze-drying services.

16

Effects of FD on Human Milk Constituents

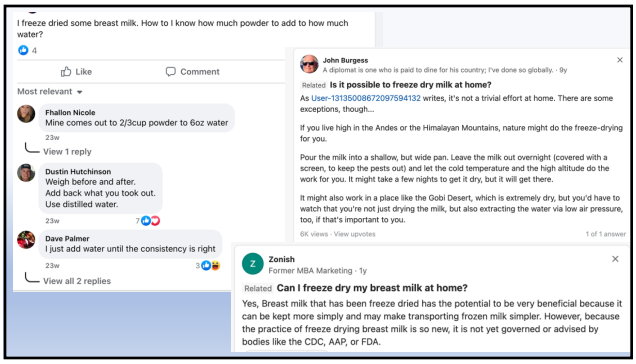
- No significant overall effect on free fatty acids (FFA) or lipid profiles in most studies but some have shown increased FFA
- FD has been shown to significantly decrease the human milk fat globule size, therefore increasing its surface area which may improve its bioavailability
- Storage of FD human milk, for up to 4 months at a temperature of 4 °C (or less) has not been reported to change the composition of DHM. However, storage at 25°C or prolonged beyond 120 days has been associated with significant changes in FFA, protein and antioxidants
- No effect concentrations of B vitamins (Niacin, biotin, pantothenic acid), but a 31.5% reduction in vitamin C concentration
- Studies exploring the effect of freeze-drying on antioxidant content found total antioxidant capacity, vitamin C, and vitamin E decreased after freeze-drying, with antioxidants further lowered by storage temperatures above 4°C

17

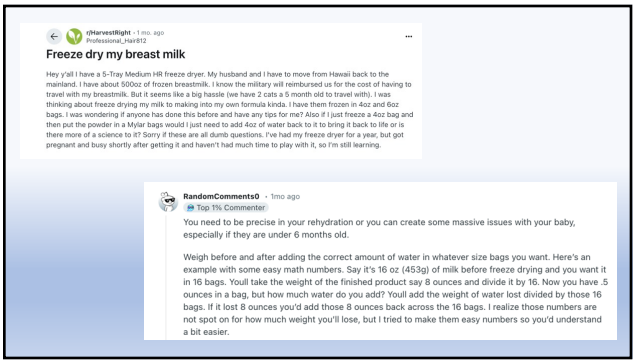
Risks of Freeze Dried Milk- Bacterial Contamination

- Bacterial contamination of powdered milk products (including commercially available powdered formulas):
  - Heat sensitive organisms such as Staphylococcus aureus and coliforms.
  - Monitoring of these microorganisms is crucial in ensuring the products are safe for preterm infants. Commercial production would require a microbiological testing program compliant with relevant national standards and suitable for small batch production. Commercial production should be conducted in a registered food grade or pharmaceutical processing plant under appropriate food safety plans to comply with local governing regulations.
  - Commercial production would require a microbiological testing program compliant with relevant national standards and suitable for small batch production. Commercial production should be conducted in a registered food grade or pharmaceutical processing plant under appropriate food safety plans to comply with local governing regulations.

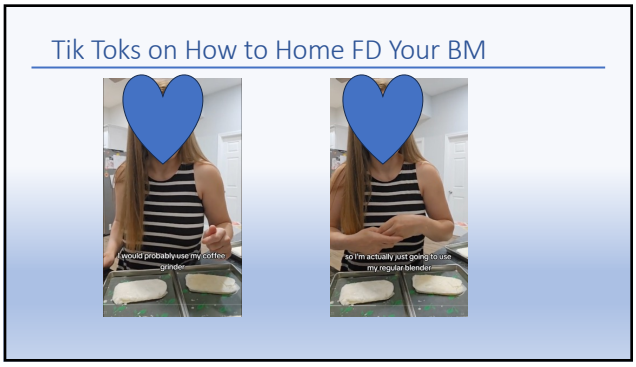
18



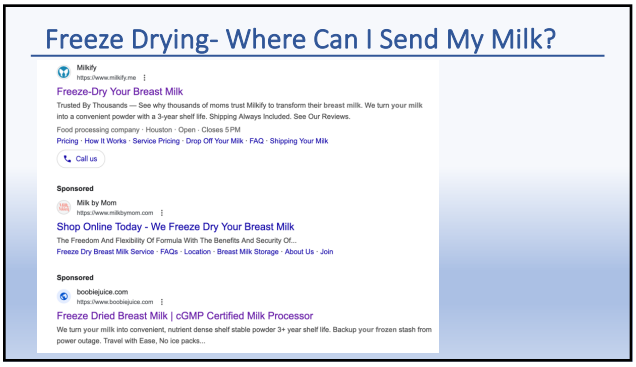
19



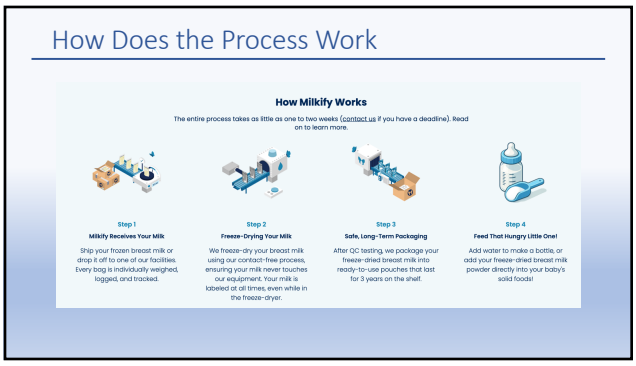
20



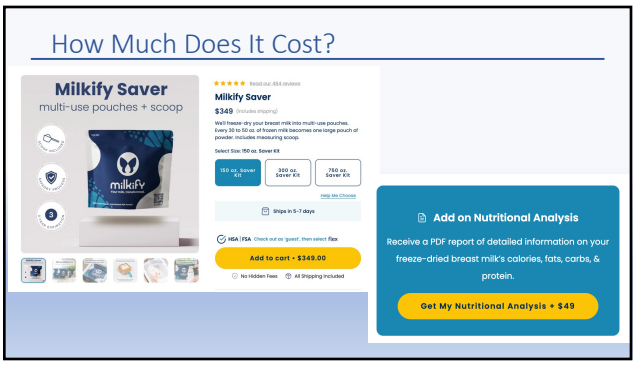
21



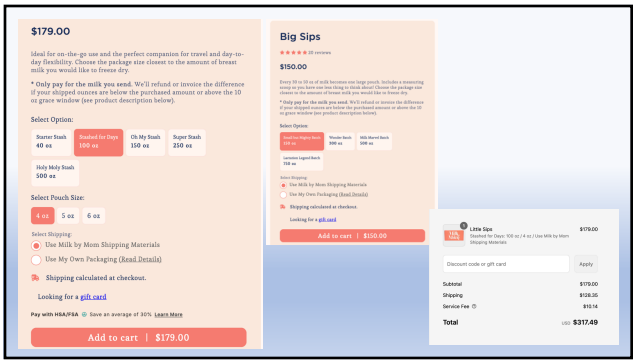
22



23



24



25

Problems in a Healthcare Setting

- Since every person's breast milk has a different water content, moms receive specific water-to-powder ratios for rehydrating their milk. For example, some bags may require three ounces of water while others may require five ounces.

Scenarios:

- You have 60 patients in a large children's hospital on human milk whose mothers bring in FD milk- each a has a different water to powder ratio, also changes per batch she brings in…
- Your milk techs reconstitute the milk and there is a mistake in preparation so it cannot be used- does the hospital have to reimburse the mother for the cost she incurred??
- A mom of a 23 week preterm infant refuses multi-nutrient Bov-HMF and wants to use her own FD breast milk as a fortifier. How do you counsel her and what do you do??

26

Nutritional Challenges in Preterm and Surgical Infants

- HM diets in preterm infants have been associated with a decreased the incidence of necrotizing enterocolitis, late-onset sepsis, increased intestinal motility and gastric emptying, improved feeding tolerance and general anti-inflammatory effects.
- HM diets in neonates with intestinal failure are associated with a decreased duration of PN, time to full feeds and Dbili, up to 50% in some recent studies
- HM has been found to stimulate the development of healthy intestinal flora, modulate inflammatory processes, and encourage bowel maturity, repair, and function.
- These studies have been confounded to some degree, however, by the use of cow-milk based fortifiers in infants that required caloric concentration for growth.
- Protein content of HM decreases rapidly over the first weeks of lactation to around 1 g protein 100 g milk, which insufficient to meet the recommended 3.5-4 g protein/ kg/day for a preterm infant consuming 150-180 mL/kg/day

Section on Breastfeeding. *PEDIATRICS*. 2012;129(3):e827–e841
Sullivan S, Schanler RJ, Kim JH, et al. *J Pediatr*. 2010;156(4):562–7.e1
Jakaitis BM, Denning PW. *Clin Perinatol*. 2014;41(2):423–435.

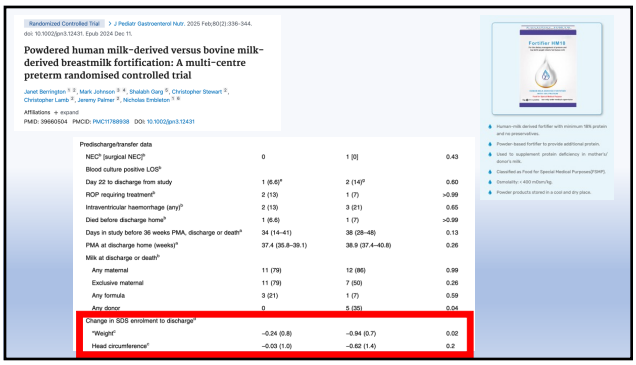
Baerg, J. E., & Munoz, A. N. *Seminars in Pediatric Surgery*, 2019; *28*(2), 118–121.
Shinnick, J. et al *Breastfeeding Medicine*, 2016; *11*(6), 286–292.

27

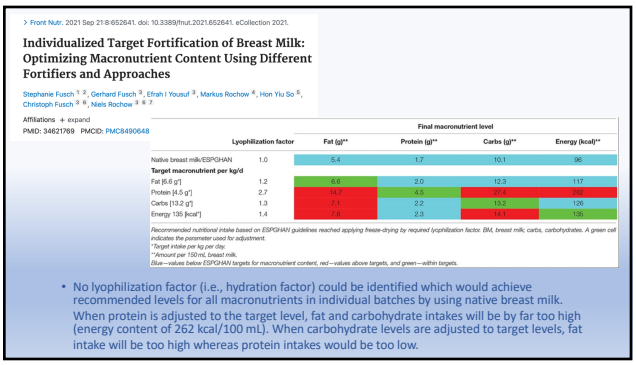
Human Milk Fortifiers

- The macro- and micronutrient content of human milk (preterm, term, or donor milk), is insufficient to meet the nutritional needs of VLBW infants without macro- and micronutrient fortification
- The micronutrient composition of human milk does not provide the recommended daily amounts of vitamins and minerals, such as iron, calcium, phosphorus, zinc, vitamins A and D for preterm/at-risk infants
- Human milk fortifiers' or 'breast milk fortifiers' are added to maternal or donor milk to increase its nutrient density, but these names can be misleading as these products are most often made from bovine (cow) milk
- Human milk derived fortifiers are are very expansive and only available in limited markets
- Lacking large-scale randomized controlled trials directly comparing donor human milk-based fortifiers to bovine-based fortifiers
- Meta-analysis of two trials involving 332 infants found that fortifiers made from human milk are associated with a decreased risk of necrotizing enterocolitis (NEC) compared with those made from cows' milk (risk ratio 0.47, 95 % Confidence Interval 0.22-0.98) (Grace et al., 2021).
- Clinical conundrum on how to provide adequate enteral nutrition to VLBW infants without increasing their risk of NEC
- The formula crisis and NEC law suits have fueled concern from parents on the potential adverse effects of bovine based fortifiers and formulas particularly in preterm infants
- Growing interest in fortifiers and supplements made from human milk (Sergius-Ronot et al., 2021).

28

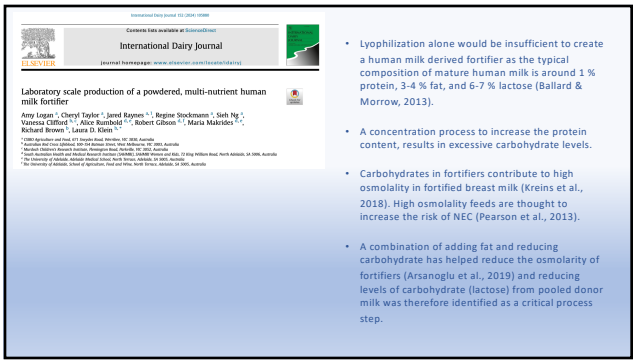


29



- No lyophilization factor (i.e., hydration factor) could be identified which would achieve recommended levels for all macronutrients in individual batches by using native breast milk. When protein is adjusted to the target level, fat and carbohydrate intakes will be by far too high (energy content of 262 kcal/100 mL). When carbohydrate levels are adjusted to target levels, fat intake will be too high whereas protein intakes would be too low.

30



- Lyophilization alone would be insufficient to create a human milk derived fortifier as the typical composition of mature human milk is around 1 % protein, 3-4 % fat, and 6-7 % lactose (Ballard & Morrow, 2013).
- A concentration process to increase the protein content, results in excessive carbohydrate levels.
- Carbohydrates in fortifiers contribute to high osmolality in fortified breast milk (Kreins et al., 2018). High osmolality feeds are thought to increase the risk of NEC (Pearson et al., 2013).
- A combination of adding fat and reducing carbohydrate has helped reduce the osmolarity of fortifiers (Arsanoglu et al., 2019) and reducing levels of carbohydrate (lactose) from pooled donor milk was therefore identified as a critical process step.

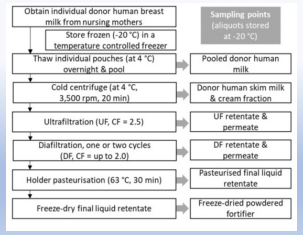
31

Large-scale production of Multi-Nutrient HMBF

- The objective of this study was to demonstrate the feasibility of producing a powdered, multi-nutrient breast milk fortifier made from donated human milk with a target energy (400 kcal 100 g^{-1} powder) and protein (40 g protein 100 g^{-1} powder) composition, supplementation of selected micronutrients, and acceptable solubility and osmolality for clinical use.
- A powdered, rather than liquid, product was desirable to avoid significant displacement of maternal breast milk
- For comparison, a commonly used cows' milk-based fortifier in Australia has 435 kcal, 36 g protein, 18.1 g fat, and 32 g carbohydrate per 100 g powder
- Ultrafiltration (UF) and diafiltration (DF) using spiral wound UF membranes is a standard process in the dairy processing industry for producing milk protein concentrates (Gwazzi-Aarril, 2018).
- .

32

Methods



- Freeze-dried powders were rehydrated with equal amounts of water to match that removed during the freeze-drying process and supplemented with calcium (calcium carbonate), phosphorus (calcium phosphate dibasic), sodium (sodium citrate) and zinc (zinc sulphate mono) to levels found in a typical commercial cows' milk-based powdered fortifier.
- The dry elements were added with stirring until dissolved, and the combined solution freeze-dried as described earlier

Logan, et al International Dairy Journal, 2024; Vol 152

33

Comparison of Freeze Dried DM Fortifiers to Cows-Milk Based Fortifier

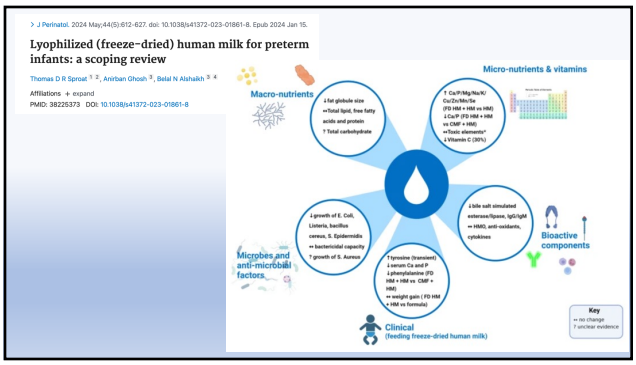
Table 5
The elemental analysis of the freeze-dried powders made from skimmed donor human milk.[a]

Component	PES-1UF-1DF	PVDF-1UF-1DF	PVDF-1UF-2DF	PVDF-1UF-2DF (supplemented)	Control
Calcium (mg 100 g^{-1})	282 ± 17[a]	241 ± 59[a]	261 ± 23[a]	1938 ± 133[b]	1890
Phosphate (mg 100 g^{-1})	127 ± 15[a]	92 ± 2[a]	85 ± 1[a]	825 ± 74[b]	1095
Sodium (mg 100 g^{-1})	87 ± 13[a]	84 ± 2[a]	71 ± 12[a]	837 ± 31[b]	918
Magnesium (mg 100 g^{-1})	33 ± 4[b]	27 ± 4[b]	22 ± 1[a]	41 ± 4[c]	100
Potassium (mg 100 g^{-1})	362 ± 62[a]	289 ± 77[a]	474 ± 12[b]	541 ± 11[b]	1210
Zinc (mg 100 g^{-1})	3.2 ± 0.5[a]	3.3 ± 0.3[a]	6.1 ± 0.5[b]	24.4 ± 0.2[c]	24
Iron (mg 100 g^{-1})	BL	BL			45
Copper (mg 100 g^{-1})	0.42 ± 0.05[a]	0.79 ± 0.12[b]			1.3
Selenium (µg 100 g^{-1})	BL	BL			70
Manganese (µg 100 g^{-1})	BL	BL			152
Iodine (µg 100 g^{-1})	140	270			317

[a] Abbreviations are: BL, below limits; PES, polyethersulphone; PVDF, polyvinylidene fluoride; UF, ultrafiltration; DF, diafiltration. A commercial cows' milk-based powdered fortifier is included as a control reference; compositional information taken from the nutritional label. A different superscript letter within each row denotes a significant difference where $p < 0.05$.

7

34



35

Trials on Fortifying MOM/DM with FD-HM

- All trials had small sample sizes from 4-42 patients
- FD HM with DHM lower protein, calcium, phosphorous, magnesium, and mixed results for sodium, copper, zinc, and iron than DHM +bovine fortifier
- Overall weight gain reported is 7-19g/kg/d which is below the goal of 20-30 g/kg/d or 30g/d when >2kg.
- No trials sufficiently designed or powered to determine effects on risks of NEC, sepsis, growth, metabolic parameters and ND outcomes

36

Key Take Aways

- Due to the lack of available evidence on the osmolar, microbial, and nutritional safety of freeze-dried human milk, its use in "at-risk" or preterm infants is not currently recommended. This includes use in reconstituted form or as a fortifier in any setting.
- Before the use of freeze-dried human milk can be recommended, more research is needed to assess osmolar, microbial, and nutritional safety and whether there are benefits
- The various proprietary methods for freeze drying human milk makes handling, reconstitution and feeding of these freeze dried human milk products problematic in the hospital setting
- Research of any type at a clinical or practice level is absent. Adequate safety and efficacy need to be established and policies developed to protect infants. Clinical trials are required that include rigorous human milk analysis, assessment of risks including NEC, bacterial contamination/sepsis
- Need for studies on safety, growth and outcomes using FD milk products, especially as a fortifier
- Mother's Own Milk provided at the breast or expressed and fed to the infant, and/or pasteurized donor human milk are currently SOC until there is sufficient data.

37

And to the babies for whom we strive to move medicine and science forward

38