



## Neonate Feeding Regimens and the Expanding Role of Lactoferrin

### Overview

Human breast milk is the gold standard, providing a myriad of health benefits. Lactoferrin, which is found in high concentration in mammalian milk, is a multifunctional glycoprotein that has antimicrobial and immunomodulatory properties. Paolo Manzoni, MD, PhD, explains its critical role in protecting neonates against infection.

Historically, lactoferrin has been low in infant formula due to lower levels in bovine milk; however, advanced technology concentrated bovine lactoferrin in formula. Data show how much lactoferrin is needed, and when it is appropriate to supplement. Lactoferrin supplementation—human or bovine—appears to have measurable clinical benefits. Although inconsistencies exist among major RCTs in terms of lactoferrin efficacy, in study results, Dr. Manzoni identifies various heterogeneity as a cause.

### Target Audience

This activity was developed for neonatologists, nurses, nurse practitioners, dietitians, hospital pharmacists, and other health care providers who have an interest in newborns, infants and toddlers.

### Learning Objectives

At the conclusion of this activity, participants should be better able to:

- Evaluate clinical research that is expanding the understanding of the physiological and developmental properties of lactoferrin
- Develop evidence-based NICU feeding regimens with lactoferrin.

### Faculty

#### **Paolo Manzoni, MD, PhD**

Division of Pediatrics and Neonatology  
Department of Maternal-Infant Medicine  
Nuovo Ospedale degli Infermi  
Ponderano (Biella), Italy

### Accreditation and Certification

The Annenberg Center for Health Sciences at Eisenhower is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians.

The Annenberg Center for Health Sciences at Eisenhower designates this enduring material for a maximum of 1.0 *AMA PRA Category 1 Credit™*. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

The American Medical Association has an agreement of mutual recognition of Continuing Medical Education (CME) credits with the European Union of Medical Specialists (UEMS), the accreditation body for European countries. Physicians interested in converting *AMA PRA Category 1 Credit™* to UEMS-European Accreditation Council for Continuing Medical Education (EACCME®) CME credits should contact the UEMS at [mutualrecognition@uems.eu](mailto:mutualrecognition@uems.eu)



Annenberg Center for Health Sciences at Eisenhower is accredited by the American Association of Nurse Practitioners as an approved provider of nurse practitioner continuing education. Provider number: 040207.

This program is accredited for 1.0 contact hour.  
Program ID #5729-EM

Annenberg Center for Health Sciences is accredited as a provider of continuing nursing education by the American Nurses Credentialing Center's Commission on Accreditation.

A maximum of 1.0 contact hour may be earned for successful completion of this activity.

Provider is approved by the California Board of Registered Nursing, Provider #13664, for 1.0 contact hour. *To receive credit for education contact hours outside of the state of California, please check with your state board of registered nursing for reciprocity.*

## Neonate Feeding Regimens and the Expanding Role of Lactoferrin

Annenberg Center for Health Sciences at Eisenhower is a Continuing Professional Education (CPE) Accredited Provider with the Commission on Dietetic Registration (CDR). Registered dietitians (RDs) and dietetic technicians, registered (DTRs) will receive 1.0 continuing professional education unit (CPEU) for completion of this program/material.

Provider number: AC857

Activity number: 155851

Learners may submit additional evaluations of the quality of this program/material at [cdr@eatright.org](mailto:cdr@eatright.org).

### Disclosure Statement

It is the policy of the Annenberg Center for Health Sciences to ensure fair balance, independence, objectivity, and scientific rigor in all programming. All faculty and planners participating in sponsored programs are expected to identify and reference off-label product use and disclose any relationship with those supporting the activity or any others with products or services available within the scope of the topic being discussed in the educational presentation.

The Annenberg Center for Health Sciences assesses conflict of interest with its instructors, planners, managers, and other individuals who are in a position to control the content of CE/CME activities. All relevant conflicts of interest that are identified are thoroughly vetted by the Annenberg Center for fair balance, scientific objectivity of studies utilized in this activity, and patient care recommendations. The Annenberg Center is committed to providing its learners with high-quality CE/CME activities and related materials that promote improvements or quality in health care and not a specific proprietary business interest of a commercial interest.

In accordance with the Accreditation Council for Continuing Medical Education Standards, parallel documents from other accrediting bodies, and Annenberg Center for Health Sciences policy, the following disclosures have been made:

### Faculty

Paolo Manzoni, MD, PhD

*Speakers Bureau* Sodilac—clinical area:  
Lactoferrin use in infants

Mead Johnson Nutrition—clinical area: infant nutrition

The faculty for this activity has disclosed that there will be discussion about the use of products for non-FDA approved applications.

### *Additional content planners*

Victoria Anderson (Medical writer)

*Individual* Abbott—clinical area: N/A

*Stockholder* AbbVie—clinical area: N/A

The following have no significant relationship to disclose:

Coy Flowers, MD (Peer Reviewer)

Erin Allen, MS, RD, LDN (Dietitian Reviewer)

Heather M. Jimenez, FNP (Nurse Planner)

### *Annenberg Center for Health Sciences*

Staff at the Annenberg Center for Health Sciences at Eisenhower have no relevant commercial relationships to disclose.

The ideas and opinions presented in this educational activity are those of the faculty and do not necessarily reflect the views of the Annenberg Center and/or its agents. As in all educational activities, we encourage practitioners to use their own judgment in treating and addressing the needs of each individual patient, taking into account that patient's unique clinical situation. The Annenberg Center disclaims all liability and cannot be held responsible for any problems that may arise from participating in this activity or following treatment recommendations presented.

This activity is supported by an independent educational grant from **Mead Johnson Nutrition**.

This activity is an online enduring material. It has been edited to meet requirements for online learning. Successful completion is achieved by reading and/or viewing the materials, reflecting on its implications in your practice, and completing the assessment component.

The estimated time to complete the activity is 1.0 hour.

This activity was released on June 4, 2020 and is eligible for credit through June 4, 2022.

## Neonate Feeding Regimens and the Expanding Role of Lactoferrin



Obtain your CE/CME credit at:  
<https://pnce.org/lactoferrin>

### Contact Information

For help or questions about this activity please contact  
Continuing Education:  
[ce@annenberg.net](mailto:ce@annenberg.net)

*Editor's Note: This is a transcript of an audio webcast presented on May 20, 2020. It has been edited and condensed for clarity*

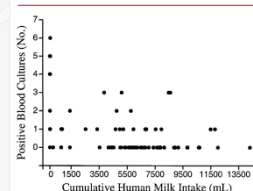


**Paolo Manzoni, MD, PhD:** It's my pleasure to be here today and to go through this presentation of lactoferrin with the most recent updates and with an overview of the role of lactoferrin in nutrition of preterm infants, specifically whether lactoferrin has a role, thanks to its properties, in good development of the neonatal period and of the period following birth.

The benefits of human milk are well known by everyone. Fresh human milk is reported to prevent bronchopulmonary disease (BPD), chronic lung disease, retinopathy of prematurity (ROP), necrotizing enterocolitis (NEC), and especially infections in neonates. This activity against infection is noteworthy because it is related to the intake of human milk.<sup>1-6</sup>

that these fascinating properties of human milk should be attributable to a number of bioactive factors that can be retrieved in human milk.

**Figure.** The beneficial effects of fresh human milk are linearly associated with the intake volume.<sup>[1]</sup>



**Table.** Reduced logistic regression model for infection in relation to confounding variables<sup>[a],[2]</sup>

Variable	Odds Ratio	95% CI	P Value
Gestational age (wk)	0.80	(0.68-0.95)	.009
Apgar score at 5 mins	0.93	(0.77-1.14)	.494
Days without enteral feeding (NPO)	1.03	(0.99-1.07)	.153
Mechanical ventilator days	1.01	(0.99-1.03)	.184
Human milk fed	0.43	(0.23-0.81)	.010

a. The total for the regression model is 212 cases. The number of cases with imputed values on any single variable is 11 (5.2%).

Fresh human-milk feeding prevents infections in neonates.

CI, confidence interval; NPO, nil per os (nothing by mouth).

1. Schanler RJ, et al. *Pediatrics*. 1999;103:1150-1157. Reproduced with permission from *Journal Pediatrics*. Vol. 103, Pages 1150-1157. Copyright © 1999 by the AAP. 2. Hylander MA, et al. *Pediatrics*. 1998;102:838.

### Slide 2

You see in this slide [Slide 2], and in the next couple of slides, that the list of putative actors in prevention of infection is truly long [Slides 3-5], with several actions and several modalities that, of course, cannot but be interactive between each other. Lactoferrin stands as one of the most likely important bioactive factors with specific anti-infective activity.<sup>6,7</sup>

### Benefits of Human Milk

Fresh human milk prevents:

- Bronchopulmonary disease (BPD)/ chronic lung disease (CLD)<sup>[1]</sup>
- Retinopathy of prematurity (ROP)<sup>[2],[3]</sup>
- Necrotizing enterocolitis (NEC)<sup>[4],[5]</sup>
- Infections in neonates<sup>[4],[6]</sup>



1. Furman L, et al. *Arch Pediatr Adolesc Med*. 2003;157:66-71. 2. Hylander MA, et al. *J Perinatol*. 2001;21:356-362. 3. Manzoni P, et al. *Early Hum Dev*. 2016;90 Suppl 1:560-5. 4. Lucas A, et al. *Lancet*. 1990;336:1519-1523. 5. Corpeleijn WE, et al. *JAMA Pediatr*. 2016;170:654-661. 6. Hylander MA, et al. *Pediatrics*. 1998;102:838.

### Slide 1 – Benefits of Human Milk

It has been estimated, according to a series of neonates fed with human milk, that babies need to receive at least 50 ml/kg/day of fresh human milk, and this translates into a 50%-60% reduction of the odds of developing sepsis. In this view, we know

### Major Bioactive Factors in Human Milk

Compound	Function
<b>Cells</b>	
Macrophages	Protection against infection, T-cell activation
Stem cells	Regeneration and repair
<b>Immunoglobulins</b>	
IgA/IgA	Pathogen binding inhibition
IgG	Antimicrobial, activation of phagocytosis (IgG1, IgG2, IgG3); anti-inflammatory, response to allergens (IgG4)
IgM	Agglutinators, complement activation
<b>Cytokines</b>	
IL-6	Stimulation of the acute phase response, B cell activation, proinflammatory
IL-7	Increased thymic size and output
IL-8	Recruitment of neutrophils, proinflammatory
IL-10	Repressing Th1-type inflammation, induction of antibody production, facilitation of tolerance
IFN $\gamma$	Proinflammatory, stimulates Th1 response
TGF $\beta$	Anti-inflammatory, stimulation of T cell phenotype switch
TNF $\alpha$	Stimulates inflammatory immune activation

Adapted from: Ballard O, Morrow AJ. *Pediatr Clin North Am*. 2013;60:49-74.

### Slide 3 – Major Bioactive Factors in Human Milk

## Neonate Feeding Regimens and the Expanding Role of Lactoferrin

### Major Bioactive Factors in Human Milk (continued)

Compound	Function
<b>Chemokines</b>	
G-CSF	Trophic factor in intestines
MIF	Macrophage Migratory Inhibitory Factor: prevents macrophage movement, increases antipathogen activity of macrophages
<b>Cytokine Inhibitors</b>	
TNFR1 and II	Inhibition of TNF $\alpha$ , anti-inflammatory
<b>Growth Factors</b>	
EGF	Stimulation of cell proliferation and maturation
HB-EGF	Protective against damage from hypoxia and ischemia
VEGF	Promotion of angiogenesis and tissue repair
NGF	Promotion of neuron growth and maturation
IGF	Stimulation of growth and development, increased RBCs and hemoglobin
Erythropoietin	Erythropoiesis, intestinal development
<b>Hormones</b>	
Calcitonin	Development of enteric neurons
Somatostatin	Regulation of gastric epithelial growth

Adapted from: Ballard O, Morrow AJ. *Pediatr Clin North Am*. 2013;60:49-74.

Slide 4 – Major Bioactive Factors in Human Milk (continued)

### Major Bioactive Factors in Human Milk (continued)

Compound	Function
<b>Antimicrobial</b>	
<b>Lactoferrin</b>	<b>Acute phase protein, chelates iron, antibacterial, antioxidant</b>
Lactadherin/MFG E8	Antiviral, prevents inflammation by enhancing phagocytosis of apoptotic cells
<b>Metabolic and Hormones</b>	
Adiponectin	Reduction of infant BMI and weight, anti-inflammatory
Leptin	Regulation of energy conversion and infant BMI, appetite regulation
Ghrelin	Regulation of energy conversion and infant BMI
Milk Fat Globule Membranes (MFGM)	Myelination, immunitory
<b>Oligosaccharides &amp; Glycans</b>	
HMOS	Prebiotic, stimulating beneficial colonization, reducing colonization with pathogens; reduced inflammation
Gangliosides	Brain development; anti-infectious
Glycosaminoglycans	Anti-infectious
<b>Mucins</b>	
MUC1	Block infection by viruses and bacteria
MUC4	Block infection by viruses and bacteria

Adapted from: Ballard O, Morrow AJ. *Pediatr Clin North Am*. 2013;60:49-74.

Slide 5 – Major Bioactive Factors in Human Milk (continued)

And this is true for lactoferrin, also, thanks to its iron-binding characteristics, which we'll be elucidating later on. As a matter of fact, lactoferrin is multifunctional, and it's very widely represented in human milk because, just as in mammal milk, it's a considerable part for all the human milk whey-proteins portfolio. It's a glycoprotein and can also be found in other secretions, such as saliva, plasma, and neutrophils [Slide 6].<sup>8,9</sup>

### Lactoferrin Defined

Lactoferrin (LF) is a multifunctional, glycoprotein found in high concentration in mammalian milk with antimicrobial and immunomodulatory properties:

- Iron-binding characteristics
- Critical role protecting neonates and infants against infection



LF, lactoferrin.

Jiang R, Lönnemdal B, et al. *J Pediatr Gastroenterol Nutr*. 2014;59:642-652. Telang S. *Nutrients*. 2018;10, pii: E1228.

Slide 6 – Lactoferrin Defined

Historically, lactoferrin is very low in formula milk because lactoferrin in cow's milk is not very concentrated, with respect to colostrum in human milk. It's very important, however, [to note] that bovine and human lactoferrin share a very strong homology in biochemical structure. Most of all, they have the same antimicrobial peptide called N-lactoferricin. It's an 11 amino-acidic peptide placed on the N-terminal side of the protein.<sup>10-16</sup>

### Lactoferrin: A Multifunctional Milk Protein



BSA, bovine serum albumin; IgG, Immunoglobulin G.

- Lactoferrin is a biologically active protein with potentially important health benefits<sup>[1]</sup>
- Glycoprotein (80kDa), with iron-binding property
- Major whey protein in human milk<sup>[2]</sup>
- Also found in other mucosal secretions, such as tears and saliva,<sup>[3,4]</sup> as well as in plasma, neutrophils, and epithelial cells
- Resistant to degradation in the newborn digestive tract<sup>[5]</sup>
- LF is historically low in infant formulas due to low levels in bovine milk<sup>[2]</sup>; newer technology allows LF to be concentrated for addition to infant formula
- Bovine and human lactoferrin share strong (77%) sequence homology<sup>[6]</sup> and the same antimicrobial peptide (n-Lactoferricin)
- Bovine LF is resistant to proteolytic digestion<sup>[7]</sup>

1. Kanwar JR, et al. *Molecules*. 2015;20:9703-9731. 2. de Witte JN. *J Dairy Sci*. 1998;81:597-608. 3. Reetano S, et al. *Histochemistry*. 1980;66:285-291. 4. McClellan KA. *Surv Ophthalmol*. 1997;42:233-246. 5. Spik G, et al. *Acta Paediatr Scand*. 1982;71:979-985. 6. Manzoni P, et al. *JAMA*. 2009;302:1421-1426. 7. Davidson LA, et al. *Acta Paediatr Scand*. 1987;76:733-740.

Slide 7 – Lactoferrin: A Multifunctional Milk Protein

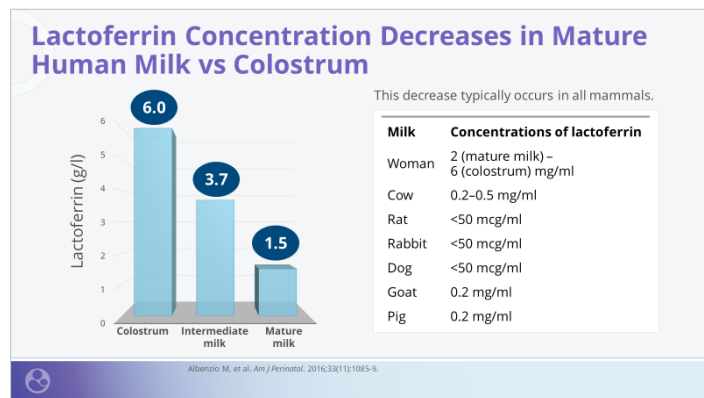
### Critical role in immune response

Lactoferrin, as I was alluding to, is not only a milk protein, because it's a part of a more complex antimicrobial protein network of inflammatory markers that have a critical role in immune response during infections. In fact, lactoferrin is released by neutrophils, by mucosal secretion. It's highly represented in the ocular liquid during

## Neonate Feeding Regimens and the Expanding Role of Lactoferrin

ophthalmitis. It's highly represented in saliva. It's highly represented in all places where an inflammatory response occurs.<sup>17</sup>

It's very important to underline the typical pattern, the typical trend of the decreasing concentration of lactoferrin during different stages of lactation. In colostrum, lactoferrin is abundant with the concentration estimated around 6 g per liter. But this concentration decreases to 1.5 g/l in mature milk. And this decrease occurs typically in all mammals.<sup>18</sup>



Slide 8 – Lactoferrin Concentration Decreases in Mature Human Milk vs Colostrum

That means the action and the role of lactoferrin is even more critical in the periods in which all human beings—and I would dare to say, all mammalian beings—receive colostrum.

### Anti-infective activity

This is a representation of all the different actions exerted by lactoferrin in terms of anti-infective activity [Slide 9]. There are direct mechanisms of action that can be defined antibiotic-like by targeting different epitopes of the pathogens: [Lipopolysaccharide] LPS with gram-negatives, [Lipoteichoic acid] LTA with gram-positives, as an example. But there are also indirect mechanisms; one of them would be in iron-sequestering ability, ultimately prompting to bacteriostasis.

An indirect mechanism—and probably a very important one—is the ability to modulate the function and the development of the intestinal enterocyte by mixing actions of proliferation and differentiation, thus enhancing the gut barrier in a period in which the gut is terribly leaky. An indirect mechanism, [also includes] the ability to promote a good bifidogenic microflora in the gut.

**Lactoferrin and Its Mechanisms for Anti-Infective Activity**

<p><b>Direct Mechanisms—antibiotic-like action</b></p> <ul style="list-style-type: none"> <li>• Anti-LPS (vs Gram-negatives)</li> <li>• Anti-LTA (vs Gram-positives)</li> <li>• Anti-Candida cell-wall components</li> </ul>	<p><b>Indirect Mechanisms</b></p> <ul style="list-style-type: none"> <li>• Iron-sequestering (→bacteriostasis)</li> <li>• Functional modulation of intestinal proliferation and differentiation (→enhancement of gut barrier)</li> <li>• Bifidogenic action on gut microflora</li> </ul>
<p><b>Immunomodulatory Actions in the Gut Lymphoid Tissues (GALT)</b></p> <ul style="list-style-type: none"> <li>• IL-18 production, NK cell activity</li> <li>• Maturation and differentiation of T-lymphocytes—Th1/Th2 balance</li> <li>• CD8<sup>+</sup>/4<sup>-</sup> DCs maturation</li> <li>• Recruitment and activation of APCs</li> </ul>	<p><b>Antiflogistic Mechanisms</b></p> <ul style="list-style-type: none"> <li>• Inhibition of formation of reactive oxygen species (ROS) by suppressing free-radical activity</li> <li>• Decrease the levels of oxidative products when medicinal iron is present in a formula</li> </ul>

APCs, antigen-presenting cells; NK cell, natural killer cells; Th1, T-helper 1; Th2, T-helper 2.

Prother R, et al. *Biochem Cell Biol*. 2006;84:303-311; Kulkarni T, et al. *J Interferon Cytokine Res*. 2006;26:489-499; de la Rosa G, et al. *Immunol*. 2008;180:668-687; Ishikawa K, et al. *Immunol Res*. 2003;32:236-238; Sherman MP, et al. *Neurology*. 2011;76:424-411; van der Gaag RA, et al. *Immunol*. 2012;140:356; Gonzalez-Chaves SA, et al. *Int J Antimicrob Agents*. 2009;33:301-310; Lorenzetti B, et al. *J Pediatr*. 2011;156:2349-2353; Lorenzetti B, et al. *J Pediatr Gastroenterol Nutr*. 2013;56:643-644; England G, et al. *Immunol*. 2010;138:570-576; Naghizadeh T, et al. *Pediatr Res*. 2012;72:964-972; Sherman MP, et al. *Immunol*. 2004;17:289-299.

Slide 9 – Lactoferrin and Its Mechanisms for Anti-Infective Activity

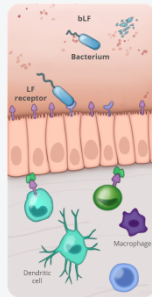
### Promoting intestinal development

We will see later on, but I want to focus once more on the ability of lactoferrin to promote intestinal development. In doing so, ultimately reaching the ability to exert true antimicrobial effects, because enterocyte developing in a correct way and proliferating rapidly soon after birth are effective in promoting the establishment of a gut barrier that prevents pathogens from translocation from the gut to the bloodstream.<sup>19-23</sup>

## Neonate Feeding Regimens and the Expanding Role of Lactoferrin

### Functions of Lactoferrin in Gut Development and Immune Defense

- **Intestinal development**
  - Promotes cell proliferation and differentiation
  - Improves intestinal mucosal structure, increased villus height, and crypt proliferation<sup>[1],[2]</sup>
- **Antimicrobial effects**
  - Antibacterial, antiviral, and antiparasitic protein
  - Inhibits growth, adhesion, translocation, and virulence of pathogens<sup>[3],[4]</sup>
  - Sequesters iron
- **Immune modulation**
  - Stimulates cells involved in innate and acquired immunity<sup>[5]</sup>



1. Roznjkov EA, et al. *J Nutr*. 2014;144(1401-1408). 2. Li Q, et al. *Mol Biol Rep*. 2014;41(2119-2128). 3. Ochoa T. *Biochimie*. 2009;91:30-34. 4. Teraguchi S, et al. *Appl Environ Microbiol*. 1995;61(4131-4134). 5. Shan T, et al. *J Anim Sci*. 2007;95(2149-2146).

Slide 10 – Functions of Lactoferrin in Gut Development and Immune Defense

### Bifidogenic factor

I was telling you that lactoferrin is bifidogenic. This is data that we obtained in Italy,<sup>24</sup> thanks to the group in Rome [Slide 11]. This group was able to measure content of lactoferrin and characteristics of microbiota in breast milk and in stools of infants who had been breastfed. By studying 48 mother-infant pairs at birth and after 30 days of life, investigators came to the conclusion, and to the evidence, that the fecal count of *Bifidobacteria* and *Lactobacilli* were significantly associated with the concentration of fecal lactoferrin after birth. The concentration of fecal lactoferrin was significantly associated with the concentration of milk lactoferrin in the human milk of the mothers. So, the [correlation] towards human milk lactoferrin and good microbiota has been demonstrated.

### Lactoferrin Is Bifidogenic

LF is able to promote growth of gut microbiota and establish/restore “healthy” microbiota

- Mastromarino<sup>[1]</sup> et al 2014 measured content of LF and microbiota of breast milk and feces of infants
- n=48 mother-infant pairs (34 full-term and 14 preterm) at birth and 30 DoL
- LF had positive influence on the microbiota → fecal count of *Bifidobacteria* and *Lactobacilli* was significantly associated with the concentration of fecal LF at 3 DoL ( $p= 0.01$ )

DoL, days of life.

1. Mastromarino P, et al. *Biomaterials*. 2014;22:1077-1086.

Slide 11 – Lactoferrin Is Bifidogenic

### Human and bovine lactoferrin similarities

It is important to know that human and bovine lactoferrin are very similar in biochemical structure, just as I was telling you. Also, in several experiments, it has been tested that bovine and human lactoferrin actually have the same actions on the nascent gut. In particular, both of them are fully able to promote the proliferation and differentiation of the enterocyte, just as I was telling you. Commercial bovine lactoferrin might even be (in several experiments) a little more active than human lactoferrin on enterocyte *in vitro*. Therefore, we suspect, and we presume that once added to infant formula, bovine lactoferrin may actually develop the same actions as in human milk.<sup>8,25</sup>

### Bovine and Human Lactoferrin Similarities

- Bovine and human lactoferrin have 77% biochemical homology
- Bovine and human lactoferrin provide similar actions on the nascent gut:
  - Commercial bLF is biologically active, as well as purified bLF and hLF
  - Commercial bLF exerts several bioactivities if added to infant formula



bLF, bovine lactoferrin; hLF, human lactoferrin.

Lonnerdal B, et al. *J Pediatr Gastroenterol Nutr*. 2011 Dec;53(6):606-614. Jiang R, et al. *J Pediatr Gastroenterol Nutr*. 2014;59(5):642-652.

Slide 12 – Bovine and Human Lactoferrin Similarities

### Lactoferrin clinical trials

We go now to discuss clinical studies. This has been a matter and a target of a number of studies conducted in the last 10 years. Some 15 randomized clinical trials, both multicenter and single center, exploring the ability of lactoferrin to promote the prevention of sepsis, to prevent necrotizing enterocolitis, and ultimately to enhance the well-being of a neonate and infant over the first weeks of life.



## Neonate Feeding Regimens and the Expanding Role of Lactoferrin

### Lactoferrin Clinical Studies

Study (date)	Patients (n=)	Objective	Dosing	Started	Duration	Result
Manzoni P, et al. <i>JAMA</i> . 2009. Multicenter RCT, 10 months	n=472 VLBW (<1500g) infants bLF alone (n=153) bLF + LGG (n=151) or placebo (n=168)	Evaluate effectiveness of LF alone or in combo w/LGG, compared with placebo; reduces incidence of LOS in VLBW neonates	<b>Group A1</b> – bLF 100 mg <b>Grp A2</b> – bLF 100 mg + LGG, 6 x 109 CFU/day <b>Placebo</b> (2 ml of 5% glucose sol. to milk feeding, daily 4-6 weeks)	birth until day 30 of life (day 45 for neonates <1000 g at birth)		Compared with placebo, bLF supplementation alone or in combo w/LGG reduced incidence of a first episode of late-onset sepsis in VLBW neonates.
Manzoni P, et al. <i>Pediatrics</i> . 2012.	n=472 neonates (<1500g) infants bLF alone (n=153) bLF + LGG (n=151) or placebo (n=168)	Secondary analysis of data from multicenter RCT where preterm VLBW neonates	<b>Group A1</b> – bLF 100 mg <b>Grp A2</b> – bLF 100 mg + LGG, 6 x 109 CFU/day <b>Placebo</b> (2 ml of 5% glucose sol. to milk feeding)	4.0 ± 1.4 DoL	daily 4 to 6 weeks	Supplemental lactoferrin reduced incidence of late-onset sepsis in VLBW infants. Prophylactic oral administration of bLF reduces incidence of fit in preterm VLBW neonates. No effect is seen on colonization. <b>LF decreased infections but not colonization rates in the gut.</b>
Ochoa T, et al. <i>J Pediatr</i> . 2013. (Peru)	n=277 lactoferrin n=278 placebo	Determine effect of bLF on prevention of diarrhea in children	Infants received 0.5 g twice/day bLF or placebo (diluted in 25 ml of water)	12 to >18 months old; 6 days/wk, twice daily	91,446 child/days of observation; 46,545 bLF 44,901 placebo	No difference diarrhea: 5.4 vs 5.2 episodes/child/year for lactoferrin and placebo, respectively (p=0.375). Although no decrease in diarrhea incidence, longitudinal prevalence and severity were decreased with lactoferrin.
Study from Jan 2009–May 2011	randomly assigned					

bLF, bovine lactoferrin; DoL, days of life; IFI, invasive fungal infection; LGG, *Lactobacillus GG*; LOS, late-onset sepsis; VLBW, very low birth weight.

Manzoni P, et al. *JAMA*. 2009;302:1421-1428. Manzoni P, et al. *Pediatrics*. 2012;129(11):16-23. Ochoa T, et al. *J Pediatr*. 2013;162:349-356.

### Slide 13 – Lactoferrin Clinical Studies

The first study was conducted by our group. It was published in *JAMA*.<sup>15</sup> Probably many of you know it from 2009 [Slide 13]. It was the first demonstration that bovine lactoferrin, when supplemented to the regular feeds, was able to reduce the incidence of late-onset sepsis in very low-birth-weight infants.

After this study, many more [have been added]...[Slide 24] with most of them providing results similar to what we could provide. Groups in Peru, groups in Turkey, groups in India, groups in Canada, explored different dosing regimens, different categories of premature infants.<sup>26-30</sup> Most of them, up to last year, were able to show similar ability of lactoferrin to promote well-being of the infants and to prevent late-onset sepsis.

Study (date)	Patients (n=)	Objective	Dosing	Started	Duration	Result
Manzoni P, et al. <i>Early Hum Dev</i> . 2014.	n=743 VLBW neonates	Studies have shown reduction of NEC in animal models, enhanced by LGG. This study assessed whether bLF alone or w/probiotic LGG, has a similar effect in human infants.	bLF (100 mg/day) alone (n=247) or bLF w/LGG (at 6-10/09) CFU/day; n=238 <b>Placebo</b> (control group; n=258)	birth until 30 DoL	assessed until discharge for development of NEC	Compared with placebo, bLF supplementation alone or in combo w/LGG reduced the incidence of a stage 2 NEC and of death within a stage 2 NEC in VLBW neonates. <b>Decreased incidence of NEC. bLF might be a promising strategy to prevent NEC in NICU settings.</b>
Akin IM, et al. <i>Am J Perinatol</i> . 2014.	n=50 VLBW or born <32 weeks • placebo (n=25) • 200 mg LF (n=25)	Does oral LF (200 mg/d) reduce nosocomial sepsis episodes and NEC in premature infants? Evaluate possible effects of LF on Treg levels.	200 mg LF daily throughout hospitalization		daily throughout hospitalization	Fewer sepsis episodes observed in LF treated infants (4.4 vs 17.3/1,000 patient days, p=0.007) with none developing NEC. LF prophylaxis reduced nosocomial sepsis episodes. Increase of Treg levels under LF prophylaxis was observed.
Kaur G, et al. <i>J Trop Pediatr</i> . 2015. (India)	LBW infants (BW less than 2,000 g)	Evaluate efficacy of bLF to prevent first episode of LOS in LBW neonates.	bLF 100-200 mg/day, according to increasing BW 1000-2000g	<48 hrs of life	First 30 DoL	bLF supplementation in LBW neonates reduced incidence of first episode of LOS. <b>Note:</b> Ideal sample size would have been 114 per arm to have 80% power.
<b>NEOLACTO study</b> (NCT01525316, Peru)	n=190 neonates; 80 (42.1%) had <1500 g BW	Determine the effect of bovine LF on the prevention of first episode of LOS in Peruvian infants	bLF (200mg/kg/day) <b>Placebo</b> (maltodextrin) given in 3-divided doses/day	500-2500 g at birth	4 wks since enrollment	Sepsis occurred less frequently in LF grp than in control grp. Although, primary outcome did not reach statistical significance, confidence interval is suggestive of an effect that justifies a larger trial.

bLF, bovine lactoferrin; DoL, days of life; LGG, *Lactobacillus GG*; LOS, late-onset sepsis; NEC, necrotizing enterocolitis; VLBW, very low birth weight.

Manzoni P, et al. *Early Hum Dev*. 2014;90:1560-65. Akin IM, et al. *Am J Perinatol*. 2014;31(11):1111-1120. Kaur G, et al. *J Trop Pediatr*. 2015;61:370-376. Ochoa T, et al. *Pediatr Infect Dis J*. 2015;34(9):571-576.

### Slide 14

The last published Cochrane review, in 2017,<sup>31</sup> included 6 randomized clinical trials, with more than

1000 preterm infants, and was able to establish that there was a 41% reduction attributed to lactoferrin in late-onset sepsis [Slide 15]. However, the evidence was graded as low-to-moderate quality with significant heterogeneity between trials. There is clearly room for additional trials warranted in order to confirm or discard the ability of lactoferrin to prevent sepsis and also NEC.

Study (date)	Patients (n=)	Objective	Dosing	Started	Duration	Result
Trial of lactoferrin for prevention of infections in very premature babies (LACTUNA) trial (2016) Barrington et al. <i>J Perinatol</i> . 2016.	neonates >23 <30.6 wks GA First 48 hrs of age (n=79) Control group: milk w/o LF	Determine tolerability of bLF in VP, and if intervention can be adequately masked	bLF 100 mg/day, 2 doses per day until 36 wks GA or discharge	<48 hours of life	36 wks GA or discharge	bLF is well tolerated, easy to administer, and its presence in prepared milk is not evident
Sherman et al. <i>J Pediatrics</i> . 2016.	TLF (n=60) or placebo (n=60)	Evaluate safety and efficacy of recombinant human LF (TLF) to reduce infection	TLF 150 mg/kg every 12 hours	Day 1-28 DoL	28 days	No clinical or lab toxicity; trend toward less infectious morbidity in infants treated with TLF
<b>Cochrane Review</b> 6 RCTs Effect of LF on Late-Onset Sepsis Parnami M, et al. <i>Cochrane Database Syst Rev</i> . 2017.	Six RCTs in 1071 preterm infants; Risk ratio 0.59 (95% CI 0.40-0.87; P=0.008)	Three co-primary outcomes: • LOS (n=886) • NEC - stage II (n=750) • Hospital mortality (n=1071)	Clarification regarding optimal dosing regimens, types of LF (human or bovine), and long-term outcomes is needed.			• Oral LF prophylaxis with two probiotics decreases LOS and NEC stage II in preterm infants without adverse effects • Current available evidence graded as 'low-moderate quality'; P = 0.05 indicates significant heterogeneity between trials • Completed ongoing trials will provide data from more than 6500 preterm neonates, which may enhance the quality of the evidence

bLF, bovine lactoferrin; DoL, days of life; GA, gestational age; LOS, late-onset sepsis; NEC, necrotizing enterocolitis; TLF, Talactoferrin; VLBW, very low birth weight.

Barrington KJ, et al. *J Perinatol*. 2016;36:666-669. Sherman MP, et al. *J Pediatr*. 2016;175:68-73.e3. Parnami M, et al. *Cochrane Database Syst Rev*. 2017;6:CD007137.

### Slide 15

### Lactoferrin tackling cytomegalovirus

In the meanwhile, several studies have been experimentally assessing the ability of lactoferrin to tackle cytomegalovirus (CMV) [Slide 16]. You all know that cytomegalovirus in human milk is of concern, especially in premature infants.

This group, led by David Kaufman in Virginia,<sup>32</sup> has performed several assays assessing the ability of different types of lactoferrin, compared with cytomegalovirus, to inhibit the growth and proliferation of cytomegalovirus. And the findings are very interesting because lactoferrin actually neutralizes cytomegalovirus *in vitro*. And bovine lactoferrin is even more potent than human. What's disappointing is that the naturally occurring concentrations of lactoferrin in breast milk and saliva are likely too low for effective neutralization *in vivo*. And this explains why, even in breastfeeding, the concern of transmitting cytomegalovirus is still there. Therefore, these findings advocate for assessing the opportunity to give extra lactoferrin to

## Neonate Feeding Regimens and the Expanding Role of Lactoferrin

preterm neonates who are breastfed, when there is a risk to transmit the cytomegalovirus.

### Lactoferrin and Cytomegalovirus (CMV)

- Lactoferrin (LF) neutralizes CMV in vitro
- Bovine LF has more potency than human
- LF concentrations in breast milk and saliva are likely too low for effective neutralization in vivo
- Breastfed, preterm neonates might need extra-LF to prevent CMV acquisition

Neutralization potency of human and bovine lactoferrin against human cytomegalovirus. Neutralization of HCMV AD169 by human purified, human recombinant, and two bovine-purified lactoferrin preparations. The dotted lines represent the IC<sub>50</sub> and IC<sub>90</sub> (ng/mL). HCMV, human cytomegalovirus.

Weimer KED, et al. *Am J Perinatol*. 2020. [published online ahead of print February 18, 2020] Used by permission of Thieme. © Georg Thieme Verlag KG.

Slide 16 – Lactoferrin and Cytomegalovirus (CMV)

Getting back to the Cochrane review, I was telling you that 41% [reduction in LOS] was seen, but this was clearly in need, and pending the results of 2 major trials conducted in the last 2 years—one in the UK and the other in Australia: the ELFIN trial and the LIFT trial [Slide 18].

### Benefits of Prophylaxis Oral Lactoferrin (Cochrane Database Syst Rev)

- 6 RCTs; n=1071 preterm infants
- 3 co-primary outcomes:

	n=	Risk Ratio	
<b>Late-onset sepsis</b>	886	0.59 (95% CI 0.40 to 0.87; P= 0.008)	NNT 17 Current available evidence graded as "low-moderate quality"
<b>NEC ≥ stage II</b>	750	0.40 (95% CI 0.18 to 0.86; P= 0.02)	NNT 25 Current available evidence graded as "low quality"
<b>Hospital mortality</b>	1071	0.65 (95% CI 0.37 to 1.11; P= 0.12)	

CI, confidence interval; NNT, Number Needed to Treat.

Meyer MP, et al. *J Neonatal Perinatal Med*. 2017;10:249-255. Pammis M, et al. *Cochrane Database Syst Rev*. 2017;6: CD007137.

Slide 17 – Benefits of Prophylaxis Oral Lactoferrin

Both of them have been published, and I will go through the results in order to show what is happening now, and how the results of these very recent large trials can be reconciled with existing literature.

### Year 2018–2019–2020 — Two Mega Trials: ELFIN and LIFT Lactoferrin RCTs

Study	Population	Intervention Group	Control Group	Primary Outcome
Enteral Lactoferrin in Neonates (ELFIN); ISRCTN88261002	UK Neonates <32 wks GA First 72 h of age (n=2,200)	Bovine LF 150 mg/kg/day (max: 300 mg) until discharge	Milk with placebo	Culture-proven or clinically suspected LOS from trial entry until discharge
Lactoferrin Infant Feeding Trial (LIFT) to prevent sepsis and death in preterm infants; ACTRN12611000247976	AUSTRALIA, INDIA, CANADA, ITALY Neonates with BW <1,500 g GA 22-28 wks First 7 DoL (n=1,100)	Bovine LF 200 mg/kg/day until 34 weeks GA corrected or discharge	Breast milk or formula without bLF	Incidence of sepsis or brain injury or CLD or NEC or severe ROP

CLD, chronic lung disease; DoL, days of life; ELFIN, Enteral Lactoferrin in Neonates; GA, gestational age; LIFT, Lactoferrin Infant Feeding Trial; LOS, late-onset sepsis; NEC, necrotizing enterocolitis; ROP, retinopathy; RCT, randomized controlled trial.

Slide 18 – Year 2018-2019-2020—Two Mega Trials: ELFIN and LIFT Lactoferrin RCTs

### ELFIN trial

The ELFIN trial was published in the *Lancet* one year ago, and it was absolutely disappointing because the goal investigators had was not achieved.<sup>33</sup> Lactoferrin supplementation, 150 mg/kg/day until 34 weeks of post-menstrual age was not able to prevent or reduce the risk of late-onset infection. The reduction was only 6%, [which is] not significant.

### ELFIN Trial (The Lancet, Jan 2019)

Primary and Secondary Outcomes	Lactoferrin group (n=1098)	Control group (n=1101)	Unadjusted risk ratio or median difference (95% CI or 95% CI*)	Adjusted risk ratio or median difference (95% CI or 95% CI**)	p value†
Microbiologically confirmed or clinically suspected late-onset infection	316/1093(29%)	334/1086(31%)	0.94(0.83 to 1.07)	0.95 (0.86 to 1.04)	0.233
Microbiologically confirmed late-onset infection	100/1031(10%)	100/1081(10%)	1.05(0.82 to 1.34)	1.05 (0.87 to 1.26)	0.460
All-cause mortality	71/1076(7%)	66/1076(6%)	1.06(0.69 to 1.59)	1.05 (0.68 to 1.63)	0.782
NEC (all stage I or II)	63/1085(6%)	56/1084(5%)	1.12 (0.71 to 1.77)	1.13 (0.68 to 1.89)	0.538
Severe ROP (retreated medically or surgically)	54/1080(5%)	52/1080(5%)	0.99(0.58 to 1.26)	0.99 (0.62 to 1.26)	0.483
ROP ≥ 36 weeks postmenstrual age	356/1033(34%)	355/1027(34%)	1.01 (0.87 to 1.18)	1.01 (0.90 to 1.13)	0.867
Dead before 36 weeks postmenstrual age	64	60	-	-	-
Infections, NEC, ROP, BPD, or mortality	525/1062(49%)	521/1094(48%)	1.01 (0.90 to 1.13)	1.01 (0.94 to 1.08)	0.743
Total number of days of administration of antimicrobials from commencement of intravenous medical product until 34-week postmenstrual age, median (IQR)	59(40 to 85)	58(40 to 84)	1 (-2 to 4)	1 (-1 to 3)	0.446
Length of hospital stay (days) to discharge, median (IQR)	8(4 to 16)	8(4 to 16)	0 (-1 to 1)	0 (-1 to 1)	0.963
Days in level 1 (intensive care, median (IQR))	10(3 to 30)	9(3 to 29)	0 (-1 to 1)	1 (-1 to 3)	0.420
Days in level 2 (special care, median (IQR))	29(21 to 39)	30(22 to 39)	-1 (-2 to 1)	-1 (-3 to 1)	0.216

Unless otherwise stated, data are n/N (%) when N is not equal to the total number of infants in the group it means that data are missing for some of the infants. NEC, necrotizing enterocolitis; BPD, bronchopulmonary dysplasia; ROP, retinopathy of prematurity. \*Risk ratios for binary outcomes and median differences for continuous outcomes. 95% CI for microbiologically confirmed or clinically suspected late-onset infection. 95% CI for all other outcomes. †Adjusted for randomization factors (i.e., collaborating hospital, sex, gestational age at birth, and origin of mother twins). \*\*Adjusted for baseline characteristics, whether adjusted risk ratios in question or adjusted median differences in question.

“...enteral lactoferrin supplementation (150 mg/kg/d until 34-weeks postmenstrual age) does not reduce the risk of late-onset infection, other morbidity, or mortality in very preterm infants...”

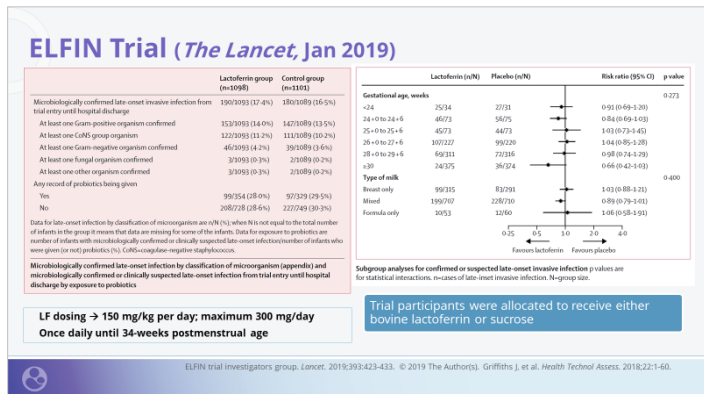
ELFIN trial investigators group. *Lancet*. 2019 Feb 23;393:423-433. Used under terms of a Creative Commons Attribution License. © 2019 The Author(s). Griffiths J, et al. *Health Technol Assess*. 2018;22:1-60.

Slide 19 – ELFIN Trial

This was true, more or less, in all categories of preterm infants, and in all feeding types of the infants. However, please consider that there were different conditions in this trial that might explain these disappointing results.

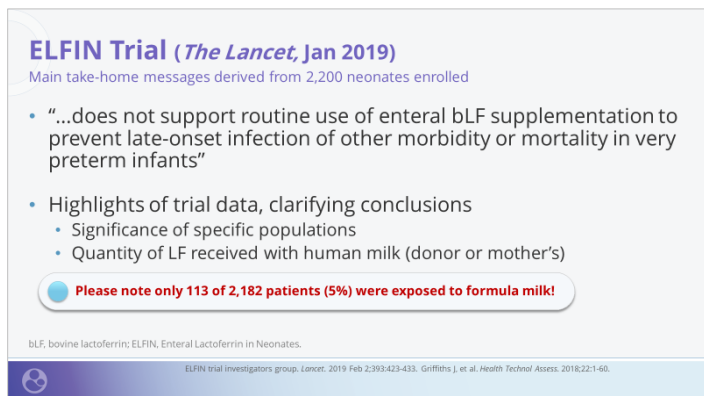


## Neonate Feeding Regimens and the Expanding Role of Lactoferrin



Slide 20 – ELFIN Trial

In any case, the ELFIN trial has a take-home message that is clearly a pre-final word against lactoferrin. Lactoferrin in this trial was not able to reduce late-onset sepsis, and therefore, it's not recommended as supplementation.<sup>34</sup>

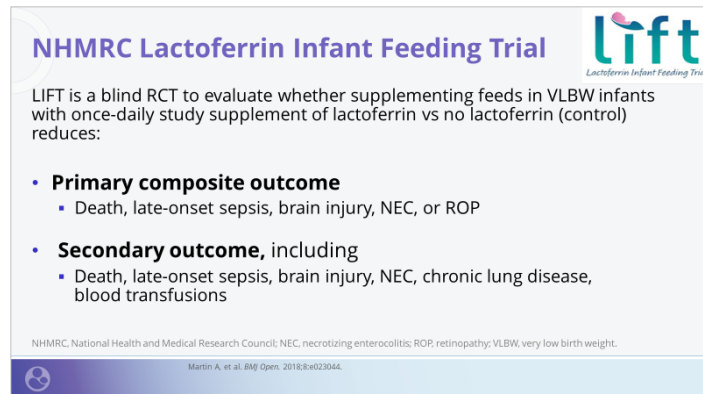


Slide 21 – ELFIN Trial

### LIFT trial

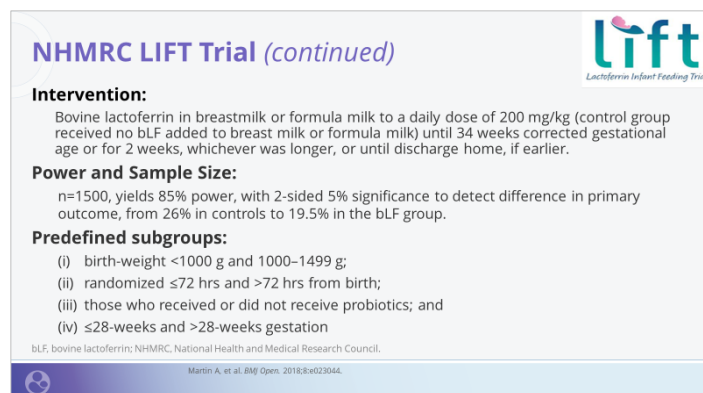
The LIFT trial was conducted during the same years and was published just a few days ago in the *Lancet Child & Adolescent Health*.<sup>35</sup> I will go through the results, and you will see there is room for further consideration.

Differently from other trials, the LIFT was targeting a composite outcome, which was the occurrence of death, and/or late-onset sepsis, and/or brain injury, and/or NEC, and/or ROP. So, the ultimate goal of this trial was to see whether lactoferrin improves the general health of the preterm infants.



Slide 22 – NHMRC Lactoferrin Infant Feeding Trial

This trial recruited infants less than 1500 g; they were allocated to lactoferrin or placebo; lactoferrin dose was 200 mg/kg/day [Slide 23]. Feeds with lactoferrin were commenced up to 7 days of life. These are the characteristics of compliance, when we started treatment.




Slide 23 – NHMRC LIFT Trial (*continued*)

As you see [Slide 24], most infants—almost 80% of the infants—completed the study treatment. So, the results are reliable, and we have a very preliminary piece of it...very interesting information here.

The proportion of infants fed with mother's milk was 95% in both investigational groups. In addition, those who received probiotics at any time during the study were 85%. So, the vast majority received compounds, such as human milk or probiotics, which can interfere with the prevention of sepsis—clearly can prevent sepsis.

## Neonate Feeding Regimens and the Expanding Role of Lactoferrin

**NHMRC LIFT Trial (continued)**



**Table. Compliance With Study Treatment**


Characteristic N (%)	Lactoferrin n=770	Control n=771
Days of study treatment median (IQR)	29 (16 to 40)	29 (17 to 40)
≥7 days of study treatment	719 (93.4%)	734 (95.2%)
≥14 days of study treatment	638 (82.9%)	665 (86.3%)
Study treatment completed	603 (78.3%)	634 (82.2%)
Study treatment incomplete	167 (21.7%)	137 (17.8%)

LIFT, Lactoferrin Infant Feeding Trial; NHMRC, National Health and Medical Research Council.  
Martin A, et al. BMJ Open. 2018;8:e023044.

Slide 24 – NHMRC LIFT Trial (continued)

Please note that the exclusively formula milk-fed infants were only 8%, so, really few.

**NHMRC LIFT Trial (continued)**



**Table. Descriptive Characteristics: Nutrition**


Characteristic N (%)	Lactoferrin n=770	Control n=771
Any mother's milk	733 (95.2%)	725 (94.0%)
Any donor breast milk	54 (7.0%)	53 (6.9%)
Any formula milk	64 (8.3%)	63 (8.2%)
Received probiotics at any time	660 (85.7%)	658 (85.3%)

LIFT, Lactoferrin Infant Feeding Trial; NHMRC, National Health and Medical Research Council.  
Martin A, et al. BMJ Open. 2018;8:e023044.

Slide 25 – NHMRC LIFT Trial (continued)

As I was telling you, the primary outcome was not met. There was a reduction by 5%, which is absolutely negligible.

**NHMRC LIFT Trial (continued)**



**Table. Primary Composite Outcome**


Primary Outcome	Lactoferrin n=770	Control n=771	Relative Risk (95% CI)	P
Death or major morbidity	162 (21.0%)	170 (22.0%)	0.95 (0.79 to 1.14)	0.60

CI, confidence interval; LIFT, Lactoferrin Infant Feeding Trial; NHMRC, National Health and Medical Research Council.  
Martin A, et al. BMJ Open. 2018;8:e023044.

Slide 26 – NHMRC LIFT Trial (continued)

When we cluster the analysis for the different compounds of the composite outcome [Slide 27], we see that late-onset sepsis was reduced by 17%, from 14% to 11.6%. It's not significant, but there is a solid trend towards reduction, which is different from all the other outcomes that were not affected by lactoferrin.

**NHMRC LIFT Trial (continued)**



**Table. RESULTS: Components of Primary Outcome**

Primary Outcome	Lactoferrin n=770	Control n=771	Relative Risk (95% CI)	P
Death before discharge	32 (4.2%)	29 (3.8%)	1.12 (0.68 to 1.84)	0.66
Necrotizing enterocolitis	26 (3.4%)	25 (3.2%)	1.09 (0.63 to 1.9)	0.75
Late-onset sepsis	89 (11.6%)	108 (14.0%)	0.83 (0.64 to 1.08)	0.16
Brain injury	50 (6.5%)	47 (6.1%)	1.06 (0.72 to 1.54)	0.78
Treated retinopathy	29 (3.8%)	20 (2.6%)	1.43 (0.84 to 2.44)	0.19

**Not significant; 17% reduction of sepsis in LF-treated infants.**

Martin A, et al. BMJ Open. 2018;8:e023044.

Slide 27 – NHMRC LIFT Trial (continued)

This may make sense because, as I was showing you in the first part of my talk, the activities of lactoferrin are mainly towards infections, and mainly based on several actions that target pathogens, by targeting them directly or by promoting a new modulation. So, it's not surprising that brain injury or retinopathy could not be affected by lactoferrin. But in my opinion, **it's still important that the 17% reduction in sepsis occurs.**

Well, anyway, this trial was not meeting the primary outcome. The trend in reduction of sepsis is still not significant. The power of the trial was enough to detect a 25% decrease or increase in primary outcome; however, a more moderate effect cannot be excluded. What we can say is that the treatment was absolutely well-tolerated, and this occurred also in the ELFIN trial. So, safety concerns are not at all applicable with lactoferrin, based on the evidence.

## Neonate Feeding Regimens and the Expanding Role of Lactoferrin

### LIFT Trial Summary Overview

- LF did not reduce the primary outcome, death, or major morbidity (Risk Ratio 0.95, 95% CI 0.79–1.14).
- Although a trend was observed, LF failed to reduce late-onset sepsis significantly (RR 0.83, 95% CI 0.64–1.08).
- LF did not affect other primary outcome components.
- The trial had 85% power to detect a 25% decrease or increase in primary outcome, but more moderate effects are not excluded.
- Treatment was well tolerated with good compliance, and there were no safety concerns with LF.

CI, confidence interval; LF, lactoferrin; RR, risk ratio.

### Why Are There Inconsistencies Between the 3 Major RCTs in Terms of LF Efficacy?

	LIFT n=1541	ELFIN n=2199	Manzoni n=472	Ratio
Only mother's milk	86%	92%	24%	>3
Only formula milk	<8%	<5%	15%	~0.5
Any probiotic	86%	75%	32%	>2.5
Was bLF flash pasteurized?	Yes	Yes	No	

bLF, bovine lactoferrin; ELFIN, Enteral Lactoferrin in Neonates; LF, lactoferrin; LIFT, Lactoferrin Infant Feeding Trial.

Martin A, et al. BMJ Open. 2018;8:e020046. ELFIN trial investigators group. Lancet. 2019 Feb;2:393-423-433; Manzoni P, et al. JAMA. 2009;301:1421-1428.

#### Slide 28 – LIFT Trial Summary Overview

The LIFT results appear inconsistent with the Cochrane review and with the ELFIN [trial]. Maybe with the ELFIN even more, but both of them are not confirming the ability of lactoferrin to prevent sepsis. **This raises the question about whether exactly the same product has been tested in the different trials or the same population has been tested in the different trials.** We'll go through these 2 pending questions in the next slide [Slide 29].

#### Slide 30 – Why Are There Inconsistencies Between the 3 Major RCTs in Terms of LF Efficacy?

Only formula milk, vice versa was received by 15% in the original trial vs 5% to 8% in the 2 recent trials. Probiotic exposure was 80%, or something like this, in the LIFT and ELFIN, whereas only 32% of our kids in the original trial received probiotics. And finally, bovine lactoferrin was flash pasteurized in the 2 recent trials owing to safety reasons—but was not flash pasteurized in ours.

### LIFT Trial Summary Overview (continued)

- The LIFT results appear inconsistent with the previous Cochrane review of 6 RCTs.
- While some differences between LIFT and the other trials may reflect the play of chance, there was significant heterogeneity, both amongst earlier studies and between LIFT and earlier studies.
- This raises questions about whether exactly the same product was being tested, or the same population was tested.

CI, confidence interval; LF, lactoferrin; LIFT, Lactoferrin Infant Feeding Trial; RR, risk ratio.

### Pending Issues Related to Supplementation Strategies With Lactoferrin

- LF levels vary in maternal milk during lactation
- Infant's GA and time of study sampling affect LF levels
- LF ranges in various breast milk types
  - Mother's milk
  - Stored, refrigerated mother's milk
  - Donor milk
- Clarification regarding optimal dosing regimens, types of lactoferrin (human or bovine), and long-term outcomes is needed.

GA, gestational age.

Albenzio M, et al. Am J Perinatol. 2016;33:1085-1089.

#### Slide 29 – LIFT Trial Summary Overview (continued)

### Trial inconsistencies

My first very provoking slide is this one [Slide 30]. In the different trials that have opposite results—stop lactoferrin in red, green-light to lactoferrin in green—you see that only mother's milk was received by 90% in LIFT and ELFIN, compared with 24% only in the original trial in Italy.<sup>15,33,35</sup>

#### Slide 31 – Pending Issues Related to Supplementation Strategies With Lactoferrin

I think that, and I suggest for your consideration, that these 4 main differences can actually impact why the results are so strikingly different.

Please remember the **lactoferrin levels vary in maternal milk during lactation**. Please remember that these levels are varying not only in maternal milk but also when maternal milk is stored or refrigerated, or even more, if it is pasteurized as

## Neonate Feeding Regimens and the Expanding Role of Lactoferrin

donor milk.<sup>18</sup> It's clearly a matter of clarifying which lactoferrin we are using and clarifying which regimens we need to use.

If the evidence before 2020 was suggesting that lactoferrin is effective, these recent results, based on the differences in the population that have been studied [Slide 32], might suggest that further studies are needed to understand not only whether lactoferrin is effective in preventing sepsis, but ultimately **what is the actual intake of lactoferrin providing a beneficial effect.**<sup>31</sup>

### How Do We Correctly Interpret These Data? How Do We Reconcile Apparently Contrasting Findings?

- The evidence BEFORE 2020 was suggesting that lactoferrin supplementation to enteral feeds decreases late-onset in preterm infants without adverse effects.
- The recently completed large-sized trials have provided data from >6000 preterm neonates and should enhance the quality of the evidence.
- However, the populations studied in these 2 recent RCTs are likely different from those studied in the earlier trials!

Farroni M, et al. Cochrane Database Syst Rev. 2017;6: CD007137.

*Slide 32 – How Do We Correctly Interpret These Data? How Do We Reconcile Apparently Contrasting Findings?*

### Significance of lactoferrin intake

It is possible that the issue here is not supplementation of lactoferrin simply, but it is rather how much lactoferrin is this kid getting from human milk or externally. I will guide you through these considerations, thanks to a number of studies that have been published in the last years, most clinical and *in vitro*.

### How Do We Correctly Interpret These Data? How Do We Reconcile Apparently Contrasting Findings?

- The key for a better understanding is NOT THE SUPPLEMENTATION OF LACTOFERRIN, but rather THE ACTUAL INTAKE OF LACTOFERRIN
- Evidence from 3 clinical studies:
  1. Trend<sup>[1]</sup> et al. (Australia)—2015
  2. Ochoa<sup>[2]</sup> et al. (Peru)—2020
  3. Manzoni<sup>[3]</sup> et al. (Italy & NZ)—2019
- Evidence from 2 Lab studies...

1. Trend S, et al. PLOS One. 2015;10: e0117038.  
2. Ochoa J, et al. Neonatology. 2020;1-6.  
3. Manzoni P, et al. Am J Perinatol. 2019;36:5120-5125.

*Slide 33 – How Do We Correctly Interpret These Data? How Do We Reconcile Apparently Contrasting Findings?*

This one is very important. It is from a group in Australia.<sup>36</sup> Five years ago, they assessed the concentration of lactoferrin and the outcomes of preterm infants in a number of mothers' and infants' diet. They noted, and they demonstrated that lactoferrin was the only antimicrobial peptide that limited pathogen growth more than 50% when added to formula at a concentration equivalent to that present in breast milk. But more importantly, the addition of lactoferrin to formula milk was able to inhibit growth of all pathogens, and the effect was dose dependent. Meaning that **if you want to have an impact on pathogens, you should really care about the amount, about the intake of lactoferrin.**

### Antimicrobial Protein and Peptide Concentrations and Activity in Human Breast Milk Consumed by Preterm Infants at Risk of Late-Onset Neonatal Sepsis<sup>[1]</sup>

This study assessed the levels and antimicrobial activity of antimicrobial proteins and peptides, including lactoferrin, in breast milk consumed by preterm (<32 wks) infants, and whether deficiencies of these factors were associated with late-onset neonatal sepsis

- Breast milk from mothers of preterm infants (32 wks GA) was collected on days 7 (n=88) and 21 (n=77) postpartum.
- Concentrations of lactoferrin, LL-37, beta-defensins 1 and 2, and alpha-defensin 5 were measured by ELISA.
- The antimicrobial activity of breast milk samples against *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus agalactiae* was compared to the activity of infant formula, alone or supplemented with physiological levels of AMPs.
- Samples of breast milk fed to infants with and without subsequent LOS were compared for levels of AMPs and inhibition of bacterial growth.

GA, gestational age; ELISA, enzyme-linked immunosorbent assay; LOS, late-onset sepsis; AMPs, antimicrobial proteins.

1. Trend S, et al. PLOS One. 2015;10(2):e0117038.

*Slide 34 – Antimicrobial Protein and Peptide Concentrations and Activity in Human Breast Milk Consumed by Preterm Infants at Risk of Late-Onset Neonatal Sepsis*

## Neonate Feeding Regimens and the Expanding Role of Lactoferrin

And the final proof of this comes from the assessment of the clinical findings from all infants in these studies. Those babies who had a late-onset sepsis episode during the course of their stay in the NICU had actually consumed and received lower intakes of lactoferrin both on day 7 of life and on day 21 [Slide 35].

### Results<sup>[1]</sup>

- Levels of most AMPs, including LF, and antibacterial activity in preterm breast milk were higher at day 7 than at day 21.
- The range of total daily LF consumed by infants ranged from 0–794 mg/kg on days 7 and 21 postpartum.
- Lactoferrin was the only AMP that limited pathogen growth >50% when added to formula at a concentration equivalent to that present in breast milk.**
- Levels of AMPs were similar in the breast milk fed to infants with and without LOS, however, infants who developed LOS consumed significantly less breast milk and lower doses of milk AMPs than those who were free from LOS.

Levels of LF in breast milk are higher at 7 days than at 21 days ( $P < 0.001$ ).

AMPs, antimicrobial proteins; LF, lactoferrin; LOS, late-onset sepsis.

1. Trend S, et al. PLOS One. 2015;10(2):e0117038. Used under terms of a Creative Commons Attribution License. © 2015 Trend et al.

Slide 35 – Results

Please note that the lactoferrin received in a septic infant was one-fourth or one-third less than the lactoferrin received by those babies who did not develop sepsis.

### Antimicrobial Activity of Lactoferrin When Added to Infant Formula

- In a secondary experiment, physiological milk levels of individual AMPs, including LF, were independently capable of inhibiting bacterial growth in LBWF.
- The addition of LF to LBWF** at doses equivalent to the median concentration measured in preterm breast milk (3.8 mg/mL) had >50% bacteriostatic effect against all bacterial species, with >97% inhibition of growth for *S. epidermidis*, *S. aureus*, and *E. coli*, and 67% for *S. agalactiae* (Fig. 3, Trend<sup>[1]</sup> 2015).
- The effect was dose-dependent, with inhibition of all species >97% when 9.5 mg/mL LF (equivalent to the highest concentration detected in preterm breast milk) was used.
- No significant effect on growth inhibition was seen when the lowest concentration detected in preterm breast milk (0.5 mg/mL LF) was added to LBWF.
- The other AMPs tested did not show similar efficacy in inhibiting pathogens.

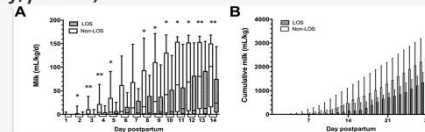
LF, lactoferrin; LBWF, low birth weight formula.

1. Trend S, et al. PLOS One. 2015;10(2):e0117038.

Slide 36 – Antimicrobial Activity of Lactoferrin When Added to Infant Formula

### Late-Onset Sepsis in Infants and Levels/Intakes of LF

- The concentration of LF in breast milk showed negative correlation with the colony forming units of *E. coli* and *S. aureus* after incubation with breast milk
- The median doses of LF consumed by LOS cases were lower on day 7 (14 mg/kg LF in LOS cases and 52 mg/kg in controls, respectively;  $p = 0.03$ ) and day 21 (131 mg/kg LF in LOS cases and 298 mg/kg LF in controls, respectively;  $p = 0.04$ ).



Trend S, et al. PLOS One. 2015 Feb 2;10(2):e0117038. Used under terms of a Creative Commons Attribution License. © 2015 Trend et al.

Slide 37 – Late-Onset Sepsis in Infants and Levels/Intakes of LF

The same finding was retrieved by our group when we received a secondary analysis from the data of our original trial [Slide 38]. With the post-hoc study, we showed that the effect of lactoferrin was not significant in those babies who had received only human milk.<sup>7,15,37</sup>

### In Light of ELFIN and LIFT Results... Let's Look Into JAMA 2009<sup>[1]</sup> Trial Results

- N=472; 361 (76%) infants were not exclusively human-milk fed
- Remaining 111 (24%) HM-fed infants had mean daily intake of 79 ml/kg/day (current evidence suggests >50 ml/kg/day is protective) (Schanler<sup>[2]</sup> 1999)
- A post-hoc analysis shows the effect of bLF was not significant in the HM-only subgroup ( $p = 0.15$  vs  $p < 0.001$ )<sup>[3]</sup>**
- Why is this the case?**

bLF, bovine lactoferrin; DoL, days of life; HM, human milk; LF, lactoferrin.

1. Manzoni P, et al. JAMA. 2009;302:1421-1428.  
2. Schanler B, et al. Pediatrics. 1999;103:1150-1157.  
3. Manzoni P, et al. Am J Perinatol. 2019;36:5120-5125.

Slide 38 – In Light of ELFIN and LIFT Results... Let's Look Into JAMA 2009 Trial Results

Not only were we able to show that the exposure of human milk-fed infants in our trial was...the exposure to lactoferrin was seen in ranges spanning between 160 to 280 mg/kg/day [Slide 39]. This means that if we give 100 mg of lactoferrin a day to a baby who is already receiving 200 or more mg, maybe this is not effective because the effect is already there. It's completely different to give 100 mg of lactoferrin to a baby who is not receiving anything because he's getting formula milk.<sup>37</sup>

## Neonate Feeding Regimens and the Expanding Role of Lactoferrin

### Why Was the Effect of bLF Not Significant in the Subgroup of Infants Fed HM?

Assuming a mean concentration of hLF comprised between 2–3.5 mg/ml, HM-fed infants in the LIFT study were likely exposed to 160–280 mg/kg/day during their stay in NICU, which is more than the bLF supplement (100 mg/kg/day) given to the experimental arms

Post-hoc analysis on the 2009 RCT patients

Is Lactoferrin More Effective in Reducing Late-Onset Sepsis in Preterm Neonates Fed Formula Than in Those Receiving Mother's Own Milk? Secondary Analyses of Two Multicenter Randomized Controlled Trials

Paolo Manzoni, MD<sup>1,2</sup>, Maria Angela Milillo, MD<sup>1</sup>, Stefano Rizzolo, MD<sup>3</sup>, Elena Tavella, MD<sup>2</sup>, Alessandra Mariani, MD<sup>4</sup>, Maria Perinelli, MD<sup>5</sup>, Sara Cusi, MD<sup>6</sup>, Maria Carla Cusi, MD<sup>7</sup>, Eleonora Tognato, MD<sup>8</sup>, Roberta Spola, MD<sup>9</sup>, Anna Perinelli, MD<sup>10</sup>, Milena Maria Rizzo, MD<sup>11</sup>, Roberto Caracciolo, MD<sup>12</sup>, Maria Monica, MD<sup>13</sup>, Sara Storti, MD<sup>14</sup>, Lorenza Pagni, MD<sup>15</sup>, Hubert Messner, MD<sup>16</sup>, Silvia Cattari, MD<sup>17</sup>, Pinella Maria Betta, MD<sup>18</sup>, Luigi Menici, MD<sup>19</sup>, Lidia De Santis, MD<sup>20</sup>, Sara Bellini, MD<sup>21</sup>, Matteo Ronchi, MD<sup>22</sup>, Maria Fioretti, MD<sup>23</sup>, Michele Quercia, MD<sup>24</sup>, Chryssola Tsolia, MD<sup>25</sup>, Nicola Laforgia, MD<sup>26</sup>, Fabio Mosca, MD<sup>27</sup>, Rosario Magalhães, MD<sup>28</sup>, Michael Mostert, MD<sup>29</sup>, Daniele Farina, MD<sup>30</sup>, William Tarone-Morici, MD<sup>31</sup>, on behalf of the Italian Task Force for the Study Prevention of Neonatal Fungal Infections; the Italian Society of Neonatology

bLF, bovine lactoferrin; HM, human milk; hLF, human lactoferrin.

Manzoni P, et al. *Am J Perinatol*. 2019;36(5):5120-5125.

Slide 39 – Why Was the Effect of bLF Not Significant in the Subgroup of Infants Fed HM?

The same findings have been found recently by Theresa Ochoa in Peru [Slide 40].<sup>38</sup> Her group again, and once more, conducted a retrospective study assessing which was the mother-milk cumulative intake in the first weeks of life in babies developing or not developing sepsis, and drew the conclusion that the daily human lactoferrin intake in babies not featuring sepsis was 334 mg/kg/day. In contrast, it was only 89 mg/kg/day, which makes a great difference and probably explains why the addition and supplementation of external lactoferrin may not be critical when a baby is already receiving such a big amount of natural lactoferrin.

### Is Mother's Own Milk Lactoferrin Intake Associated With Reduced Neonatal Sepsis, Necrotizing Enterocolitis, and Death?

**OBJECTIVES** → to determine the association of maternal LF intake and mother's own milk intake in the first 10 days of life on the prevention of late-onset sepsis (LOS), necrotizing enterocolitis (NEC), or death in the first 8 weeks of life in newborns with a birth weight <2,000 g.

**METHODS** → retrospective cohort study on 240 mother/infant pairs. Intakes of maternal milk, and content of LF in the maternal milk feeds, were measured.

**RESULTS** →

1. The average daily human LF intake over days 4–10 of life was 283 mg/kg/day
2. The adjusted hazard ratio (HR) of mother's own milk LF intake  $\geq 100$  mg/kg/day in days 4–10 for LOS, NEC, or death 0.752 (95% CI 0.301–1.877,  $p = 0.541$ )
3. The adjusted HR of mother's own milk cumulative intake (days 4–10) of 54–344 mL/kg (25–75 quartiles) for LOS, NEC, or death was 0.414 (95% CI 0.196–0.873,  $p = 0.02$ ). **Infants who developed an event (LOS, NEC, or death) had significantly less median daily human LF intake than those that did not (89 vs 334 mg/kg/day, respectively,  $p < 0.0001$ ).**

**CONCLUSION** → Consumption of higher amounts of mother's own milk in the first days of life is associated with less infection, NEC, and death.

Ochoa T, et al. *Neonatology*. 2020;118.

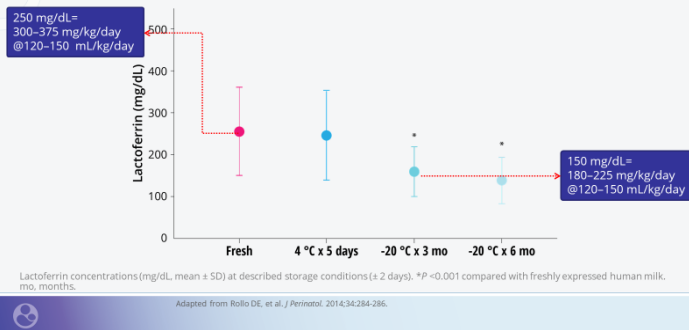
Slide 40 – Is Mother's Own Milk Lactoferrin Intake Associated With Reduced Neonatal Sepsis, Necrotizing Enterocolitis, and Death?

### Lactoferrin stability

Two additional findings: please consider that lactoferrin decreases in concentration in stored

human milk. If you are feeding a baby with stored human milk, you cannot rely on the same intakes of lactoferrin as if it was in fresh human milk. With breastfeeding, compared to giving stored human milk, there is a reduction of 50%,<sup>39</sup> and even more,

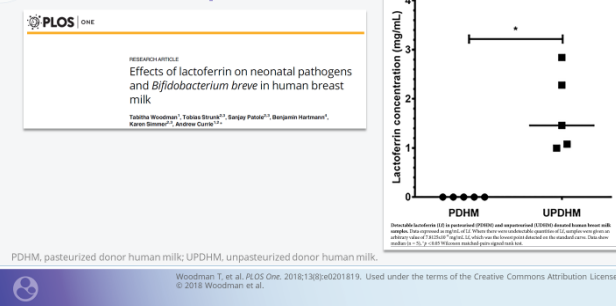
### Stability of Lactoferrin in Stored Human Milk



Slide 41 – Stability of Lactoferrin in Stored Human Milk

if lactoferrin undergoes pasteurization. Donated human milk undergoing pasteurization, as it occurs regularly and routinely, has a dramatic decrease in concentration of lactoferrin. Something that does not enable us to provide a protective effect of lactoferrin in this human milk sample.<sup>40</sup>

### Detectable Lactoferrin Is Significantly Lower in Pasteurized vs Unpasteurized Donated Human Breast Milk Samples



Slide 42 – Detectable Lactoferrin Is Significantly Lower in Pasteurized vs Unpasteurized Donated Human Breast Milk Samples

### Beneficial feeding regimens with lactoferrin

This is summarizing the flow of the last slides, and we are getting to the conclusion. If the goal of this presentation is to give advice about which are the most beneficial feeding regimens with lactoferrin,

## Neonate Feeding Regimens and the Expanding Role of Lactoferrin

please note that according to 3 different studies on 3 different populations [Slide 43],<sup>36-38</sup> infants without sepsis in the first 4 weeks of life receive 300 mg/kg/day of lactoferrin as a mean daily intake. Infants with sepsis, in contrast, receive no more than 100 mg/kg/day. That is only 30%, a two-thirds reduction. This might be the key to understand what we need to do.

**Let Us Focus on the Figures...  
LF Mean Daily Intakes and Neonatal Sepsis**

Study	Infants WITHOUT sepsis	Infants WITH sepsis
Manzoni P, et al. 2019 <sup>[1]</sup>	160-280	100
Trend S, et al. 2016 <sup>[2]</sup>	298	131
Ochoa RJ, et al. 2020 <sup>[3]</sup>	334	89

Lactoferrin intakes are expressed in mg/kg

1. Manzoni P, et al. *Am J Perinatol*. 2019;36:S120-S125.  
2. Trend S, et al. *PLoS One*. 2015;10:e0117038.  
3. Ochoa RJ, et al. *Neonatology*. 2020;1-8.

Slide 43 – Let Us Focus on the Figures... LF Mean Daily Intakes and Neonatal Sepsis

And, once more, we had already foreseen and designed this issue when the application for the LIFT trial was placed 7 years ago. In fact, we had nicely estimated the actual daily intakes of human lactoferrin in babies who are regularly and routinely fed with fresh human milk. It's already around 200 mg daily [term], and it's getting up to more than 300 mg when the baby is 2-weeks old. I dare say that these intakes are the correct intakes we need to target in order to have a protective effect.

### How Much Lactoferrin Do We Need? The Natural Model<sup>[a]</sup>

**Table.** Typical intake of hLF in a 1000g infant after starting trophic feeding with breast milk

Day	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16
ml feed	0.5	1	2	3	4	5	6	8
No. of feeds	6-8	8-12	12	12	12	12	12	12
Mean daily volume of feeds	3-4	8-12	24	36	48	60	72	96
hLF concentration [mg/ml]	7	6.5	6	5.5	5	5	4.5	4
Presumed weight in grams <sup>[b]</sup>	1000	900	850	870	870	890	920	950
Mean daily hLF (mg/kg)	21-27	47-66	130	172	209	267	298	365

a. Patterns of mean daily human lactoferrin amounts for a 1000 g birth weight preterm infant in the first 2 weeks of life.  
b. Assuming a typical weight loss of up to 15% in the first week.

hLF, human lactoferrin.

LIFT application, NHHMRC, 2014—unpublished

Slide 44 – How Much Lactoferrin Do We Need? The Natural Model

Once these intakes are achieved—and this is another summary of what I was telling you—remember these figures because when these intakes are achieved, thanks to fresh human milk, it's probably not useful to give additional lactoferrin.

### Dosing Summary: Ideal Ingestion of Lactoferrin

Studies suggest ideal scenario...

A newborn with exposure to fresh maternal milk since birth would ingest the following daily amounts of bioactive LF:

- At least 50 mg/kg at DoL 3
- At least 150 mg/kg at DoL 7
- Around 300 mg/kg at DoL 15 to 21

**Please remember these figures!**

DoL, days of life.

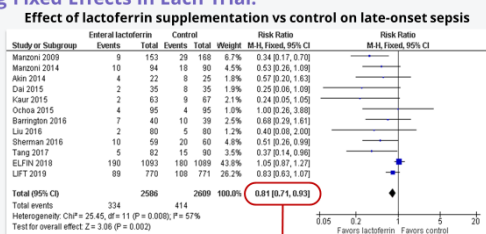
Slide 45 – Dosing Summary: Ideal Ingestion of Lactoferrin

I want to leave you with this very recent (included in the LIFT paper) meta-analysis, which has been able to include, not only the previous studies but also the ELFIN and the LIFT trials [Slide 46]. Now we have more than 5,000 infants; very low-birth-weight infants were randomized to lactoferrin or placebo over 10 years coming from 11 different studies.<sup>41</sup>

The evidence is solid enough to say that lactoferrin prevents infections with a decrease by 19%. It's not a great decrease, but it's actually significant within a very narrow interval of confidence. And this might be the final word prompting speculations.

## Neonate Feeding Regimens and the Expanding Role of Lactoferrin

### A Final Word: Prespecified Synthesis of Effects of Lactoferrin Supplementation in LIFT and 11 Other Trials (up to August 31, 2019), Assuming Fixed Effects in Each Trial.



**Lactoferrin provides a 19% reduction in Late-Onset Sepsis**

Tarnow-Mordi WO, et al. *Lancet Child Adolesc Health*. 2020. pii: S2352-4642(20)30093-6. [published online ahead of print May 12, 2020]

Slide 46 – A Final Word: Prespecified Synthesis of Effects of Lactoferrin Supplementation in LIFT and 11 Other Trials

I would dare to say once more that bovine lactoferrin supplementation may give clinically measurable benefits only when lactoferrin intakes of human milk are below a certain threshold, and this occurs with pasteurized, stored, refrigerated human milk or with donor milk, or even less with formula milk.

### Speculation Rising From Current Data

- Lactoferrin (either human or bovine) supplementation appears to have clinically measurable benefits *only* when LF intakes from HM are below a certain threshold (see LIFT; Manzoni<sup>11</sup> et al. *JAMA*. 2009; Sherman<sup>12</sup> et al. *J Pediatr*. 2016.).
- Possible protective threshold levels of LF intake—according to the experimental data and to natural breastfeeding trends—could be comprised between 50–150 mg/kg at 7 DoL, and between 300–400 mg/kg at 21 DoL.
- When breastfeeding already provides these intakes, an external supplementation might not be needed, nor effective, nor confer additional advantages.
- However, in all situations where LF intake is not needed, (including processed HM and donor-banked HM), LF external supplementation could be considered.

DoL, days of life; HM, human milk; LF, lactoferrin.

1. Manzoni P, et al. *JAMA*. 2009;302:1421-1428.  
2. Sherman MP, et al. *J Pediatr*. 2016;175:68-73.e3.

Slide 47 – Speculation Rising From Current Data

We were able to capture the current protective threshold levels of lactoferrin intake, thanks to the 3 studies I was showing you, and thanks to the analysis of the findings of different trials that assessed different populations with different dosing regimens. Therefore, my advice is that in all situations where breastfeeding is already providing these intakes, an external supplementation with lactoferrin might not be needed or, in any case, might not be able to provide additional advantages.

In contrast, in all situations where lactoferrin intake is not the one that we estimate, that we need as preventative, is not optimal... we need to consider supplementation with external lactoferrin.

### Ongoing Issues With Supplemental Lactoferrin

- Quality control
- Correct intakes
- Optimal dosing regimens
- Types of LF: human or bovine, milk and formulas
- Interactions with probiotics
- Long-term outcomes

LF, lactoferrin.

Slide 48 – Ongoing Issues With Supplemental Lactoferrin

We need to understand that giving bovine lactoferrin is safe (as of today), and that we can expect the results only when human lactoferrin is not given in the correct amounts.

And finally, before going further, we need also to clarify further points about the quality control, about the dosing regimens, and about the interactions with probiotics because all these areas are critical when we want to establish a feeding regimen with a supplementation that might enhance the protective effect of human milk.

### QUESTION & ANSWER

*Editor's Note: This is a transcript of audience questions together with Dr. Manzoni's responses from the May 20, 2020 audio webcast.*

### Is there a role for lactoferrin in COVID-19 prevention management or treatment in preterm or term infants?

**Dr. Manzoni:** This is a very timely question. We actually have some scattered data on the ability of lactoferrin to interfere with the MERS coronavirus. The MERS coronavirus is closely similar to the COVID-19, so we may expect that some action of lactoferrin could be envisaged on coronavirus.



## Neonate Feeding Regimens and the Expanding Role of Lactoferrin

Clearly, we need time and studies to understand whether this antiviral effect of lactoferrin is actually there.

### **How does lactoferrin promote growth of intestinal microbiota to establish and restore healthy microbiota?**

Well, lactoferrin acts like a prebiotic. Prebiotics are defined as food for probiotics. In other terms, they are compounds, both chemical or other natural compounds, that are critical to allow probiotics to proliferate. Lactoferrin does this by stimulating the proliferation of specific colonies of probiotics, specific strains namely, *bifidobacteria* and *lactobacilli*. These strains have been most implicated with the benefits in human beings, especially in the first weeks of life.

### **What is the most significant benefit of lactoferrin for neonates?**

Well, certainly the most significant one is the prevention of sepsis, which is a result of the varying and several anti-infective actions of lactoferrin. Of course, human milk is a very complex compound. We cannot extrapolate one single compound of human milk and attribute the benefit of human milk to that single compound. But it's presumable in a complex context, like the one I was showing you. Some compounds have different roles, and in this view, lactoferrin has a critical role in preventing infection.

### **How much does human breast milk vary in the amount of lactoferrin produced? And is the production of lactoferrin consistent in human breast milk?**

Yes. There are 2 different trends that can also mix with each other and interfere with each other. The first trend you mention, lactation. Usually more lactoferrin in the first days, in colostrum in the first days, then decreasing intermediate, and final decrease in natural milk. And another trend is related to the gestational age of the baby. These

mothers of premature infants may maintain higher levels of lactoferrin over the duration of their breastfeeding compared with what occurs should the baby be born at term. This might be an interesting mechanism of Mother Nature to provide benefits of lactoferrin even longer than what occurs for those babies more at risk of infection because they have been born prematurely.

### **Is lactoferrin retained in pasteurized donor milk after processing at the milk bank?**

This is a critical point because it's known that pasteurization, also Holder pasteurization decreases by 30% to 60% the amount of lactoferrin contained in the sample because pasteurization has a denaturing ability. So, lactoferrin does not escape this ability of pasteurization. The point is to identify methods of pasteurization that could be more gentle towards critical proteins, such as lactoferrin, or, in contrast, to design strategies of supplementation of lactoferrin whenever the milk is pasteurized.

### **When should breast milk be supplemented with bovine lactoferrin?**

We need to be very accurate with the wording here because if we speak about breast milk—and we mean milk taken by an infant through breastfeeding—we mean fresh human milk. And therefore, supplementation with bovine lactoferrin probably is not needed, thanks to all the data and all the considerations that I was showing you. But if breast milk is expressed human milk that goes to storage and/or to refrigeration, because mothers are expressing milk and storing it in the refrigerator for 20 days because they have to go back to work, this might be a different issue. And in my opinion, in these cases, the decrease of lactoferrin content compared with the original samples could be much higher that it could be applicable to supplement lactoferrin in those samples.

## Neonate Feeding Regimens and the Expanding Role of Lactoferrin

### **What is the minimum threshold or intake of human lactoferrin that requires bovine lactoferrin supplementation?**

Well, again we could speculate on that, and we could identify threshold levels of 70, 80, 90 mg/kg/day. Below these levels, infants are significantly more likely to develop sepsis. If a baby is getting less than these intakes, in my opinion, this baby would need an external supplementation.

### **In the ELFIN trial, there are 2 serious adverse events that authors reported as possibly associated with lactoferrin use. What are your thoughts?**

Well, I think I cannot respond to that. If they were reporting on possibly regular lactoferrin, this might be the case. In any case, it's curious that the lactoferrin proved pace in all the other 11 trials, which means 9,000 infants were randomized, so far. In addition, let's consider that lactoferrin is naturally given to neonates since the Stone Age. So, it would be absolutely unreasonable that there would be a safety issue for this protein, which is naturally occurring within human beings for such a long time.

It is true that we are talking about bovine lactoferrin, but anyway, the homology of biochemical structure is very high. And notably there is, to my knowledge and to the knowledge of the most of us, no reported allergy to lactoferrin. Lactoferrin is not a serum protein of milk; it's not a lactic casein. It's a different kind of protein; it's a glycoprotein. In pediatrics, we never saw allergies to lactoferrin.

### **Lactoferrin has iron-binding characteristics. What do you recommend in terms of lactoferrin use and iron supplementation for example, dose of iron timing of starting the supplementation if an infant is given lactoferrin supplement?**

Well, this is a very important question. Since lactoferrin's iron-binding ability is there to steal iron from the pathogens, it's clear that if we provide iron to the medium, and lactoferrin saturates with the

iron that we are providing externally, probably there's no more room for lactoferrin to steal iron from the pathogens. My recommendation and the recommendation of everyone is not to give lactoferrin together with iron. It's not only in several studies, especially in women during pregnancy, it has been seen that lactoferrin itself has good activity promoting iron storage and restoring anemia in sideropenic anemia during pregnancy. My strong suggestion is absolutely not to give iron to infants treated with lactoferrin, or to consider giving iron for different periods.

### **Is there any research showing the role of lactoferrin for infants who were not born early?**

Yes, there are. There is data showing that lactoferrin is able to provide the benefits of several types, also in term infants and in infants during the first year of life, in toddlers, in infants between 1 and 2 years of age. In these other populations, the effect of lactoferrin might be both for respiratory tract infection, as shown in a study from 2007, and in gastrointestinal morbidity, as shown in several studies conducted by the Peruvian group.

It's important to remember that lactoferrin is there when phlogistic reaction occurs. Lactoferrin has an anti-inflammatory activity that might justify a role also in an immunocompetent infant who actually needs management over their immunocompetence, rather than a replacement of their competency.

### **Are there drug supplements we give infants—iron and others—that could interfere with the apo- or holo-forms of lactoferrin and, in turn, the results of studies?**

Yes, this is a very interesting point. I can tell you... I can answer this question by addressing the more general issue of stability and the pharmaceutical form of lactoferrin. This is something we absolutely need. The problem is that when you rely on lactoferrin produced by manufacturers, you may

## Neonate Feeding Regimens and the Expanding Role of Lactoferrin

find different rates of saturation and different balances for apo- and holo-forms of lactoferrin. This is a problem, because these products are not comparable. I advocate for new studies and new regulations clearly re-forming [those] that are the commercial preparations of lactoferrin when this is given as a supplement. Otherwise, we cannot expect the full pattern of benefits to be displayed.

### Do you support a role of lactoferrin to protect from bronchiolitis as suggested in Ochoa's trial?

Yes. More generally speaking, there are data, at least from 3 studies so far, showing that in the first year of life supplementation on a daily basis with lactoferrin given through formula milking, which through lactoferrin is able to prevent upper and lower respiratory tract infections, and it heals bronchiolitis, too. Of course, this is not probably attributable to a specific antiviral action, nor to a specific anti-RSV [respiratory syncytial virus] action of lactoferrin, but rather to immunomodulation or mucosal secretion of lactoferrin, just like it occurs in the tears or in saliva.

### Is there any cohort of neonates who cannot tolerate lactoferrin?

No. This has not yet been described. And again, I would be very surprised should these kinds of neonates exist, because if we think once to the history of mankind and humanity, lactoferrin has been there. It's not a new compound. It's not something we created in a laboratory 20 years ago. [It is] something that existed forever; it's something that existed both in human milk and in cow milk or in goat milk since forever. So, the point is only to understand whether a little more concentrated

intake of lactoferrin might exert issues in terms of toleration. But it would be quite unlikely to expect serious issues about that.

### Does lactoferrin have any effect on growth velocity?

No. This is not occurring, and this is absolutely consistent with the characteristics of lactoferrin. Lactoferrin is not acting as a nutritional compound but rather as anti-infective or bioactive compound, better to say. This is something different.

### When can we anticipate long-term outcomes from bovine lactoferrin and low-birth-weight formula?

Well, long-term outcomes might be a little difficult to target and to show because the only long-term outcome I can envision is a composite quality of the neurodevelopmental, neurocognitive patterns attributable to the fact that the infant was not affected by sepsis during the NICU stay. It has been shown that infections occurring in the NICU stay in very low-birth-weight infants, impact the quality of neurodevelopmental patterns, determining impairments both in neurological and in cognitive areas. However, it has not yet been shown that by preventing sepsis, there can be any improvement. This is a more complex issue, and I'm sincerely a little less skeptical on the possibility to show long-term outcomes significantly affected by a single compound given in the very first weeks of life. This is true for lactoferrin, but for whichever compound.

### Abbreviations

<b>BPD</b>	Bronchopulmonary disease	<b>MERS</b>	Middle East Respiratory Syndrome
<b>CMV</b>	Cytomegalovirus	<b>NEC</b>	Necrotizing enterocolitis
<b>LOS</b>	Late-onset sepsis	<b>NICU</b>	Neonatal Intensive Care Unit
<b>LPS</b>	Lipopolysaccharide	<b>ROP</b>	Retinopathy of prematurity

## Neonate Feeding Regimens and the Expanding Role of Lactoferrin

<b>LTA</b>	Lipoteichoic acid	<b>RSV</b>	Respiratory syncytial virus
------------	-------------------	------------	-----------------------------

### References

1. Furman L, Taylor G, Minich N, Hack M. The effect of maternal milk on neonatal morbidity of very low-birth-weight infants. *Arch Pediatr Adolesc Med.* 2003;157(1):66-71.
2. Hylander MA, Strobino DM, Pezzullo JC, Dhanireddy R. Association of human milk feedings with a reduction in retinopathy of prematurity among very low birthweight infants. *J Perinatol.* 2001;21(6):356-62.
3. Manzoni P, Meyer M, Stolfi I, et al. Bovine lactoferrin supplementation for prevention of necrotizing enterocolitis in very-low-birth-weight neonates: a randomized clinical trial. *Early Hum Dev.* 2014;90 Suppl 1:S60-5. doi:10.1016/S0378-3782(14)70020-9.
4. Lucas A, Cole TJ. Breast milk and neonatal necrotising enterocolitis. *Lancet.* 1990;336(8730):1519-23.
5. Corpeleijn WE, de Waard M, Christmann V, et al. Effect of donor milk on severe infections and mortality in very low-birth-weight infants: The early nutrition study randomized clinical trial. *JAMA Pediatr.* 2016;170(7):654-61. doi:10.1001/jamapediatrics.2016.0183.
6. Hylander MA, Strobino DM, Dhanireddy R. Human milk feedings and infection among very low birth weight infants. *Pediatrics.* 1998;102(3):E38.
7. Schanler RJ, Shulman RJ, Lau C. Feeding strategies for premature infants: beneficial outcomes of feeding fortified human milk versus preterm formula. *Pediatrics.* 1999;103(6 Pt 1):1150-7.
8. Jiang R, Du X, Lönnerdal B. Comparison of bioactivities of talactoferrin and lactoferrins from human and bovine milk. *J Pediatr Gastroenterol Nutr.* 2014;59(5):642-52. doi:10.1097/MPG.0000000000000481.
9. Telang S. Lactoferrin: A critical player in neonatal host defense. *Nutrients.* 2018;10(9). pii: E1228. doi:10.3390/nu10091228.
10. Kanwar JR, Roy K, Patel Y, et al. Multifunctional iron bound lactoferrin and nanomedicinal approaches to enhance its bioactive functions. *Molecules.* 2015;20:9703-9731.
11. deWit JN. Marschall Rhône-Poulenc Award Lecture. Nutritional and functional characteristics of whey proteins in food products. *J Dairy Sci.* 1998;81:597-608.
12. Reitamo S, Konttinen YT, Segerberg-Konttinen M. Distribution of lactoferrin in human salivary glands. *Histochemistry.* 1980;66:285-291.
13. McClellan KA. Mucosal defense of the outer eye. *Surv Ophthalmol.* 1997;42:233-246.
14. Spik G, Brunet B, Mazurier-Dehaine C, Fontaine G, Montreuil J. Characterization and properties of the human and bovine lactotransferrins extracted from the feces of newborn infants. *Acta Paediatr Scand.* 1982;71:979-985.
15. Manzoni P, Rinaldi M, Cattani S, et al. Bovine lactoferrin supplementation for prevention of late-onset sepsis in very low-birth-weight neonates: a randomized trial. *JAMA.* 2009;302:1421-1428.
16. Davidson LA, Lönnerdal B. Persistence of human milk proteins in the breast-fed infant. *Acta Paediatr Scand.* 1987;76(5):733-40.
17. Berkestedt I, Herwald H, Ljunggren L, Nelson A, Bodelsson M. Elevated plasma levels of antimicrobial polypeptides in patients with severe sepsis. *J Innate Immun.* 2010;2(5):478-82. doi:10.1159/000317036.
18. Albenzio M, Santillo A, Stolfi I, et al. Lactoferrin levels in human milk after preterm and term delivery. *Am J Perinatol.* 2016;33(11):1085-1089. doi:10.1055/s-0036-1586105.

## Neonate Feeding Regimens and the Expanding Role of Lactoferrin

19. Reznikov EA, Comstock SS, Yi C, Contractor N, Donovan SM. Dietary bovine lactoferrin increases intestinal cell proliferation in neonatal piglets. *J Nutr.* 2014;144(9):1401-8. doi:10.3945/jn.114.196568.
20. Li Q, Hu W, Zhao J, Wang J, et al. Supplementation transgenic cow's milk containing recombinant human lactoferrin enhances systematic and intestinal immune responses in piglets. *Mol Biol Rep.* 2014;41(4):2119-28. doi:10.1007/s11033-014-3061-5.
21. Ochoa TJ, Cleary TG. Effect of lactoferrin on enteric pathogens. *Biochimie.* 2009;91(1):30-4. doi:10.1016/j.biochi.2008.04.006.
22. Teraguchi S, Shin K, Ogata T, et al. Orally administered bovine lactoferrin inhibits bacterial translocation in mice fed bovine milk. *Appl Environ Microbiol.* 1995;61(11):4131-4.
23. Shan T, Wang Y, Wang Y, Liu J, Xu Z. Effect of dietary lactoferrin on the immune functions and serum iron level of weanling piglets. *J Anim Sci.* 2007;85(9):2140-6.
24. Mastromarino P, Capobianco D, Campagna G, et al. Correlation between lactoferrin and beneficial microbiota in breast milk and infant's feces. *Biometals.* 2014;27(5):1077-86. doi:10.1007/s10534-014-9762-3.
25. Lönnerdal B, Jiang R, Du X. Bovine lactoferrin can be taken up by the human intestinal lactoferrin receptor and exert bioactivities. *J Pediatr Gastroenterol Nutr.* 2011;53(6):606-14. doi:10.1097/MPG.0b013e318230a419.
26. Ochoa TJ, Chea-Woo E, Baiocchi N, et al. Randomized double-blind controlled trial of bovine lactoferrin for prevention of diarrhea in children. *J Pediatr.* 2013;162(2):349-56. doi:10.1016/j.jpeds.2012.07.043.
27. Ochoa TJ, Zegarra J, Cam L, et al. Multicenter, RCT of oral bovine lactoferrin for prevention of sepsis in neonates at birth. *Pediatr Infect Dis J.* 2015;34(6):571-6.
28. Akin IM, Atasay B, Dogu F, et al. Oral lactoferrin to prevent nosocomial sepsis and necrotizing enterocolitis of premature neonates and effect on T-regulatory cells. *Am J Perinatol.* 2014;31(12):1111-20. doi:10.1055/s-0034-1371704.
29. Kaur G, Gathwala G. Efficacy of bovine lactoferrin supplementation in preventing late-onset sepsis in low birth weight neonates: A randomized placebo-controlled clinical trial. *J Trop Pediatr.* 2015;61(5):370-6. doi:10.1093/tropej/fmv044.
30. Barrington KJ, Assaad MA, Janvier A. The Lacuna Trial: a double-blind randomized controlled pilot trial of lactoferrin supplementation in the very preterm infant. *J Perinatol.* 2016;36(8):666-9. doi:10.1038/jp.2016.24.
31. Pammi M, Suresh G. Enteral lactoferrin supplementation for prevention of sepsis and necrotizing enterocolitis in preterm infants. *Cochrane Database Syst Rev.* 2017;6:CD007137. doi:10.1002/14651858.CD007137.pub5.
32. Weimer KED, Roark H, Fisher K, et al. Breast milk and saliva lactoferrin levels and postnatal cytomegalovirus infection. *Am J Perinatol.* 2020;10.1055/s-0040-1701609.
33. ELFIN trial investigators group. Enteral lactoferrin supplementation for very preterm infants: a randomised placebo-controlled trial. *Lancet.* 2019 Feb 2;393(10170):423-433. doi:10.1016/S0140-6736(18)32221-9.
34. Griffiths J, Jenkins P, Vargova M, et al. Enteral lactoferrin to prevent infection for very preterm infants: the ELFIN RCT. *Health Technol Assess.* 2018;22(74):1-60. doi:10.3310/hta22740.

## Neonate Feeding Regimens and the Expanding Role of Lactoferrin

35. Martin A, Ghadge A, Manzoni P, et al. Protocol for the Lactoferrin Infant Feeding Trial (LIFT): a randomised trial of adding lactoferrin to the feeds of very-low birthweight babies prior to hospital discharge. *BMJ Open*. 2018;8(10):e023044. doi:10.1136/bmjopen-2018-023044.
36. Trend S, Strunk T, Hibbert J, et al. Antimicrobial protein and peptide concentrations and activity in human breast milk consumed by preterm infants at risk of late-onset neonatal sepsis. *PLoS ONE*. 2015; 10(2): e0117038. doi.org/10.1371/journal.pone.0117038.
37. Manzoni P, Militello MA, Rizzollo S, et al. Is lactoferrin more effective in reducing late-onset sepsis in Preterm Neonates Fed Formula Than in Those Receiving Mother's Own Milk? Secondary Analyses of Two Multicenter Randomized Controlled Trials. *Am J Perinatol*. 2019;36(S 02):S120-S125. doi:10.1055/s-0039-1691807.
38. Ochoa TJ, Mendoza K, Carcamo C, et al. Is Mother's Own Milk Lactoferrin Intake Associated with Reduced Neonatal Sepsis, Necrotizing Enterocolitis, and Death? *Neonatology*. 2020;1-8. doi:10.1159/000505663.
39. Rollo DE, Radmacher PG, Turcu RM, Myers SR, Adamkin DH. Stability of lactoferrin in stored human milk. *J Perinatol*. 2014;34(4):284-6. doi:10.1038/jp.2014.3.
40. Woodman T, Strunk T, Patole S, Hartmann B, Simmer K, Currie A. Effects of lactoferrin on neonatal pathogens and *Bifidobacterium breve* in human breast milk. *PLoS One*. 2018;13(8):e0201819. doi:10.1371/journal.pone.0201819.
41. Tarnow-Mordi WO, Abdel-Latif ME, Martin A, et al. The effect of lactoferrin supplementation on death or major morbidity in very low birthweight infants (LIFT): a multicentre, double-blind, randomised controlled trial. *Lancet Child Adolesc Health*. 2020;4(6):444-454. doi:10.1016/S2352-4642(20)30093-6.