Human Milk: More than just nutrition for Preterm Infants

The Science behind MBM for preterm babies

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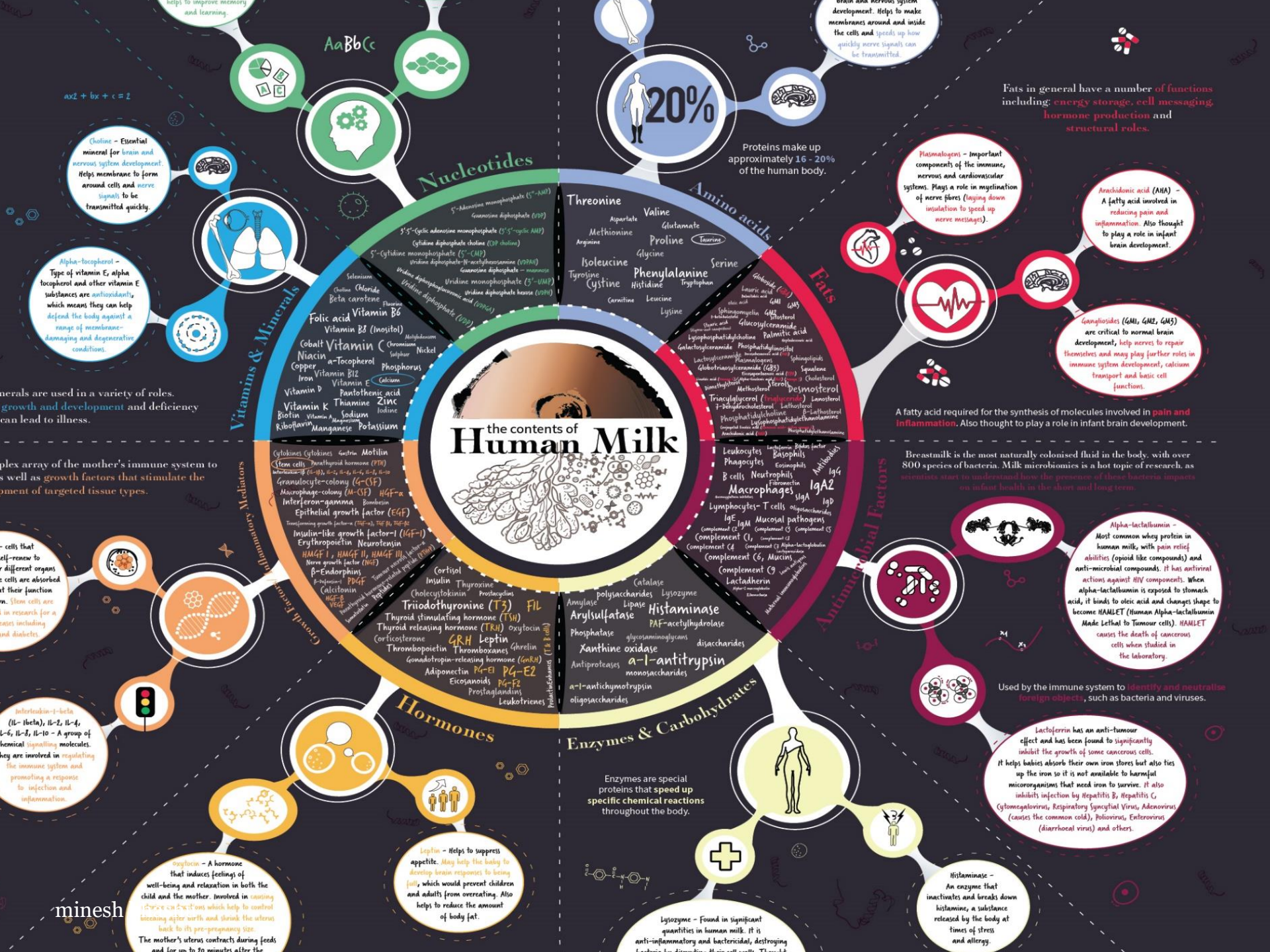
the contents of
Human Milk
Nucleotides
Amino acids
Fats
Vitamins & Minerals
Inflammatory Mediators
Antimicrobial Factors
Hormones
Enzymes & Carbohydrates
Proteins make up approximately 16 - 20% of the human body.
Fats in general have a number of functions including: energy storage, cell messaging, hormone production and structural roles.
Breastmilk is the most naturally colonised fluid in the body. with over 800 species of bacteria. Milk microbiomes is a hot topic of research, as scientists start to understand how the presence of these bacteria impacts on infant health in the short and long term.
Enzymes are special proteins that speed up specific chemical reactions throughout the body.
Choline – Essential mineral for brain and nervous system development. Helps membrane to form around cells and nerve signals to be transmitted quickly.
Alpha-tocopherol – Type of vitamin E, alpha tocopherol and other vitamin E substances are antioxidants, which means they can help defend the body against a range of membrane-damaging and degenerative conditions.
Plasmalogens – Important components of the immune, nervous and cardiovascular systems. Plays a role in myelination of nerve fibres (laying down insulation to speed up nerve messages).
Arachidonic acid (AHA) – A fatty acid involved in reducing pain and inflammation. Also thought to play a role in infant brain development.
Gangliosides (GM1, GM2, GM3) are critical to normal brain development, help nerves to repair themselves and may play further roles in immune system development, calcium transport and basic cell functions.
A fatty acid required for the synthesis of molecules involved in pain and inflammation. Also thought to play a role in infant brain development.
Alpha-lactalbumin – Most common whey protein in human milk, with pain relief abilities (opioid like compounds) and anti-microbial compounds. It has antiviral actions against HIV components. When alpha-lactalbumin is exposed to stomach acid, it binds to oleic acid and changes shape to become HAMLET (Human Alpha-lactalbumin Made Lethal to Tumour cells). HAMLET causes the death of cancerous cells when studied in the laboratory.
Used by the immune system to identify and neutralise foreign objects, such as bacteria and viruses.
Lactoferrin has an anti-tumour effect and has been found to significantly inhibit the growth of some cancerous cells. It helps babies absorb their own iron stores but also ties up the iron so it is not available to harmful microorganisms that need iron to survive. It also inhibits infection by Hepatitis B, Hepatitis C, Cytomegalovirus, Respiratory Syncytial Virus, Adenovirus (causes the common cold), Poliovirus, Enterovirus (diarrhoeal virus) and others.
Histaminase – An enzyme that inactivates and breaks down histamine, a substance released by the body at times of stress and allergy.
Lysozyme – Found in significant quantities in human milk. It is anti-inflammatory and bactericidal, destroying
Leptin – Helps to suppress appetite. May help the baby to develop brain responses to being full, which would prevent children and adults from overeating. Also helps to reduce the amount of body fat.
Oxytocin – A hormone that induces feelings of well-being and relaxation in both the child and the mother. Involved in causing uterine contractions which help to control bleeding after birth and shrink the uterus back to its pre-pregnancy size. The mother's uterus contracts during feeds and for up to 20 minutes after the
Interleukin-1-beta (IL-1beta), IL-2, IL-4, IL-6, IL-8, IL-10 – A group of chemical signalling molecules. They are involved in regulating the immune system and promoting a response to infection and inflammation.
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Tailor-made
antibodies
Your body identifies
bacteria and viruses
found in your baby's body
and environment. You
then produce antibodies
specifically tailored to
those infections, and
deliver them to your
child through
your milk.
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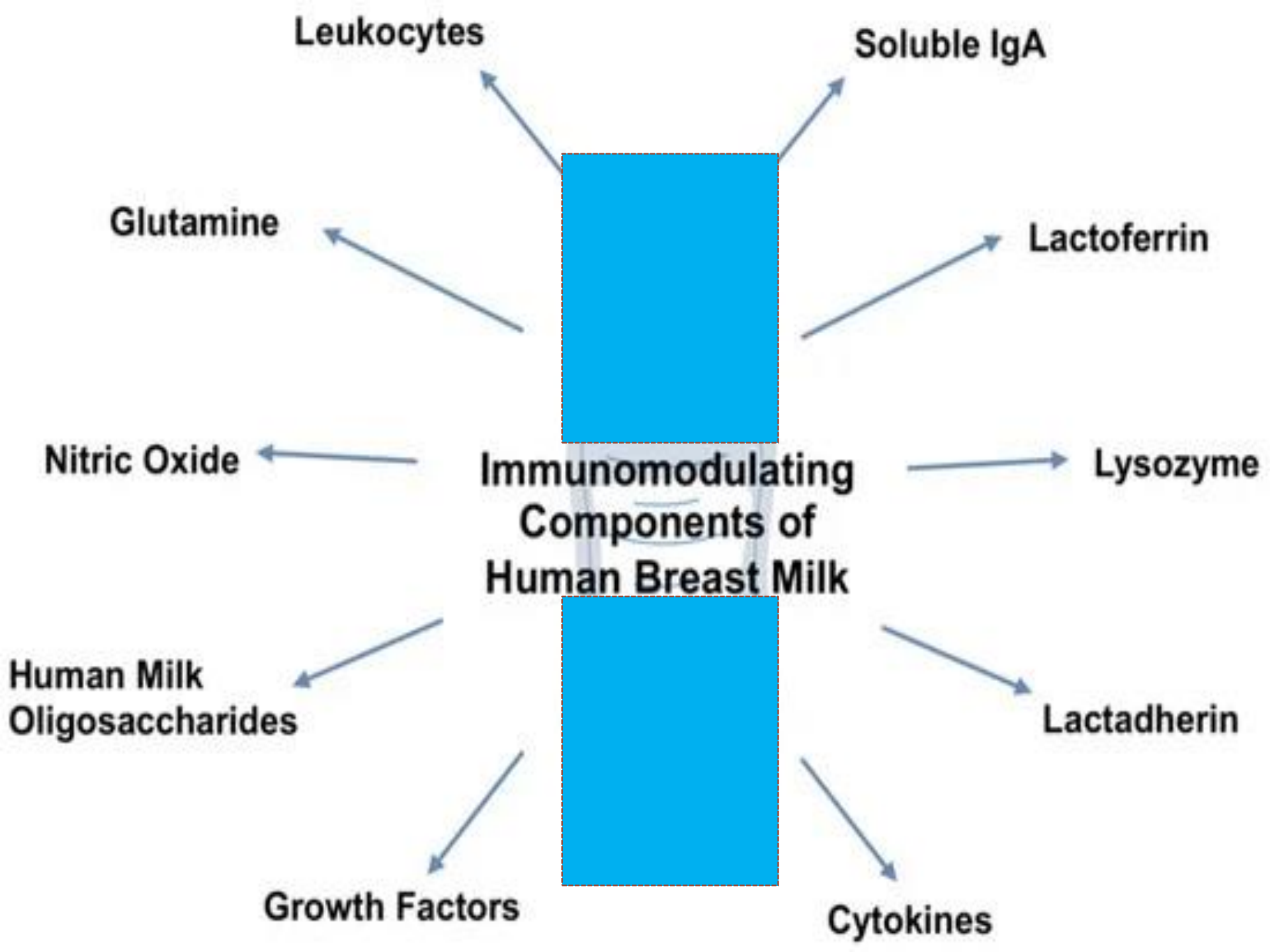
Leukocytes
Soluble IgA
Glutamine
Lactoferrin
Nitric Oxide
Lysozyme
Immunomodulating Components of Human Breast Milk
Human Milk Oligosaccharides
Lactadherin
Growth Factors
Cytokines

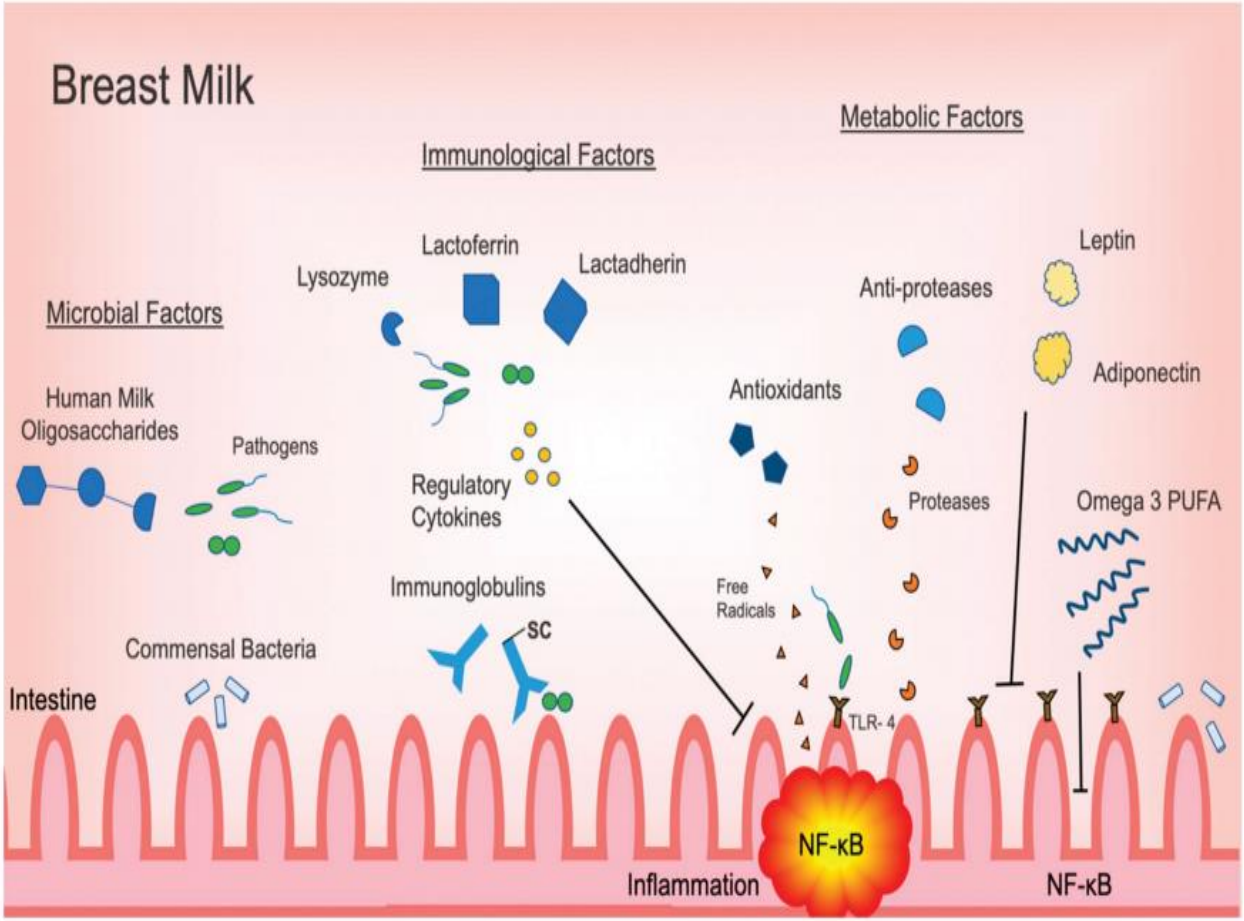

Figure 1. Summary of microbiologic, immunological and metabolic factors in breast milk with effects on regulating intestinal inflammation. Abbreviations: secretory component (SC); Toll-like receptor 4 (TLR4); nuclear factor kappa B (NF-κB); polyunsaturated fatty acid (PUFA).

Human Milk Oligosaccharides

- HMOs are not digestible by host glycosidases and yet are produced in large amounts with highly variable structures by the mother. They participate in the inhibition of bacteria, viruses or even parasites

- HMOs appear to have three important functions:
  Prebiotic : stimulation of commensal bacteria containing the bacterial glycosidases to deconstruct and consume the HMOs
  Decoy : structural similarity to the glycans on enterocytes allows HMOs to competitively bind to pathogens
  Provision of fucose and sialic acid : important in host defense and neurodevelopment respectively

- Glycosaminoglycans (GAG) appear to act as decoys providing binding sites for pathogenic bacteria to prevent adherence to the enterocyte. Premature milk is rich in GAG

- Certain human oligosaccharides interfere *in vitro* with cell-cell interactions mediated by selectins

Boehm G, Moro G. Structural and functional aspects of human milk oligosaccharides. J Nutr. (2006) 136:2127–30.

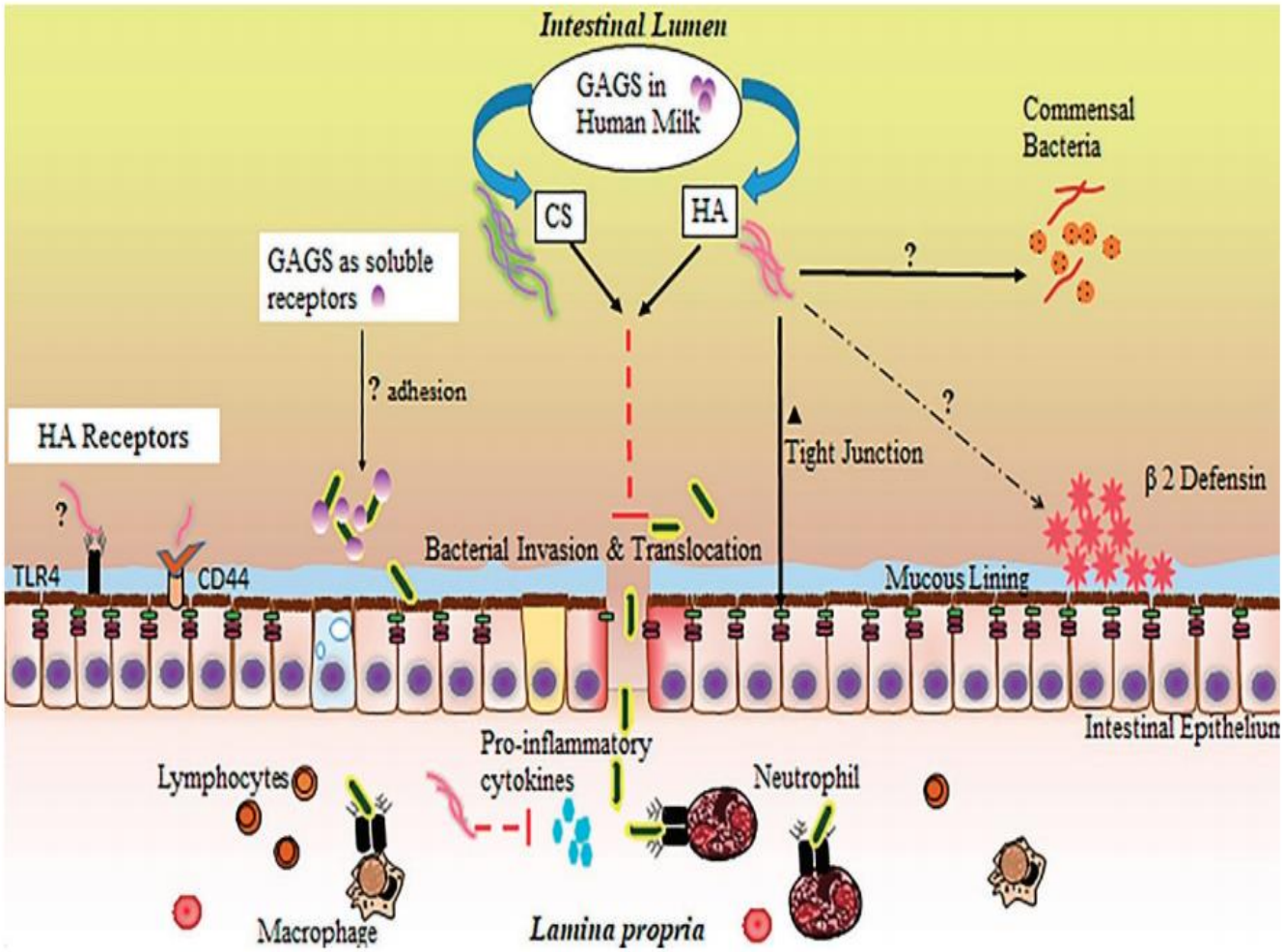

Figure 2. Schematic of potential glycosaminoglycan mechanisms of protection in necrotizing enterocolitis (NEC). CS: chondroitin sulfate; HA: hyaluronic acid; GAGS: glycosaminoglycans.

Breast Milk Lipids Enhance Neonatal Intestinal Development and Protect against Injury

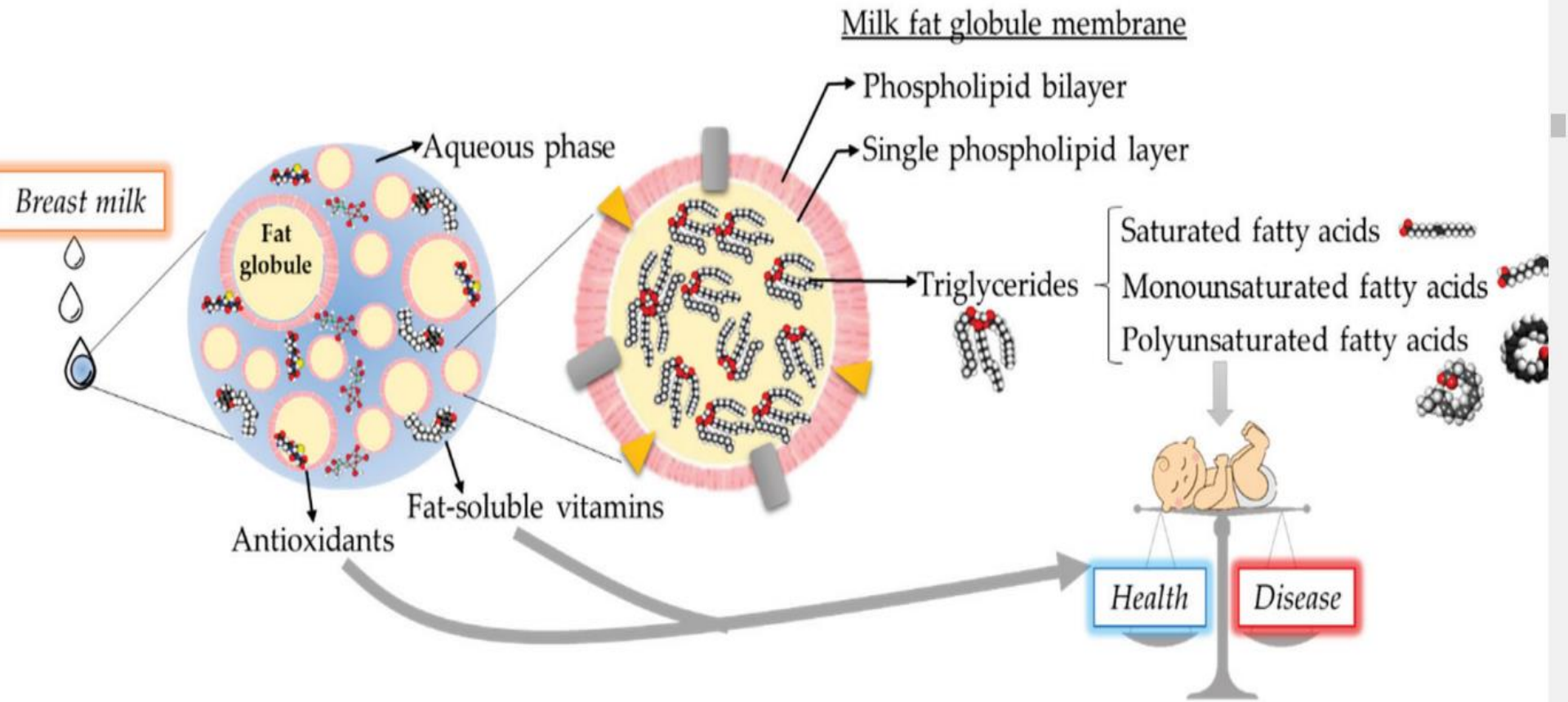

Figure 1. Breast milk fat components and relationship with neonatal health-disease balance. Scheme of fat globule illustrating of the core-shell structure.

Table 1. Bioactive Components in Breast Milk and Roles in Attenuating Intestinal Inflammation.

Bioactive Components in Breast Milk	Role in Intestinal Inflammation Regulation or Prevention	Effect	References
Microbial or microbial modulating factors			
Lactobacillus spp.	-Inhibit NF-κB pathway -decrease pro-inflammatory cytokines, TNF-α, IL-6 -reverse intestinal dysbiosis in bacterial intestinal infection	-decrease inflammatory response -Restore intestinal microbiome homeostasis	[41–44]
Bifidobacterium spp	-increase SCFA production -Decrease pro-inflammatory CK release (IL-6, CXCL-1, TNF-α, IL-23) and iNOS	-promote anti-inflammatory commensal bacteria proliferation -decrease inflammatory response	[45–48]
Human Milk Oligosaccharides	-regulate commensal bacteria -act as decoy receptors for pathogens -modulate immune signaling pathways, TLR3, TLR5, PAMP	-promote healthy intestinal microbiota with anti-inflammatory properties -prevent and decrease inflammatory response	[32,49–53]
Immunological factors			
Secretory IgA	-bind to pathogens and commensal bacteria	-prevention of typical inflammatory response, or immune exclusion -influence intestinal microbiome	[29,54]
IgG	-opsonization, agglutination of bacteria	-prevention of typical acute inflammatory response	[52,55–57]
IL-10	-inhibit Th1, NK cell, macrophages	-provide immunoregulation and prevent inflammation	[18,58–61]
TGF- β	-inhibit differentiation of naïve T cells into Th1, Th2 cells -Stabilize FOXP3 expression	-decrease pro-inflammatory cytokine expression and inflammation -inhibit immune response and decrease inflammation	[18,60,62–64]
ILRA-1 *TNFR I and II* *soluble TLR2*	-compete with IL-1 receptor for IL-1 -directly bind, inhibit TNF- α -decoy receptor to inhibit IL-8, TNF	-prevent pro-inflammatory cytokine expression and inflammation	[52,60,65–67]
EGF *HB-EGF* *VEGF*	-upregulate IL-10 expression -bind to bacteria -stimulate angiogenesis-	-decrease pro-inflammatory cytokine expression -prevent intestinal edema	[68–74]
Lactoferrin	-direct cytotoxicity on pathogens by forming lactoferricin -inhibit IL-1, IL-6, TNF-α, IL-8 -promote growth of probiotics	-eliminate trigger for acute inflammatory response -decrease pro-inflammatory cytokine expression and inflammation -regulate intestinal microbiome	[18,75–77]
Lactadherin	-enhance phagocytosis of apoptotic cells -blocks NF-κB pathway via TLR4 inhibition -promote healing during intestinal inflammation	-eliminate trigger for acute inflammatory response -prevent pro-inflammatory signaling and decreasing inflammatory response -limit degree of intestinal inflammation	[78,79]
Lysozyme	-degrades GP bacteria outer wall -kill GN bacteria with lactoferrin	-eliminate trigger for acute inflammatory response	[18,80]
Metabolic factors			
Adiponectin	-suppress mature macrophage function	-decrease inflammatory response	[52,81]
Leptin	-stimulates T cells -influence polarization of macrophages to anti-inflammatory phenotype	-regulate immune response and prevent inflammation	[81–84]
Omega 3 PUFA	-decrease NF- κB, bind to PPAR-γ -increase proliferation of *Lactobacillus* and *Bifidobacterium* -change membrane PL concentration -inhibit leukocyte migration	-downregulate pro-inflammatory genes -promote anti-inflammatory commensal bacteria proliferation -decrease degree of inflammatory response	[13,85–90]
Antioxidants	-scavenge free radicals	-prevent injury and inflammation	[60]
Anti-proteases	-metabolize proteases produced by inflammatory cells	-prevent excessive inflammatory response	[60]

Abbreviations: Nuclear factor kappa B (NF-κB); tumor necrosis factor alpha (TNF-α); interleukin (IL); short chain fatty acid (SCFA); cytokine (CK); chemokine-1 (CXCL-1); inducible nitric oxide synthase (iNOS); Toll-like receptor (TLR); pathogen-associated molecular pattern (PAMP); Immunoglobulin (Ig); T-helper (Th) cell; natural killer cell (NK); transformation growth factor beta (TGF-β); forkhead box P3 (FOXP3); interleukin receptor antagonist 1 (ILRA-1); tumor necrosis factor receptor (TNFR); epidermal growth factor (EGF); heparin-binding epidermal growth factor (HB-EGF)-like growth factor; vascular endothelial growth factor (VEGF); gram positive (GP); gram negative (GN); polyunsaturated fatty acid (PUFA); peroxisome proliferator-activated receptor gamma (PPAR-γ); phospholipid (PL).

3.2. Cytokines

Preterm infants, when compared with their term counterparts, exhibit immune immaturity, which includes lower production of cytokines and other immunological proteins during challenge with an inflammatory insult [59]. The presence of cytokines in breast milk provides passive protection and immune modulation in the infant recipient and results in absorption into the systemic circulation. In particular, these cytokines include IL-1, IL-2, IL-6, IL-8, IL-10, interferon (IFN)-γ, and TNF-α (Table 1). Breast milk produced by mothers of full-term infants contains high levels of IL-2, IL-8, and IL-10, with levels decreasing drastically by day 21 of lactation. In contrast, mothers of preterm infants have significantly lower levels of cytokines in the colostrum when compared to mothers of full-term infants [63].

Table 1. Cytokines present in human breast milk and physiologic relevance to the infant.

Cytokine	Composition in Human Milk and Significance	References
Interleukin (IL)-1	- Human milk IL-1β attenuates the activation of pro-inflammatory IL-8 and suppresses pro-inflammatory responses of nuclear factor kappa beta (NF-kB) signaling.	[53,64]
IL-2	- Highest in concentration in colostrum and reduced in later stages of lactation. - Recruits T cells to stimulate an antigen-specific immune response.	[63,65,66]
IL-6	- Detected in higher levels in term breast milk. - Pro-inflammatory properties and is present in the acute phase of infection. - Colostrum may contain anti-IL-6 antibodies that cause decreased immunoglobulin A (IgA) production by breast milk leukocytes.	[63,67,68]
IL-8	- Decreased levels of detection in later stages of lactation. - Provides chemotactic response of neutrophils. - Recombinant IL-8 may improve the viability of intestinal cells when exposed to injury.	[63,69,70]
IL-10	- Maintains anti-inflammatory mechanisms involving limiting the T_h1 response, inhibiting production of inflammatory cytokines, and promoting immunoglobulin synthesis.	[71–75]
IFN-γ	- Detected in decreasing levels with later stages of lactation. - Increases activation of intestinal macrophages and is present in higher concentrations in the ileum of infants with necrotizing enterocolitis (NEC). - Pro-inflammatory mechanism of action may provide an infant with defense against inflammation and infection.	[76–79]
TNF-α	- Detected in decreased levels in colostrum of preterm milk. - Present in breast milk in association with its soluble receptor, reducing its pro-inflammatory activity.	[63,80,81]

Factors in Human Milk Reported to Enhance Protection to NEC

Breast Milk Components
Oral IgA-IgG
PAF acetylhydrolase
Long-chain polyunsaturated fatty acids
Erythropoeitin
Arginine and glutamine supplementation
Epidermal growth factor
Probiotics
Lactoferrin
Oligosaccharides

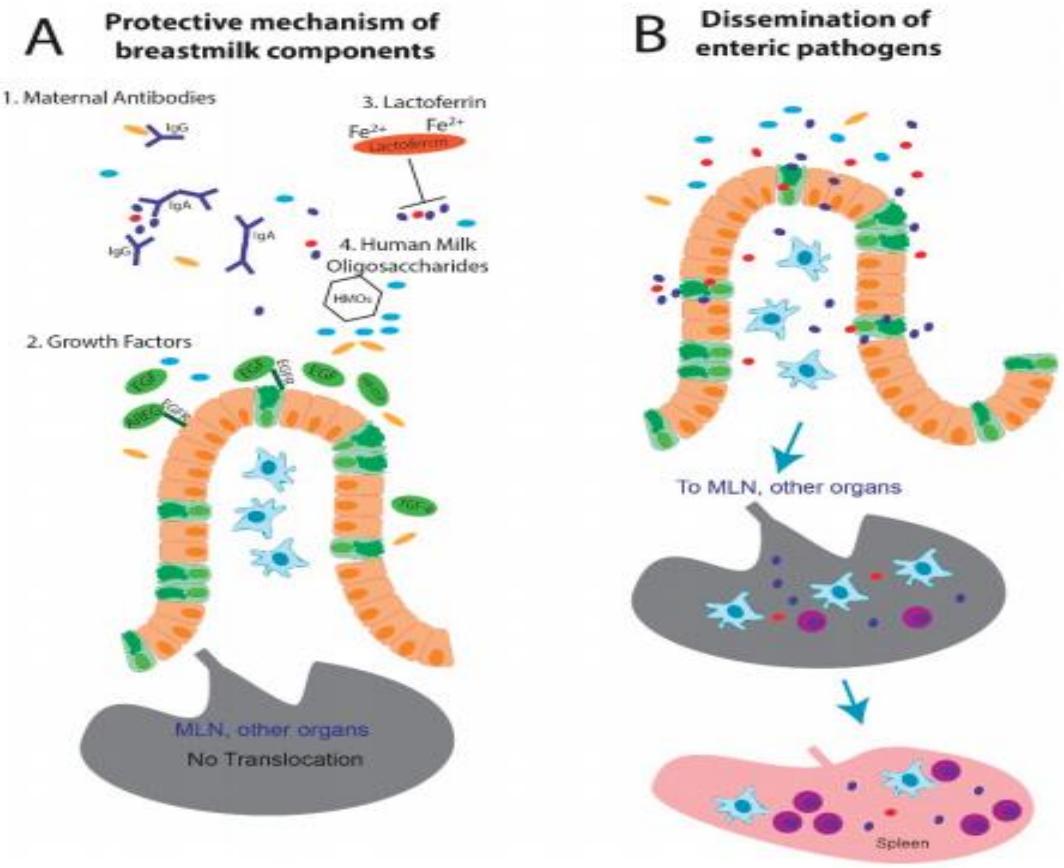

Figure 1. Maternal protection from enteric pathogens. (**A**) Components in breastmilk can limit enteric pathogen dissemination. (1) Maternal antibodies (IgG, IgA) can bind bacteria and directly inhibit pathogen adherence and invasion [62]. (2) Growth factors [epidermal growth factor (EGF), amphiregulin (AREG), heparin-binding epidermal growth factor-like factor (HB-EGF), and tumor-growth factor-alpha (TGF-α)] bind the epidermal growth factor receptor (EGFR) on epithelial cells to promote barrier function by cell proliferation and growth [63], and by limiting translocation via goblet cells [64]. (3) Lactoferrin sequesters iron which limits pathogen growth [65]. (4) Human milk oligosaccharides (HMOs) promote the development of the intestinal microbiota [66], which can offer colonization resistance to enteric pathogens [30]. (**B**) In the absence of these factors, pathogens can colonize the intestine lumen, cross the epithelium potentially through goblet cells [64], and disseminate to organs through the system, including the mesenteric lymph node (MLN) and spleen, resulting in late-onset sepsis (LOS).

A Systematic Review and Meta-Analysis of Human Milk Feeding and Morbidity in Very Low Birth Weight Infants; Jacqueline Miller et al

Nutrients **2018**, 10, 707; doi:10.3390/nu10060707

- HM provided a clear protective effect against NEC, with an approximate 4% reduction in incidence
- HM also provided a possible reduction in LOS, severe ROP and severe NEC
- Particularly for NEC, any volume of HM is better than EPTF, and the higher the dose the greater the protection

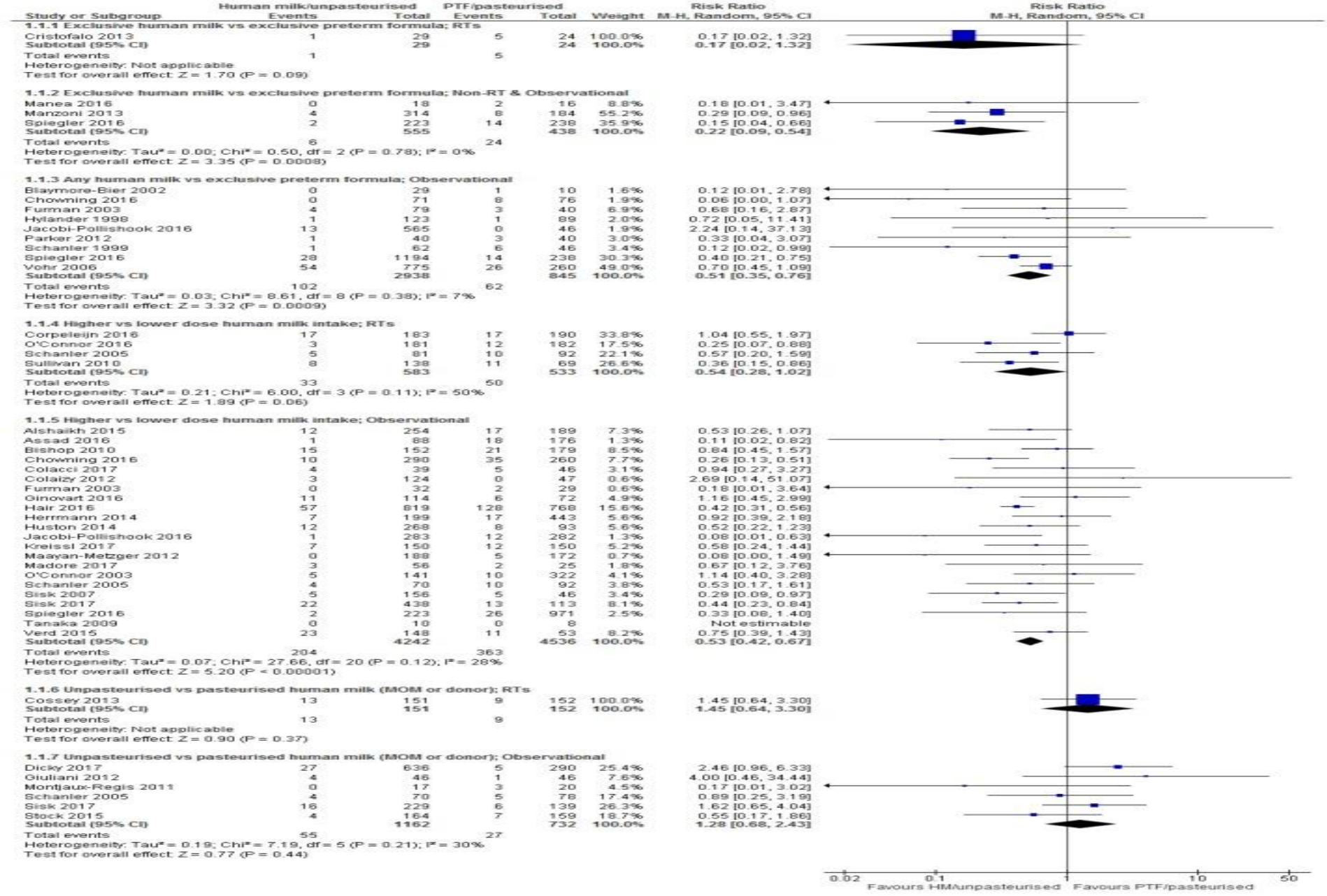

Figure 3. Forest plot of relative risk for the association between human milk use and necrotising enterocolitis. (doi: 10.3390/nu10060707)

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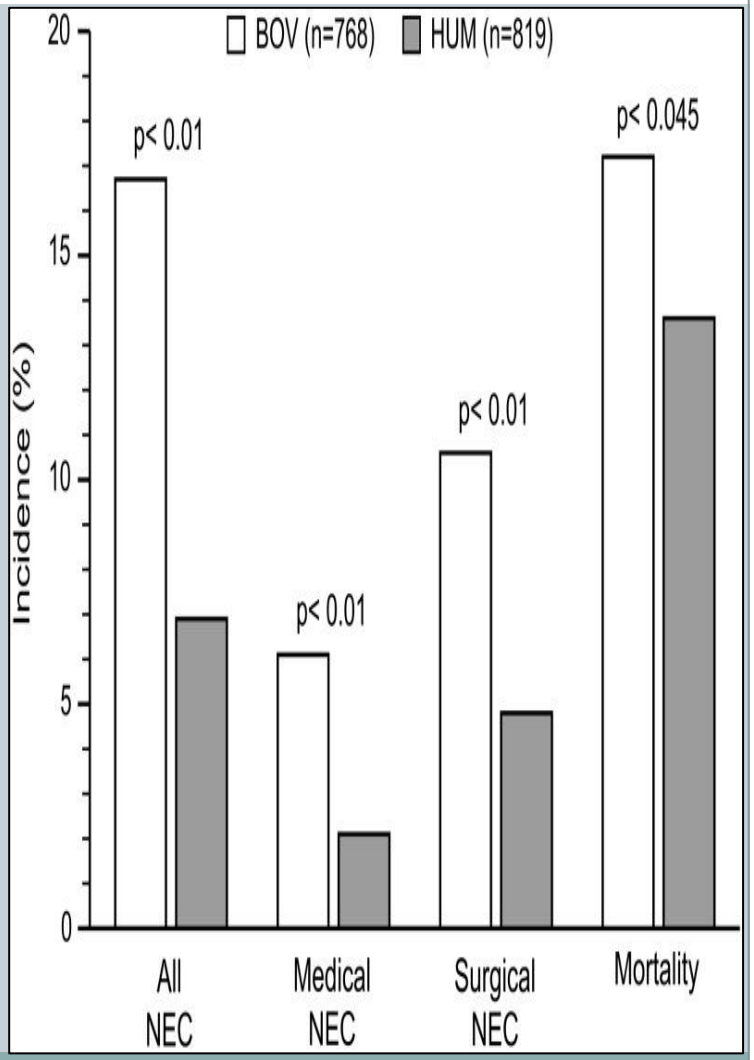
Miller et al showed in a meta-analysis that MOM provided a clear protective effect against NEC, with an approximate 4% reduction in incidence

NEC Incidence Is Significantly Lower in Human Milk-Fed Infants Compared to Formula-Fed Infants

- Several studies have shown that NEC incidence is 6- to 10-fold lower in human milk-fed infants compared to formula-fed infants
- Is it because:
  a. Components in infant formula trigger NEC
  b. Components in human milk protect from NEC, or
  c. Is it a combination of both is responsible for the gap in NEC incidence between human milk-fed and formula-fed infants.
- A significant number of infants still develop NEC although they exclusively receive human milk and are not exposed to infant formula.
  - These observations speak against the notion that components in infant formula trigger NEC and support the idea that bioactive components in human milk protect from NEC.
  - Interpersonal variation in human milk composition may explain why some infants still develop NEC despite receiving human milk.



Lucas A, Cole TJ. Breast milk and neonatal necrotising enterocolitis. *Lancet* (1990) 336:1519–23.

Meinzen-Derr J, Poindexter B, Wrage L, Morrow AL, Stoll B, Donovan EF. Role of human milk in extremely low birth weight infants' risk of necrotizing enterocolitis or death. *J Perinatol.* (2009) 29:57–62. doi: 10.1038/jp.2008.117

The Impact of Human Milk on Necrotizing Enterocolitis: A systematic Review and Meta-Analysis

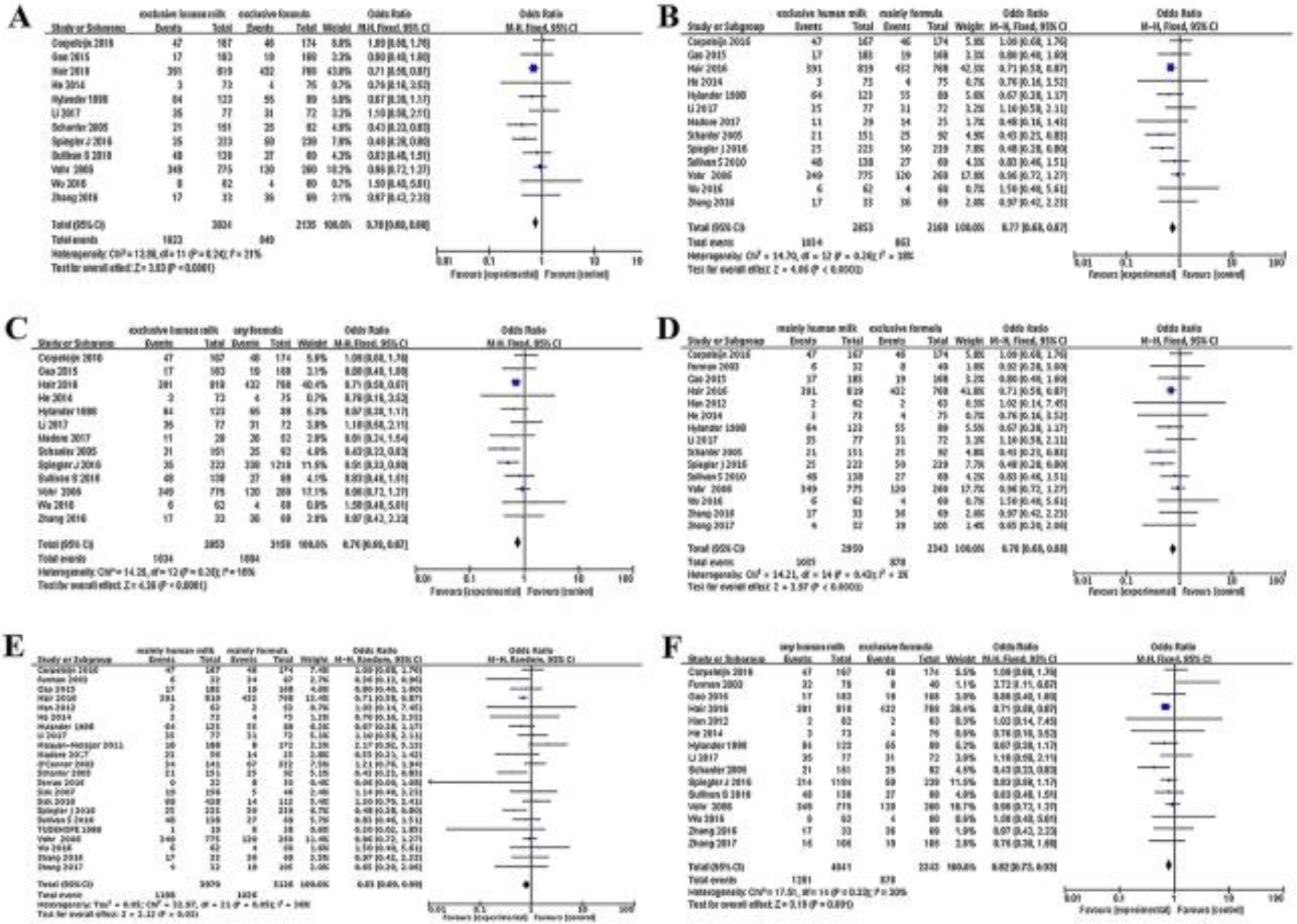
Emma Altobelli, Paolo Matteo Angeletti , Alberto Verrotti and Reimondo Petrocelli Department of Life, Health and Environmental Sciences, University of L'Aquila, 67100 L'Aquila, Italy; Published: 6 May 2020

- Results. Thirty-two papers were included in meta-analysis: 6 randomized controlled trials (RCTs) and 26 observational studies (OS)
- RCTs meta-analysis indicates a risk reduction of NEC using human milk respect to formula: Relative risk (RR) = 0.62 (0.42–0.93)
- Seven OS compared quantities high consumption of human milk against low consumption showing a risk reduction of NEC:RR = 0.51 (0.31–0.85)
- 3 OS that evaluated human milk versus mixed feeding showing that human milk has a protective role on the development of NEC:RR = 0.74 (0.63–0.91)

MBM & BPD

- Systematic review investigating the effects of MOM on BPD confirms the beneficial effects of mother's milk, at least when used as an exclusive diet

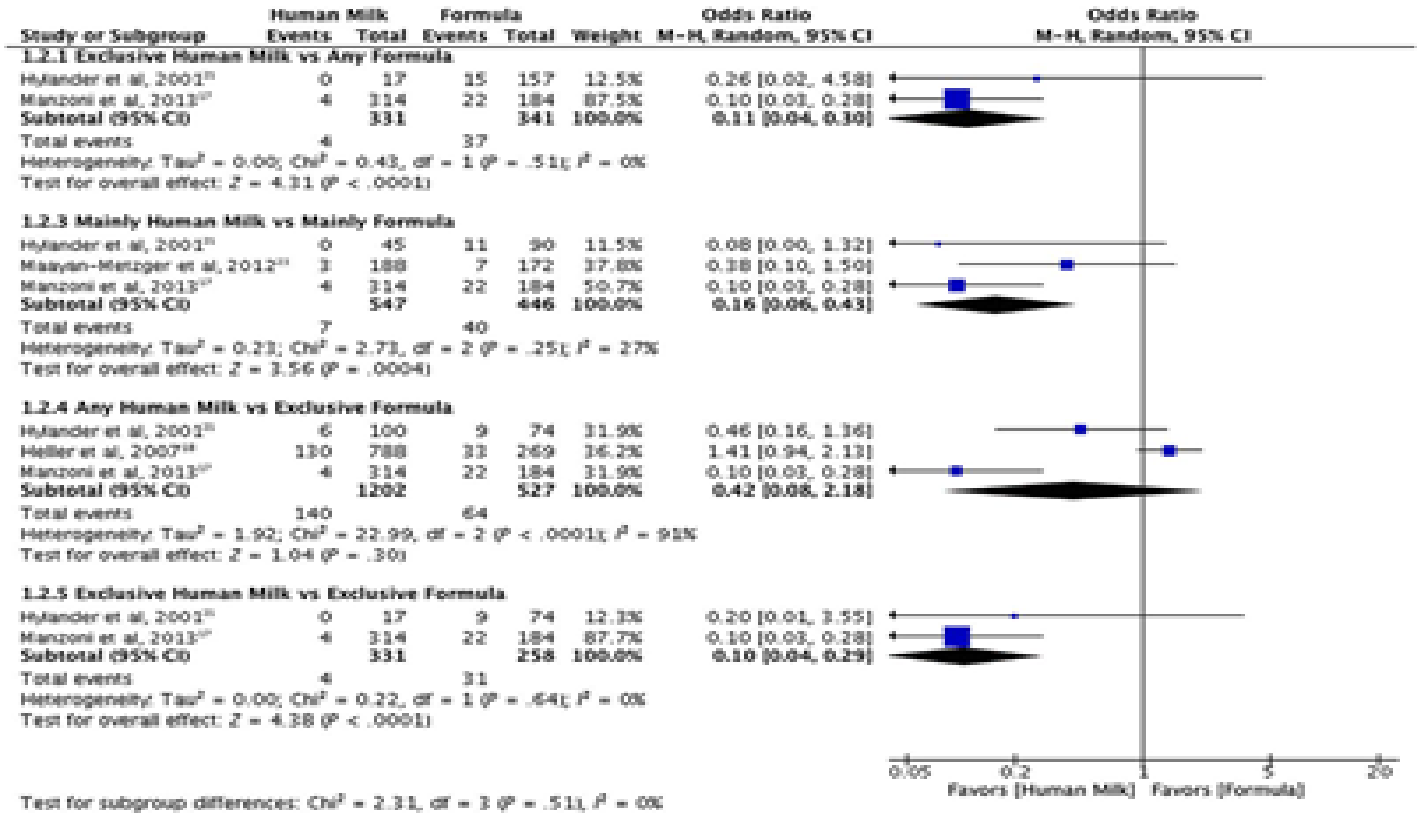
Villamor-Martínez E, Pierro M, Cavallaro G, Mosca F and Villamor E **Mother's Own Milk and Bronchopulmonary Dysplasia: A Systematic Review and Meta-Analysis.** *Front. Pediatr.* (2019) 7:224**.**



Huang, J., Zhu, L., Tang, J., Shi, J., Qu, Y., Xiong, T., & Mu, D. (2018). Human milk as a protective factor for bronchopulmonary dysplasia: a systematic review and meta-analysis. Archives of Disease in Childhood - Fetal and Neonatal Edition, fetalneonatal–2017–314205. doi:10.1136/archdischild-2017-314205

MBM & ROP

Forest plots of the summary OR value with corresponding 95% CIs for the correlation between human milk feeding and severe ROP.



Jianguo Zhou et al. Pediatrics 2015;136:e1576-e1586

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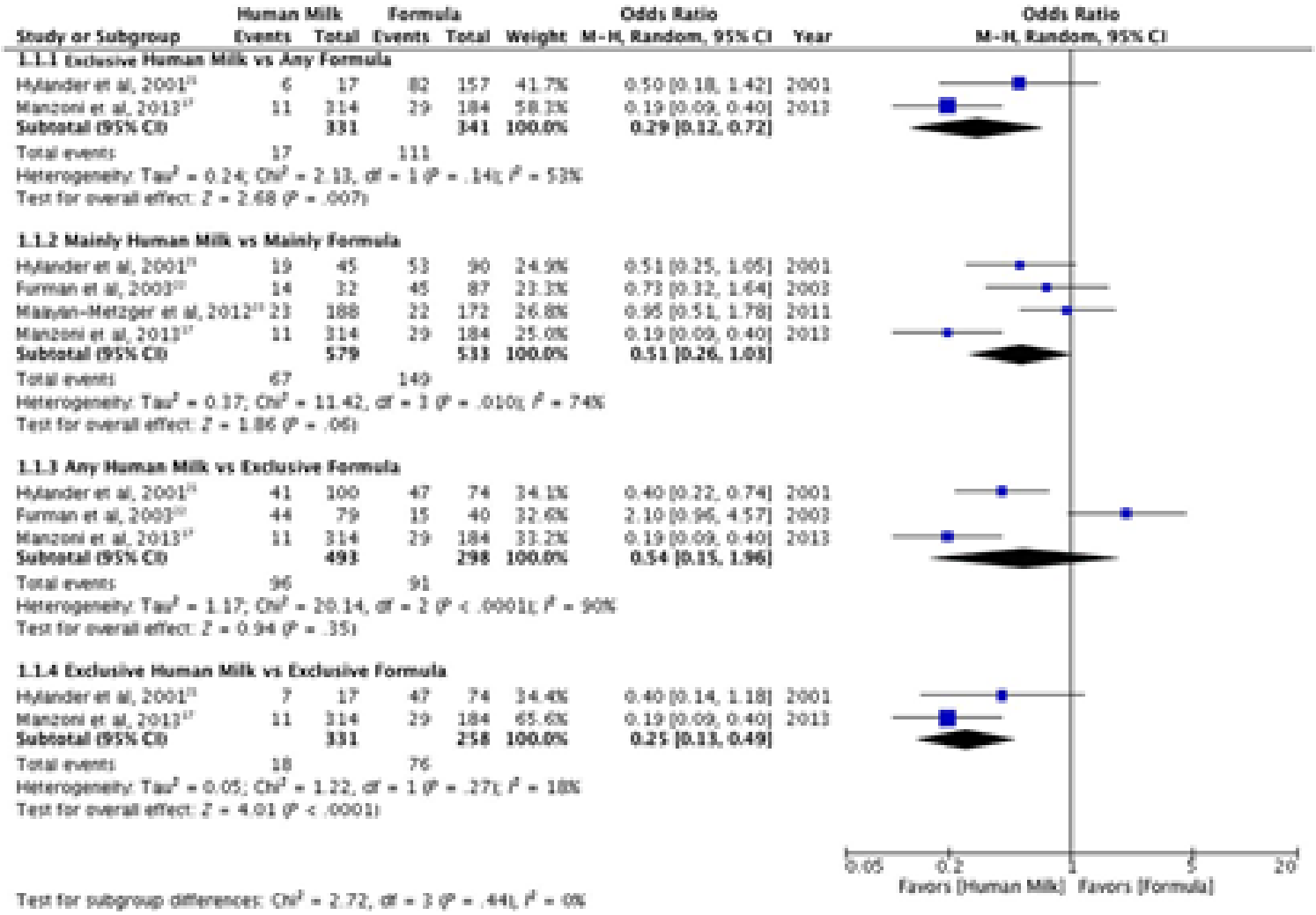
Forest plots of the summary OR value with corresponding 95% CIs for the correlation between human milk feeding and any-stage ROP.



Jianguo Zhou et al. Pediatrics 2015;136:e1576-e1586

Why MBM should be the primary enteral diet of premature infants?

The current recommendation is based on an array of benefits that human milk provides to this highly vulnerable population:

- Decreased rates of NEC , ROP and late-onset sepsis
- Fewer re-hospitalizations in the first year of life
- Improved neurodevelopmental outcomes

- Premature infants that receive human milk have lower rates of metabolic syndrome, lower blood pressure and low-density lipoprotein levels, and less insulin and leptin resistance when they reach adolescence, compared to premature infants receiving formula

Human Milk: An Ideal Food for Nutrition of Preterm Newborn.

Boquien C-Y; Front. Pediatr. (2018) 6:295

- A highly protective effect on infant mortality, with a 12% decrease in mortality risk compared to non-breastfed
- A decrease in respiratory and gastrointestinal infections during the first weeks of life of the newborn, probably related to the composition of colostrum and breast milk that confers immune protection to the child.
- In premature infants several studies show a positive relationship between the quantity of breast milk received during hospitalization and neuro development
- Breastfeeding duration “dose” effect
- Neurodevelopment advantages have been related not only to breastfeeding duration but also to the amount received, reflecting a dose response relationship

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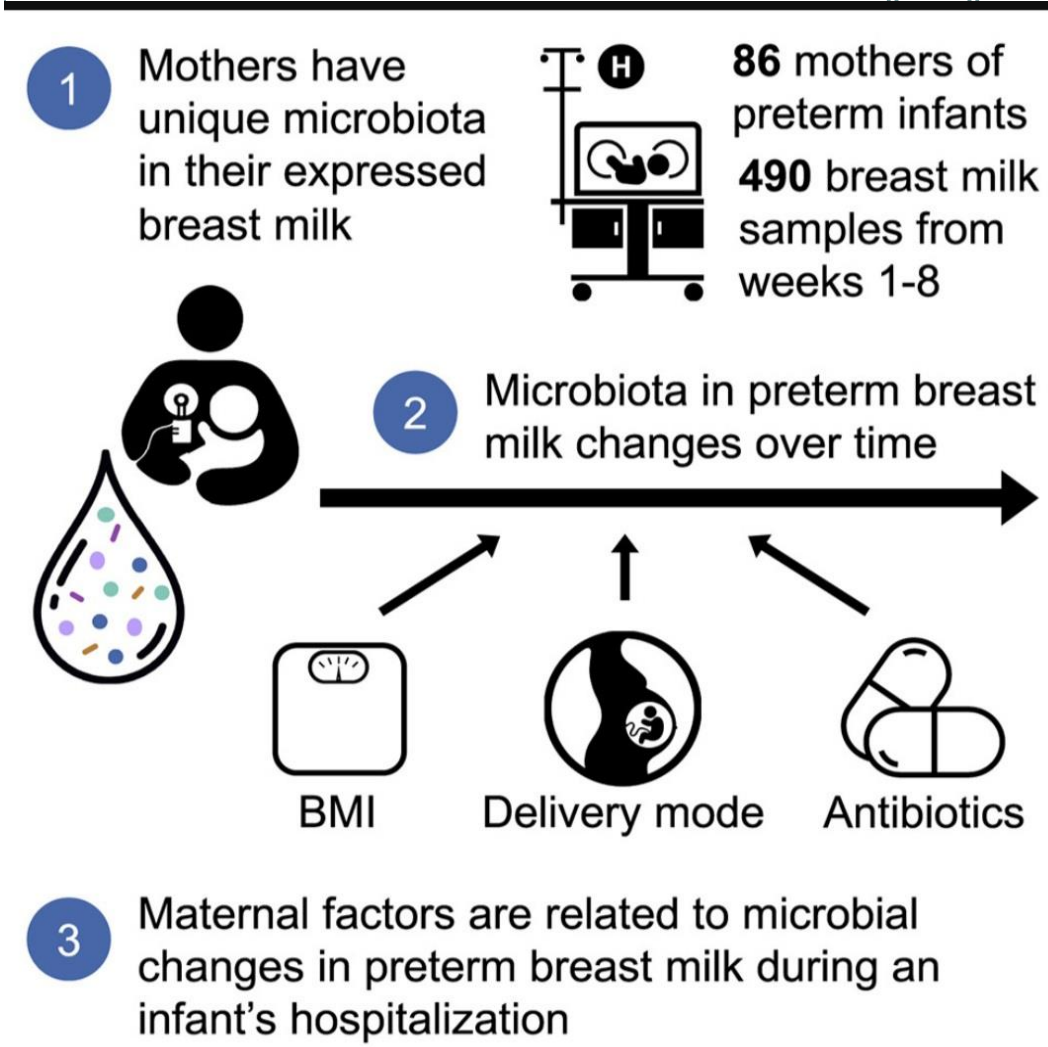
Mother's milk contains complex microbial communities thought to be important for colonizing a preterm infant's gastrointestinal tract



Mothers of Preterm Infants Have Individualized Breast Milk Microbiota that Changes Temporally Based on Maternal Characteristics, Cell Host & Microbe

Cell Host Microbe

2020 Nov 11;28(5):669-682.e4.

Michelle R. Asbury, James Butcher, Julia K. Copeland, Sharon Unger, Nicole Bando, Elena M. Comelli, Victoria Forte, Alex Kiss, Lauren LeMay-Nedjelski, Philip M. Sherman, Alain Stintzi, Christopher Tomlinson, Pauline W. Wang, Deborah L. O'Connor,

nutrients

MDPI

Article

Oropharyngeal Colostrum Positively Modulates the Inflammatory Response in Preterm Neonates

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Abstract: During the first days of life, premature infants have physiological difficulties swallowing, thereby missing out on the benefits of breastfeeding. The aim of this study is to assess the effects of oropharyngeal mother's milk administration in the inflammatory signaling of extremely premature infants. Neonates (n = 100) (<32 week's gestation and/or <1500 g) were divided into two groups: mother's milk group (n = 48), receiving 0.2 mL of oropharyngeal mother's milk every 4 h for the first 15 days of life, and a control group (n = 52), not receiving oropharyngeal mother's milk. Serum concentrations of interleukin (IL) IL-6, IL-8, IL-10, IL-1ra, tumor necrosis factor alpha (TNF-α), and interferón gamma (IFN-γ) were assessed at 1, 3, 15, and 30 days of postnatal life. Maternal and neonatal outcomes were collected. The rate of common neonatal morbidities in both groups was similar. The mother's milk group achieved full enteral feeding earlier, and showed a decrease in Il-6 on days 15 and 30, in IL-8 on day 30, and in TNF-α and INF-γ on day 15, as well as an increase in IL-1ra on days 3 and 15 and in IL-10 on day 30. Oropharyngeal mother's milk administration for 15 days decreases the pro-inflammatory state of preterm neonates and provides full enteral nutrition earlier, which could have a positive influence on the development of the immune system and inflammatory response, thereby positively influencing other developmental outcomes.

Oropharyngeal mother's milk

- Administration of oropharyngeal mother's milk contributes to decreasing the pro-inflammatory state of the preterm neonate, indicating a beneficial influence on the inflammatory response
- These findings have implications for the development of the preterm neonate, wherein inflammation plays a pathophysiological role, associated with adverse neonatal outcomes independently of the duration of gestation
- Moreover, preterm infants receiving mother's milk via oropharynx achieved complete enteral nutrition sooner than babies who did not

More robust evidence to support this intervention is required

More evidence required…

- Feeding tolerance
- Time to full enteral feeding
- Allergic/atopic outcomes
- Decrease in parental anxiety
- Improved parent-infant bonding

MBM & long term health

- Premature babies are at high risk of insulin resistance and metabolic disorders in adulthood

- A very high growth rate during this period can have deleterious effects, in terms of increased susceptibility to metabolic diseases (obesity, type 2 diabetes, cardiovascular diseases) in adulthood

Hofman PL, Regan F, Jackson WE, Jefferies C, Knight DB, Robinson EM, et al. Premature birth and later insulin resistance. N Engl J Med. (2004) 351:2179–86.
Stettler N, Zemel BS, Kumanyika S, Stallings VA. Infant weight gain and childhood overweight status in a multicenter, cohort study. Pediatrics (2002) 109:194–9. 10.1542/peds.109.2.194

MBM & miRNA

- Breast milk rich in miRNA
- MiRNAs are non-coding RNAs that regulate gene expression and control protein synthesis at the post-transcriptional level
- They play roles in the regulation of many biological and developmental processes and would be important in the development of the child's immune system
- Once the milk is ingested by the child, these maternal miRNAs resist digestion

Alsaweed M, Hartmann PE, Geddes DT, Kakulas F.
MicroRNAs in breastmilk and the lactating breast: potential immunoprotectors and developmental regulators for the infant and the mother.
Int J Environ Res Public Health (2015) 12:13981–4020.

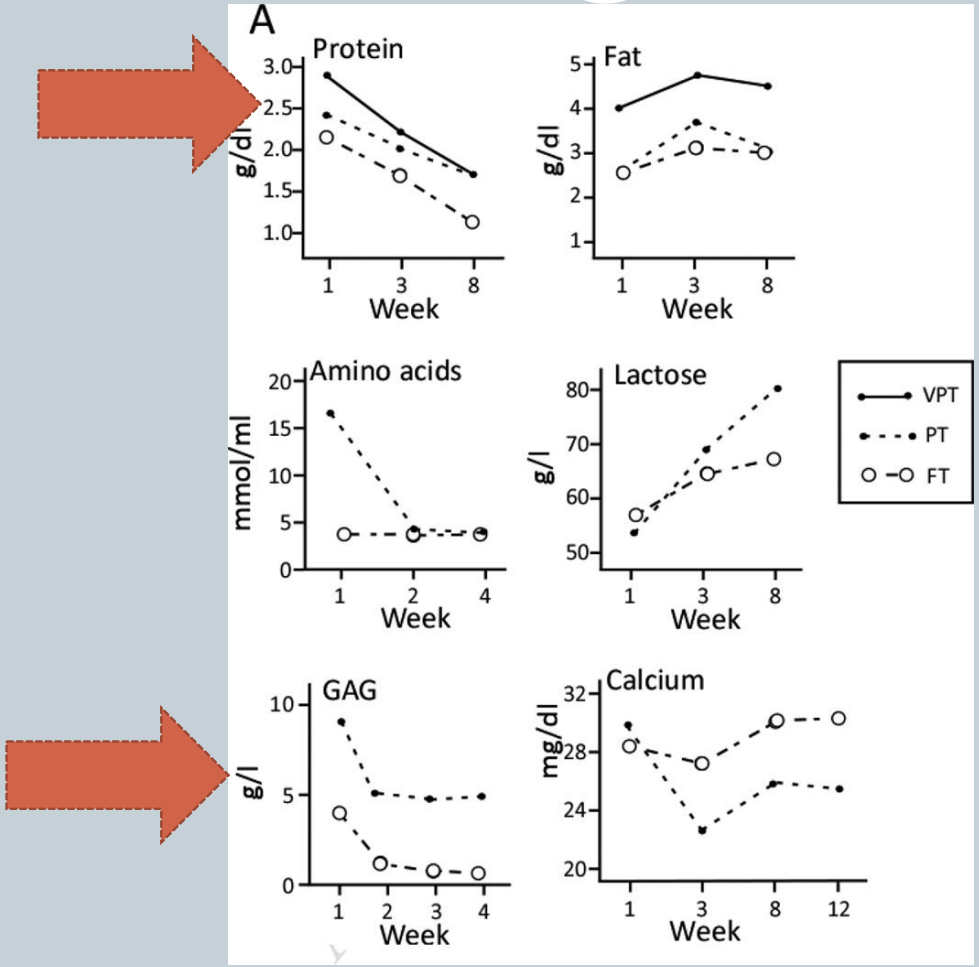
Personalised & more preterm specific

- Anti-infective components personalised
- Protein content in preterm mother's milk is higher than in term mother's milk during the first days of lactation
- Concentration of certain free amino acids, including valine, threonine and arginine is higher in preterm mother's milk
- Preterm breast milk appears also rich in sIgA and deficient in leptin

Boquien C-Y
Human Milk: An Ideal Food for Nutrition of Preterm Newborn.
Front. Pediatr. (2018) 6:295.

Changes in milk composition over time in term (37–41 weeks), preterm (30–36 weeks) and very preterm (<28–30 weeks) infants.

Data combined from multiple sources.15, 82, 115–122



Mark A. Underwood, Human Milk for the Premature Infant, Pediatric Clinics of North America, Volume 60, Issue 1, 2013, Pages 189-207,

Many areas not covered

- Collection, storage, safe handling
- Nutritional quality & Fortification

- Controversies/Debates

Moro GE and Arslanoglu S (2020) Editorial: **Human Milk in the Feeding of Preterm Infants: Established and Debated Aspects.** *Front. Pediatr.* 8:378.

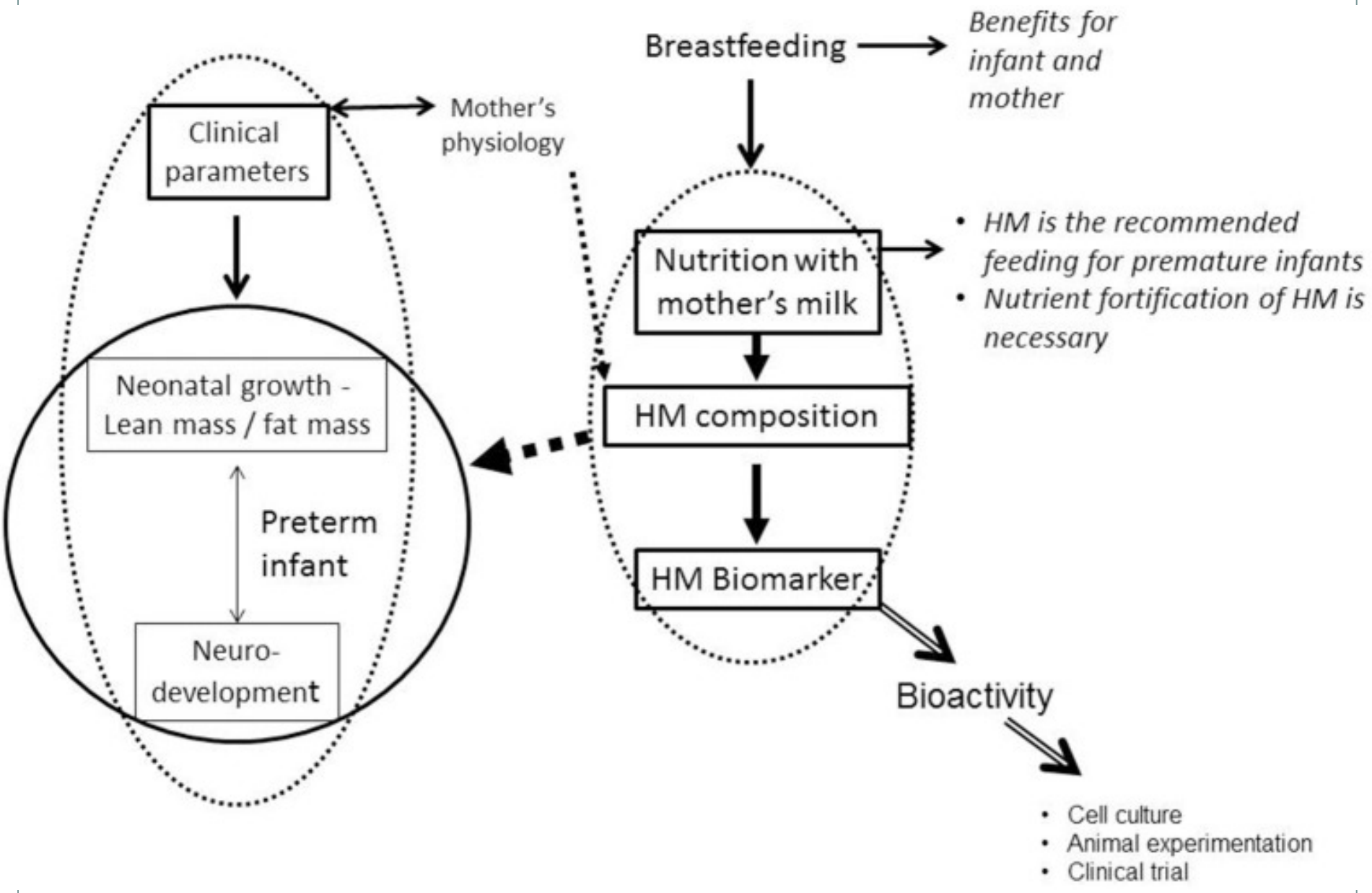
Looking ahead

- Establishing animal models that better reflect the preterm neonate as well as refinement of humanoid model systems, will be essential in bridging the gap from bench to bedside
- An improvement in the experimental model systems will iteratively close the gap in translation
- New bioinformatic tools to correlate the extensive array of metabolites and microbiota in preterms offer great promise in understanding the factors that influence health of the premature infant
- How these metabolites differ functionally in the extremely premature infant needs better study

The blood metabolome or lipidome is different between a breastmilk fed infant and a non-breastmilk fed infant

- If breast milk has certain plasticity in its composition, depending on the physiology of the mother and baby, how does this affect the physiology of the child, its growth trajectory, and long term development ?

- We need to understand this much better


Breastfeeding
Benefits for infant and mother
Clinical parameters
Mother's physiology
Nutrition with mother's milk
HM is the recommended feeding for premature infants
Nutrient fortification of HM is necessary
HM composition
Neonatal growth - Lean mass / fat mass
Preterm infant
Neuro-development
HM Biomarker
Bioactivity
Cell culture
Animal experimentation
Clinical trial

Looking Ahead

- The science of breast milk will likely open new avenues of therapeutic options to minimize the adverse health consequences of prematurity

- Methodologically better and larger trials as well as newer research methods e.g. big data may help answer many of the unanswered questions

Looking Ahead

- Further exploration of roles of various bioactive components and cells in human milk especially stem cells on infant health
- Additional studies are needed to further characterize the effects of HM-derived GAGs on the intestinal epithelium, their interactions with specific bacteria, and their influence on the neonatal intestinal microbiome, particularly in the context of prematurity
- The role of miRNAs in MBM in disease and health needs further elucidation
- The role of human milk-derived vesicles, including the human milk fat globule and exosomes, may reveal an opportunity to present multiple critical molecules simultaneously and ensuring delivery and bioavailability to the intended site
- What is the effect of maternal microbiome and diet and dietary supplements on MBM?

Figure 3. The impact of MBM on preterm babies

MBM maternal breast milk; VLBW very low birth weight; ELBW extremely low birth weight; NEC necrotising enterocolitis[18–23,28–32]

Summary

- ***"Maternal Breast Milk for preterm babies is an exceptional example of both personalised and precision medicine"***

- Early Colostrum within 6 hours of birth and MBM to be the first enteral feed given to baby (microbiome)

- Decrease in mortality & morbidity (strengthening of evidence)

- Improvement in long term outcomes including metabolic syndrome risk based on DOHaD (Developmental Origin of Health and adult Diseases) needs further study

- Maternal, Family and Societal benefits need better quantification and appreciation

COLOSTRUM

- Is the best start to your baby's life
- Is like your baby's first immunisation
- Is like liquid gold; every drop counts

Conceived by Prof. Minesh Khashu

drawn by @AnnaGeyer_NP

nutrients

Neonatal Nutrition for Inflammatory Disorders and Necrotizing Enterocolitis

Edited by
Misty Good

Printed Edition of the Special Issue Published in *Nutrients*

www.mdpi.com/journal/nutrients

MDPI

Premature labour and delivery are highly stressful to parents

- Education regarding the importance and value of breastfeeding should begin during pregnancy and be reemphasized when premature delivery seems likely
- Pumping with an electric pump should be initiated within 6 hours of delivery and continued 8–12 times per 24 hours until the milk supply is well established
- Reassurance and encouragement are valuable as new mothers are often worried and discouraged by the initial small volumes obtained
- Early assistance by a nurse or lactation consultant is helpful in establishing an effective pumping regimen
- Regular questioning by the neonatal nurse or physician regarding milk supply is valuable to encourage early intervention when milk production decreases
- Milk production decreases with maternal depression and increases with increased frequency of pumping and time spent skin-to-skin with the premature infant

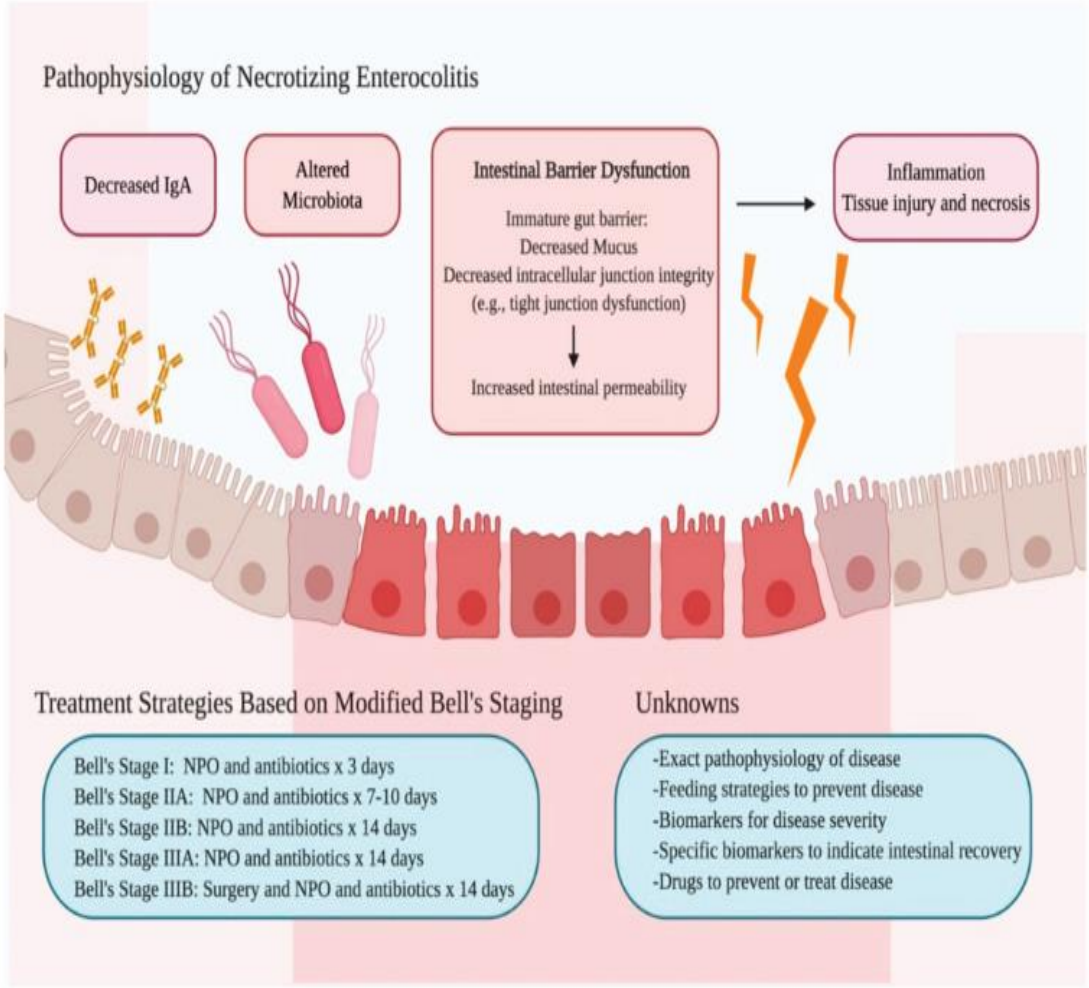


Figure 1. Summary of the Pathophysiology, Treatment Strategies, and Unknowns of Necrotizing Enterocolitis. The pathophysiology of NEC is multi-faceted, involving intestinal barrier dysfunction, decreased IgA, and altered microbiota. Current treatment strategies include stopping feeds and starting antibiotics based on disease severity, as classified by Bell's staging. Much remains unknown about disease prevention, diagnosis, and treatment. Figure created with Biorender.com. Abbreviations: Immunoglobulin A (IgA), NEC (Necrotizing enterocolitis), NPO (*nil per os*).

European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (EPSGHAN) recommendation

- Nutrition of premature infants by breast milk, and a protein enrichment of this milk, as soon as possible, at least until discharge from hospital, in order to increase weight gain and protein accretion

Agostoni C, Buonocore G, Carnielli VP, De Curtis M, Darmaun D, Decsi T, et al. .
Enteral nutrient supply for preterm infants: commentary from the European Society of Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition.
J Pediatr Gastroenterol Nutr. (2010) 50:85–91.

Alpha-lactalbumin is the most common whey protein in human milk. It has pain relief and anti-microbial abilities, and anti-viral actions against HIV components.

Human milk contains

Alpha-lactalbumin

When Alpha-lactalbumin binds to oleic acid (also found in human milk), it changes shape to become **HAMLET** (Human Alpha-lactalbumin Made Lethal to Tumour cells).

HAMLET causes the death of cancerous cells when studied in the laboratory, and work is under way to determine whether this is also true in our bodies.

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Tailor-made
antibodies
Your body identifies
bacteria and viruses
found in your baby's body
and environment. You
then produce antibodies
specifically tailored to
those infections, and
deliver them to your
child through
your milk.
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Components that kill cancerous cells

Human milk contains components that kill cancerous cells.

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oligosaccharides
disaccharides
a-1-antichymotrypsin
polysaccharides
Xanthine oxidase
Lysozyme
Histaminase
Catalase
Arylsulfatase
PAF-acetylhydrolase
Phosphatase
glycosaminoglycans
Amylase
monosaccharides
Antiproteases
Lipase
a-1-antitrypsin

Carbohydrates

Enzymes &

Enzymes are special proteins that **speed up specific chemical reactions** throughout the body.

Human milk contains enzymes and carbohydrates. Breastmilk contains over 200 sugars, either complex or simple, which provide an important energy source. Some human milk oligosaccharides can only be digested by bacteria, and help the infant to establish a healthy gut microbiome.

Histaminase – An enzyme that inactivates and breaks down histamine, a substance released by the body at times of stress and allergy.

Lysozyme – Found in significant quantities in human milk. It is anti-inflammatory and bactericidal, destroying bacteria by disrupting their cell walls. Thought to protect the infant against diarrhoeal diseases. It increases in concentration in breastmilk as babies get older and more mobile and increases further after children reach their first birthday. It is particularly effective against E.coli and salmonella.

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Human milk contains fats. Fats in general have a number of functions including: energy storage, cell messaging, hormone production and structural roles.

A fatty acid required for the synthesis of molecules involved in **pain and inflammation**. Also thought to play a role in infant brain development.

Plasmalogens – Important components of the immune, nervous and cardiovascular systems. Plays a role in myelination of nerve fibres (laying down insulation to speed up nerve messages).

Arachidonic acid (AHA) – A fatty acid involved in reducing pain and inflammation. Also thought to play a role in infant brain development.

Gangliosides (GM1, GM2, GM3) are critical to normal brain development, help nerves to repair themselves and may play further roles in immune system development, calcium transport and basic cell functions.

Fats

Lauric acid
GM2
Stigma-and campesterol
Desmosterol
Globoside (GB4)
Methosterol
Palmitic acid
Lysophosphatidylcholine
Glucosylceramide
Linoleic acid (omega 6)/ Alpha-linolenic acid (ALA) (omega 3)
Plasmalogens
Sphingolipids
Galactosylceramide
Docosahexaenoic acid (DHA)
GM1
Lactosylceramide
Eicosapentaenoic acid (EPA)
oleic acid
Globotriaosylceramide (GB3)
7-ketocholesterol
Phosphatidylinositol
7-Dehydrocholesterol
Cholesterol
Sphingomyelin
Palmitoleic acid
Dimethylsterol
Triacylglycerol (triglyceride)
Lanosterol
Heptadecenoic acid
Phosphatidylcholine
Lathosterol
Squalene
Sitosterol
Lysophosphatidylethanolamine
β-Lathosterol
Conjugated linoleic acid (Rumenic acid- active omega 6)
Stearic acid
Sterols
GM3
Arachidonic acid (AHA)
Phosphatidylethanolamine

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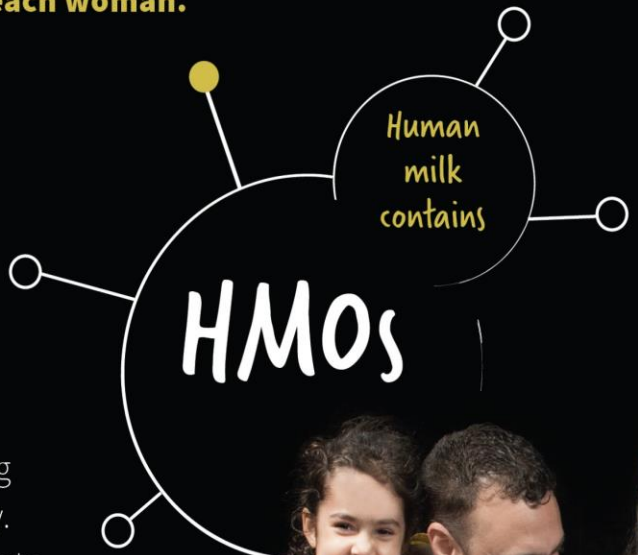
Human Milk Oligosaccharides (HMOs) are a group of complex sugars, and the third most abundant component of human milk. Over 100 different HMOs have been identified in human milk so far. Which ones are found, and in what concentration, is unique to each mother. Genetics, how many children the mother has had, the age of the nursling, and even the seasons, have been found to influence this unique HMO set in each woman.

One HMO called DSLNT has been linked to a reduction in the risk of Necrotizing Enterocolitis (NEC). NEC is a potentially fatal disorder, most often affecting premature babies, in which tissues in the gut become inflamed and start to die.

Many HMOs cannot be digested byhumans, but instead act as food for bacteria in your baby's tummy. These bacteria help your baby to resist infection from harmful bacteria, and to develop a normal gut microbiome.



What we know about HMOs is probably just the tip of the iceberg, as researchers continue to unravel their full potential.

The microbiome plays an enormous role in making sure the gut and immune system develop properly. HMOs can also trick bacteria and viruses into binding to them, instead of the gut wall, preventing them from infecting your baby. Some HMOs have the potential to reduce the risk of diarrheal diseases, one of the most common causes of infant mortality under age 5.

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Hormones

Human milk contains hormones. Hormones are chemical messengers that carry signals from one cell, or group of cells, to others via the blood. Studies in monkeys show that hormone signalling through their milk affects the feeding behaviour, temperament and weight gain of infant monkeys.

Oxytocin – A hormone that induces feelings of well-being and relaxation in both the child and the mother. Involved in causing uterine contractions which help to control bleeding after birth and shrink the uterus back to its pre-pregnancy size. The mother's uterus contracts during feeds and for up to 20 minutes after the feed. Also causes the milk-ejection reflex, or letdown.

Leptin – Helps to suppress appetite. May help the baby to develop brain responses to being full, which would prevent children and adults from overeating. Also helps to reduce the amount of body fat.

Cortisol Insulin Corticosterone FIL Prostacyclins GRH
Cholecystokinin Triiodothyronine (T3) oxytocin
Thyroxine Thyroid stimulating hormone (TSH)
Thyroid releasing hormone (TRH) Leptin PG-F2
Thrombopoietin Thromboxanes Ghrelin
Gonadotropin-releasing hormone (GnRH)
Adiponectin PG-E1 PG-E2
Eicosanoids Prostaglandins
Leukotrienes
Prolactin Enhancers (T & B cells)

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Lactoferrin is a protein with an anti-tumour effect, and has been found to significantly inhibit the growth of some cancerous cells.

Laboratory tests have shown that lactoferrin inhibits infection by Hepatitis B, Hepatitis C, Cytomegalovirus (Herpes family), Respiratory Syncytial Virus (RSV), Adenovirus (causes the common cold), Poliovirus, Enterovirus (diarrhoea virus), and others.

Human milk contains

Lactoferrin

It also helps our little ones to absorb their own iron stores.

It binds to the iron in their body, which prevents it from being accessed by harmful micro-organisms that need iron to survive.

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Antibacterial and antiviral

Human milk contains lysozyme, an antibacterial and antiviral enzyme that increases in concentration when babies are around 6 months old, and again after a year. It also contains lactoferrin, which inhibits the growth of some cancerous cells, and increases in concentration over time.

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Human milk contains nucleotides. Nucleotides are the basic building blocks of DNA and RNA. They can also form circular structures involved in cell signalling - activating or inhibiting processes within cells.

Cytidine diphosphate choline (CDP choline) – It appears to play a protective role against hypoxic brain damage, and helps to improve memory and learning.

Uridine diphosphate-N-acetylhexosamine (UDPAH) – Important for the production of essential sugars required for normal growth and development.

AaBbCc

Nucleoti

Guanosine diphosphate –

5'-Adenosine monophosphat

Uridine diphosphate (UDP)

Uridine diphosp

Uridine monophosphate (3'-UMP)

Uridine diphosphoglucuronic acid (UDPGA)

Cytidine diphosphate cho

Uridine diphosphate-N-acetylhexosamine (UDPAH)

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human milk bmf

Oxytocin is a hormone that causes feelings of well-being, relaxation and connection in both mother and child.

It also causes contractions of the uterus, which help to control bleeding after birth and shrink the uterus back to its pre-pregnancy size. The mother's uterus contracts during feeds and for up to 20 minutes after the feed.

Human milk contains

oxytocin

It causes the milk-ejection reflex, or let-down. It is carried through the bloodstream to the breast, where it causes contractions that carry the milk forward into the ducts.

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Feelings of well-being

Human milk contains oxytocin, a hormone that induces feelings of relaxation and well-being in your child, and in you.

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Human milk contains stem cells

These are cells that create and repair the body and are being researched worldwide to cure conditions like Alzheimers and diabetes.

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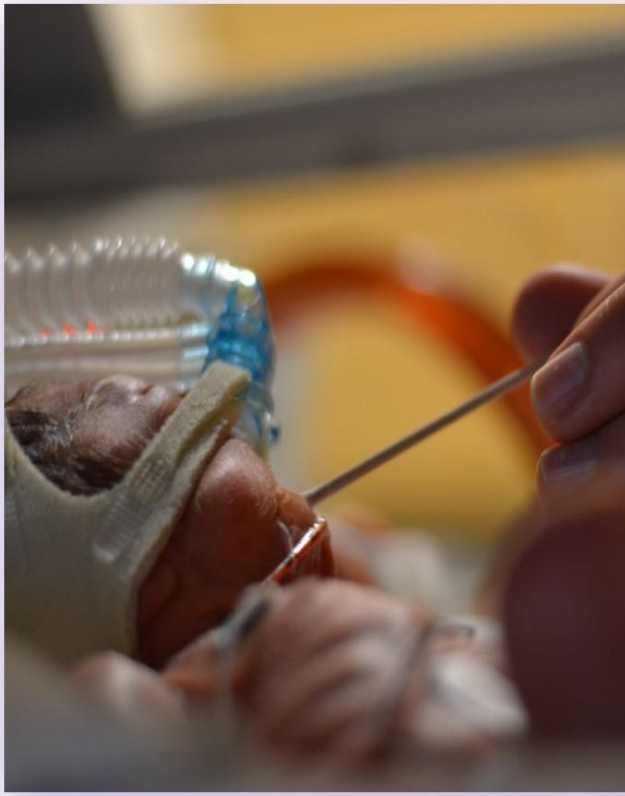
Colostrum is a baby's first immunisation as well as its first feed.

Every drop helps the immune and digestive systems of premature babies.

Breastfeeding: Foundation of Life



#preventNEC

INSTITUTO PEQUENOS GRANDES GUERREIROS

SIGNEC

NEC SOCIETY

#WBW

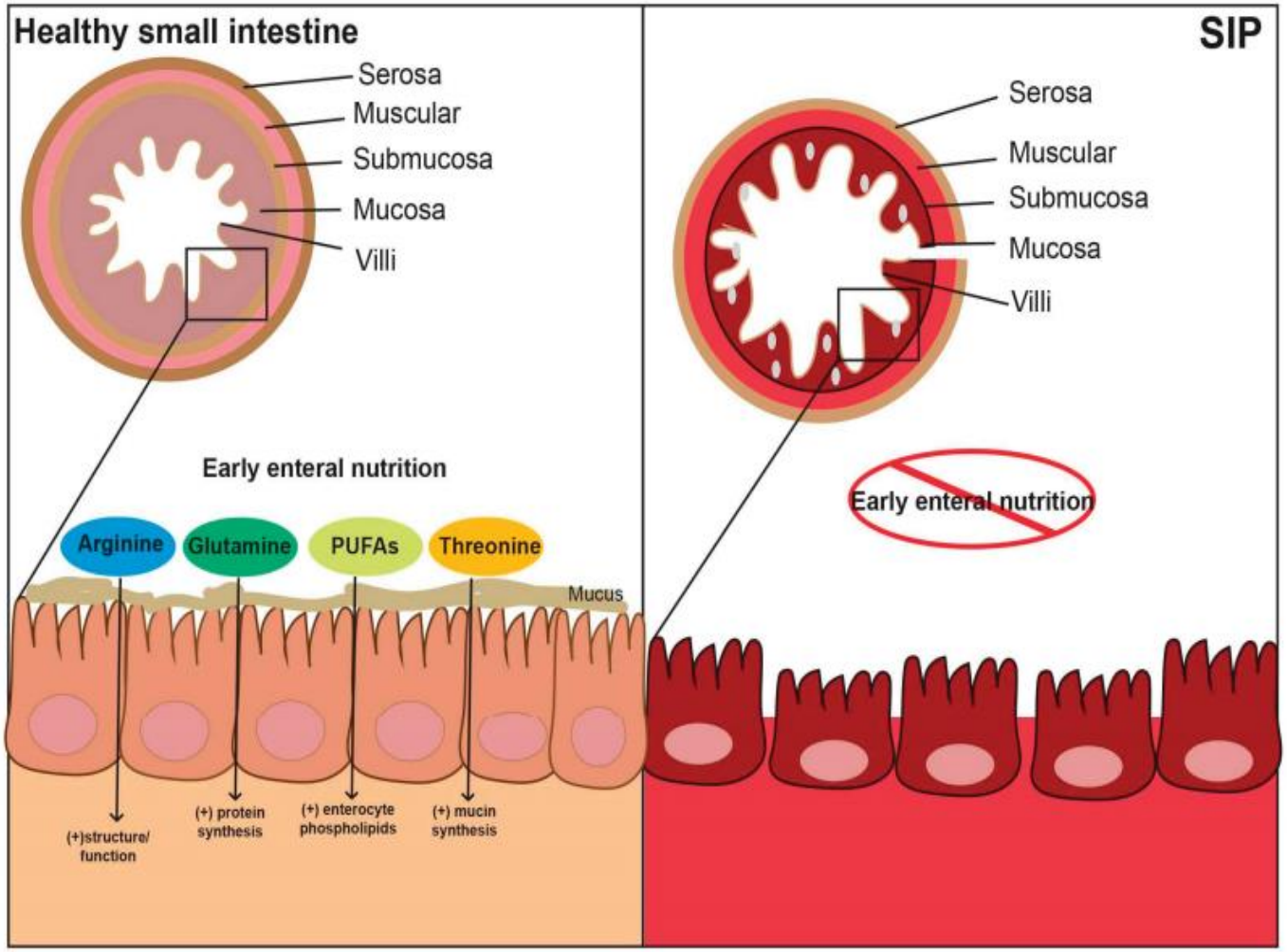


Figure 3. Relationship of early enteral nutrition to SIP. Early enteral nutrition provides arginine, threonine, glutamine and polyunsaturated fats (PUFAs) that result in improved gut structure/function, mucin synthesis and production of enterocyte phospholipids. Delayed enteral nutrition results in increased SIP susceptibility.

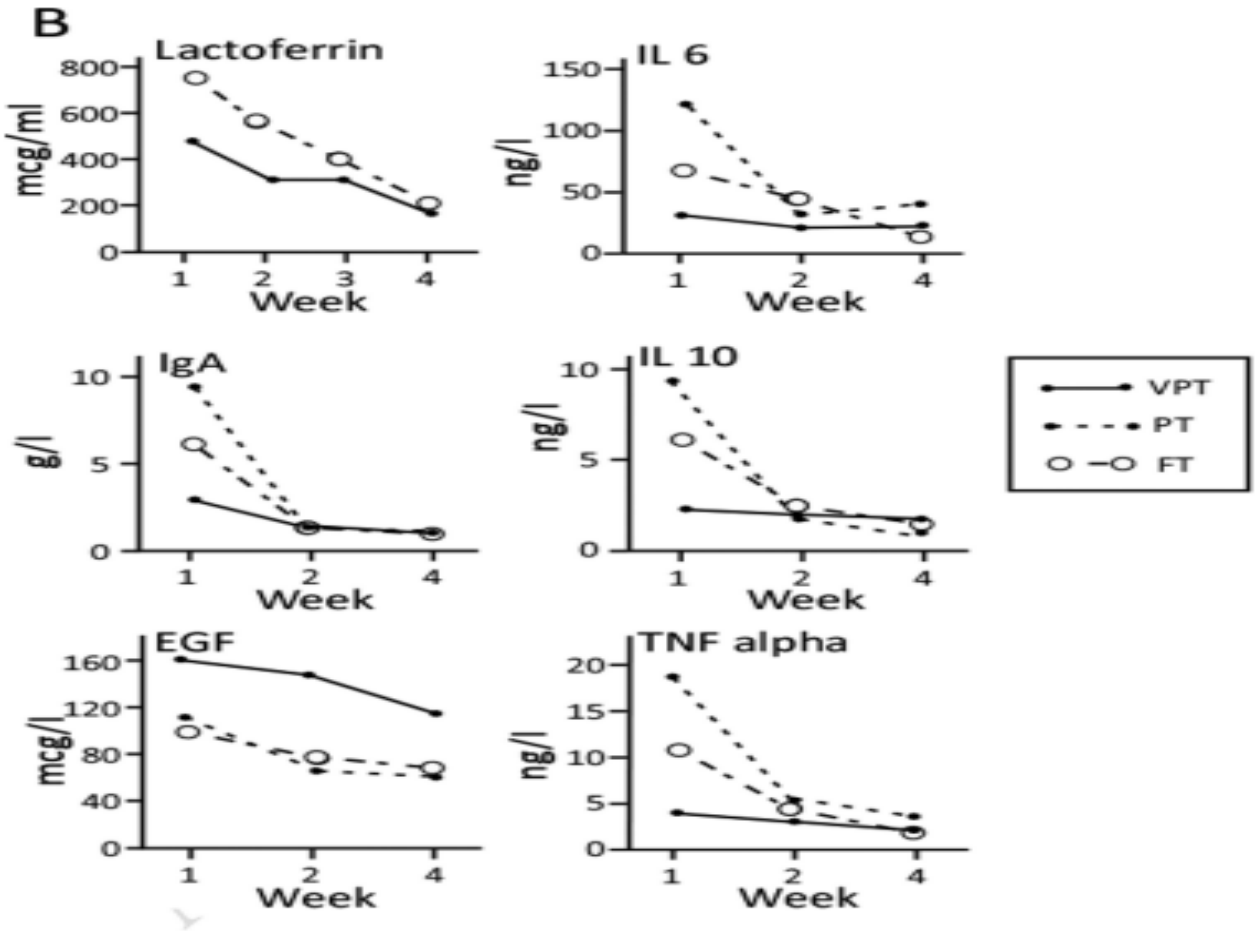
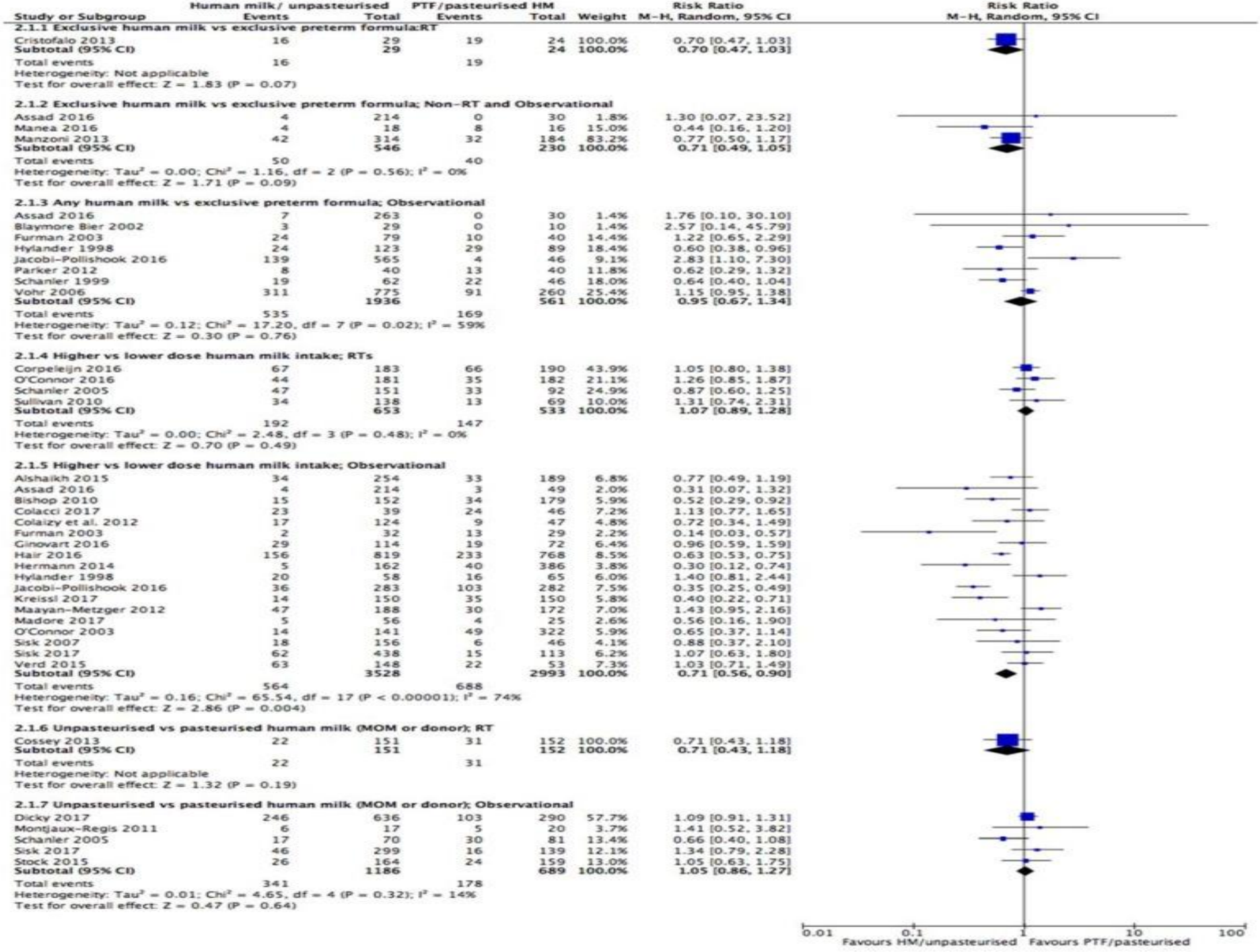


Figure 1.
Changes in milk composition over time in term (37–41 weeks), preterm (30–36 weeks) and very preterm (<28–30 weeks) infants. Data combined from multiple sources.[15, 82, 115–122] GAG glycosaminoglycans, IL 6 interleukin 6, IgA immunoglobulin A, IL 10 interleukin 10, EGF epidermal growth factor, TNF alpha tumor necrosis factor alpha.


Human milk/ unpasteurised
PTF/pasteurised HM
Risk Ratio
Risk Ratio
Study or Subgroup
Events
Total
Events
Total
Weight
M-H, Random, 95% CI
M-H, Random, 95% CI
2.1.1 Exclusive human milk vs exclusive preterm formula:RT
Cristofalo 2013 16 29 19 24 100.0% 0.70 [0.47, 1.03]
Subtotal (95% CI) 29 24 100.0% 0.70 [0.47, 1.03]
Total events 16 19
Heterogeneity: Not applicable
Test for overall effect: Z = 1.83 (P = 0.07)
2.1.2 Exclusive human milk vs exclusive preterm formula; Non-RT and Observational
Assad 2016 4 214 0 30 1.8% 1.30 [0.07, 23.52]
Manea 2016 4 18 8 16 15.0% 0.44 [0.16, 1.20]
Manzoni 2013 42 314 32 184 83.2% 0.77 [0.50, 1.17]
Subtotal (95% CI) 546 230 100.0% 0.71 [0.49, 1.05]
Total events 50 40
Heterogeneity: Tau² = 0.00; Chi² = 1.16, df = 2 (P = 0.56); I² = 0%
Test for overall effect: Z = 1.71 (P = 0.09)
2.1.3 Any human milk vs exclusive preterm formula; Observational
Assad 2016 7 263 0 30 1.4% 1.76 [0.10, 30.10]
Blaymore Bier 2002 3 29 0 10 1.4% 2.57 [0.14, 45.79]
Furman 2003 24 79 10 40 14.4% 1.22 [0.65, 2.29]
Hylander 1998 24 123 29 89 18.4% 0.60 [0.38, 0.96]
Jacobi-Pollishook 2016 139 565 4 46 9.1% 2.83 [1.10, 7.30]
Parker 2012 8 40 13 40 11.8% 0.62 [0.29, 1.32]
Schanler 1999 19 62 22 46 18.0% 0.64 [0.40, 1.04]
Vohr 2006 311 775 91 260 25.4% 1.15 [0.95, 1.38]
Subtotal (95% CI) 1936 561 100.0% 0.95 [0.67, 1.34]
Total events 535 169
Heterogeneity: Tau² = 0.12; Chi² = 17.20, df = 7 (P = 0.02); I² = 59%
Test for overall effect: Z = 0.30 (P = 0.76)
2.1.4 Higher vs lower dose human milk intake; RTs
Corpeleijn 2016 67 183 66 190 43.9% 1.05 [0.80, 1.38]
O'Connor 2016 44 181 35 182 21.1% 1.26 [0.85, 1.87]
Schanler 2005 47 151 33 92 24.9% 0.87 [0.60, 1.25]
Sullivan 2010 34 138 13 69 10.0% 1.31 [0.74, 2.31]
Subtotal (95% CI) 653 533 100.0% 1.07 [0.89, 1.28]
Total events 192 147
Heterogeneity: Tau² = 0.00; Chi² = 2.48, df = 3 (P = 0.48); I² = 0%
Test for overall effect: Z = 0.70 (P = 0.49)
2.1.5 Higher vs lower dose human milk intake; Observational
Alshaikh 2015 34 254 33 189 6.8% 0.77 [0.49, 1.19]
Assad 2016 4 214 3 49 2.0% 0.31 [0.07, 1.32]
Bishop 2010 15 152 34 179 5.9% 0.52 [0.29, 0.92]
Colacci 2017 23 39 24 46 7.2% 1.13 [0.77, 1.65]
Colaizy et al. 2012 17 124 9 47 4.8% 0.72 [0.34, 1.49]
Furman 2003 2 32 13 29 2.2% 0.14 [0.03, 0.57]
Ginovart 2016 29 114 19 72 6.4% 0.96 [0.59, 1.59]
Hair 2016 156 819 233 768 8.5% 0.63 [0.53, 0.75]
Hermann 2014 5 162 40 386 3.8% 0.30 [0.12, 0.74]
Hylander 1998 20 58 16 65 6.0% 1.40 [0.81, 2.44]
Jacobi-Pollishook 2016 36 283 103 282 7.5% 0.35 [0.25, 0.49]
Kreissl 2017 14 150 35 150 5.8% 0.40 [0.22, 0.71]
Maayan-Metzger 2012 47 188 30 172 7.0% 1.43 [0.95, 2.16]
Madore 2017 5 56 4 25 2.6% 0.56 [0.16, 1.90]
O'Connor 2003 14 141 49 322 5.9% 0.65 [0.37, 1.14]
Sisk 2007 18 156 6 46 4.1% 0.88 [0.37, 2.10]
Sisk 2017 62 438 15 113 6.2% 1.07 [0.63, 1.80]
Verd 2015 63 148 22 53 7.3% 1.03 [0.71, 1.49]
Subtotal (95% CI) 3528 2993 100.0% 0.71 [0.56, 0.90]
Total events 564 688
Heterogeneity: Tau² = 0.16; Chi² = 65.54, df = 17 (P < 0.00001); I² = 74%
Test for overall effect: Z = 2.86 (P = 0.004)
2.1.6 Unpasteurised vs pasteurised human milk (MOM or donor); RT
Cossey 2013 22 151 31 152 100.0% 0.71 [0.43, 1.18]
Subtotal (95% CI) 151 152 100.0% 0.71 [0.43, 1.18]
Total events 22 31
Heterogeneity: Not applicable
Test for overall effect: Z = 1.32 (P = 0.19)
2.1.7 Unpasteurised vs pasteurised human milk (MOM or donor); Observational
Dicky 2017 246 636 103 290 57.7% 1.09 [0.91, 1.31]
Montjaux-Regis 2011 6 17 5 20 3.7% 1.41 [0.52, 3.82]
Schanler 2005 17 70 30 81 13.4% 0.66 [0.40, 1.08]
Sisk 2017 46 299 16 139 12.1% 1.34 [0.79, 2.28]
Stock 2015 26 164 24 159 13.0% 1.05 [0.63, 1.75]
Subtotal (95% CI) 1186 689 100.0% 1.05 [0.86, 1.27]
Total events 341 178
Heterogeneity: Tau² = 0.01; Chi² = 4.65, df = 4 (P = 0.32); I² = 14%
Test for overall effect: Z = 0.47 (P = 0.64)
0.01
0.1
1
10
100
Favours HM/unpasteurised
Favours PTF/pasteurised

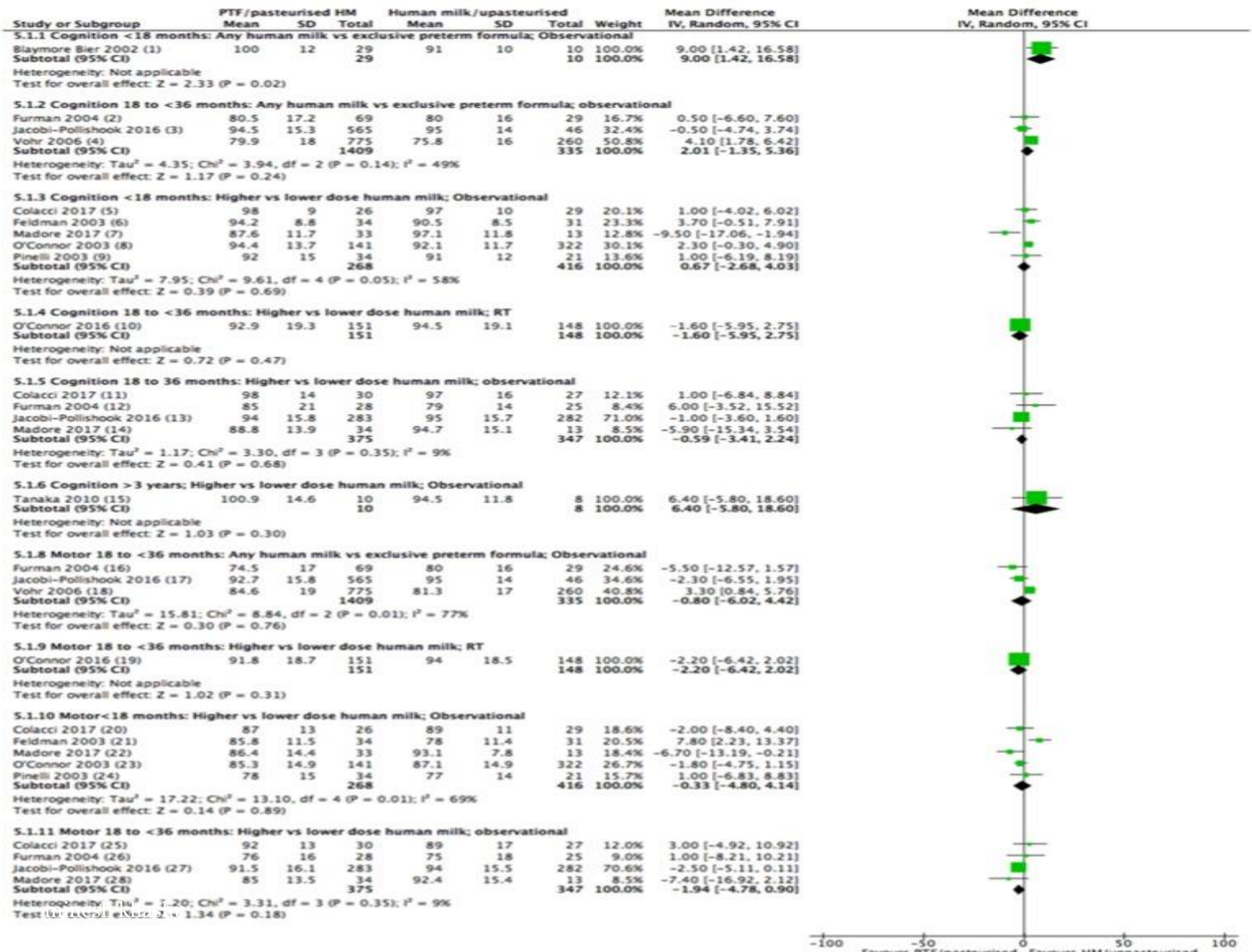

PTF/pasteurised HM
Human milk/upasteurised
Mean Difference
Mean Difference
Study or Subgroup
Mean SD Total Mean SD Total Weight IV, Random, 95% CI
IV, Random, 95% CI
5.1.1 Cognition <18 months: Any human milk vs exclusive preterm formula; Observational
Blaymore Bier 2002 (1) 100 12 29 91 10 10 100.0% 9.00 [1.42, 16.58]
Subtotal (95% CI) 29 10 100.0% 9.00 [1.42, 16.58]
Heterogeneity: Not applicable
Test for overall effect: Z = 2.33 (P = 0.02)
5.1.2 Cognition 18 to <36 months: Any human milk vs exclusive preterm formula; observational
Furman 2004 (2) 80.5 17.2 69 80 16 29 16.7% 0.50 [-6.60, 7.60]
Jacobi-Pollishook 2016 (3) 94.5 15.3 565 95 14 46 32.4% -0.50 [-4.74, 3.74]
Vohr 2006 (4) 79.9 18 775 75.8 16 260 50.8% 4.10 [1.78, 6.42]
Subtotal (95% CI) 1409 335 100.0% 2.01 [-1.35, 5.36]
Heterogeneity: Tau² = 4.35; Chi² = 3.94, df = 2 (P = 0.14); I² = 49%
Test for overall effect: Z = 1.17 (P = 0.24)
5.1.3 Cognition <18 months: Higher vs lower dose human milk; Observational
Colacci 2017 (5) 98 9 26 97 10 29 20.1% 1.00 [-4.02, 6.02]
Feldman 2003 (6) 94.2 8.8 34 90.5 8.5 31 23.3% 3.70 [-0.51, 7.91]
Madore 2017 (7) 87.6 11.7 33 97.1 11.8 13 12.8% -9.50 [-17.06, -1.94]
O'Connor 2003 (8) 94.4 13.7 141 92.1 11.7 322 30.1% 2.30 [-0.30, 4.90]
Pinelli 2003 (9) 92 15 34 91 12 21 13.6% 1.00 [-6.19, 8.19]
Subtotal (95% CI) 268 416 100.0% 0.67 [-2.68, 4.03]
Heterogeneity: Tau² = 7.95; Chi² = 9.61, df = 4 (P = 0.05); I² = 58%
Test for overall effect: Z = 0.39 (P = 0.69)
5.1.4 Cognition 18 to <36 months: Higher vs lower dose human milk; RT
O'Connor 2016 (10) 92.9 19.3 151 94.5 19.1 148 100.0% -1.60 [-5.95, 2.75]
Subtotal (95% CI) 151 148 100.0% -1.60 [-5.95, 2.75]
Heterogeneity: Not applicable
Test for overall effect: Z = 0.72 (P = 0.47)
5.1.5 Cognition 18 to 36 months: Higher vs lower dose human milk; observational
Colacci 2017 (11) 98 14 30 97 16 27 12.1% 1.00 [-6.84, 8.84]
Furman 2004 (12) 85 21 28 79 14 25 8.4% 6.00 [-3.52, 15.52]
Jacobi-Pollishook 2016 (13) 94 15.8 283 95 15.7 282 71.0% -1.00 [-3.60, 1.60]
Madore 2017 (14) 88.8 13.9 34 94.7 15.1 13 8.5% -5.90 [-15.34, 3.54]
Subtotal (95% CI) 375 347 100.0% -0.59 [-3.41, 2.24]
Heterogeneity: Tau² = 1.17; Chi² = 3.30, df = 3 (P = 0.35); I² = 9%
Test for overall effect: Z = 0.41 (P = 0.68)
5.1.6 Cognition >3 years; Higher vs lower dose human milk; Observational
Tanaka 2010 (15) 100.9 14.6 10 94.5 11.8 8 100.0% 6.40 [-5.80, 18.60]
Subtotal (95% CI) 10 8 100.0% 6.40 [-5.80, 18.60]
Heterogeneity: Not applicable
Test for overall effect: Z = 1.03 (P = 0.30)
5.1.8 Motor 18 to <36 months: Any human milk vs exclusive preterm formula; Observational
Furman 2004 (16) 74.5 17 69 80 16 29 24.6% -5.50 [-12.57, 1.57]
Jacobi-Pollishook 2016 (17) 92.7 15.8 565 95 14 46 34.6% -2.30 [-6.55, 1.95]
Vohr 2006 (18) 84.6 19 775 81.3 17 260 40.8% 3.30 [0.84, 5.76]
Subtotal (95% CI) 1409 335 100.0% -0.80 [-6.02, 4.42]
Heterogeneity: Tau² = 15.81; Chi² = 8.84, df = 2 (P = 0.01); I² = 77%
Test for overall effect: Z = 0.30 (P = 0.76)
5.1.9 Motor 18 to <36 months: Higher vs lower dose human milk; RT
O'Connor 2016 (19) 91.8 18.7 151 94 18.5 148 100.0% -2.20 [-6.42, 2.02]
Subtotal (95% CI) 151 148 100.0% -2.20 [-6.42, 2.02]
Heterogeneity: Not applicable
Test for overall effect: Z = 1.02 (P = 0.31)
5.1.10 Motor<18 months: Higher vs lower dose human milk; Observational
Colacci 2017 (20) 87 13 26 89 11 29 18.6% -2.00 [-8.40, 4.40]
Feldman 2003 (21) 85.8 11.5 34 78 11.4 31 20.5% 7.80 [2.23, 13.37]
Madore 2017 (22) 86.4 14.4 33 93.1 7.8 13 18.4% -6.70 [-13.19, -0.21]
O'Connor 2003 (23) 85.3 14.9 141 87.1 14.9 322 26.7% -1.80 [-4.75, 1.15]
Pinelli 2003 (24) 78 15 34 77 14 21 15.7% 1.00 [-6.83, 8.83]
Subtotal (95% CI) 268 416 100.0% -0.33 [-4.80, 4.14]
Heterogeneity: Tau² = 17.22; Chi² = 13.10, df = 4 (P = 0.01); I² = 69%
Test for overall effect: Z = 0.14 (P = 0.89)
5.1.11 Motor 18 to <36 months: Higher vs lower dose human milk; observational
Colacci 2017 (25) 92 13 30 89 17 27 12.0% 3.00 [-4.92, 10.92]
Furman 2004 (26) 76 16 28 75 18 25 9.0% 1.00 [-8.21, 10.21]
Jacobi-Pollishook 2016 (27) 91.5 16.1 283 94 15.5 282 70.6% -2.50 [-5.11, 0.11]
Madore 2017 (28) 85 13.5 34 92.4 15.4 13 8.5% -7.40 [-16.92, 2.12]
Subtotal (95% CI) 375 347 100.0% -1.94 [-4.78, 0.90]
Heterogeneity: Tau² = 1.20; Chi² = 3.31, df = 3 (P = 0.35); I² = 9%
Test for overall effect: Z = 1.34 (P = 0.18)
-100
-50
0
50
100
Favours PTF/pasteurised
Favours HM/unpasteurised

Table 2. Human studies of long-chain polyunsaturated fatty acid (LCPUFA) supplementation in preterm infants and necrotizing enterocolitis (NEC) risk.

Reference	Study Design	Population	n	Powerful and Prevalence of NEC	Principal Finding in NEC
Smithers et al. (2008) [86]	Systematic review	<37 GA	1333	RR = [0.62–2.04]	No benefit of n-3 LCPUFA supplemented formula
Zhang et al. (2014) [89]	Systemic review	<32 GA	900	RR = [0.23–1.10]	No benfit of n-3 LCPUFA supplementation
Double-blinded randomized clinical trials					
Carlson et al. (1998) [88]	Formula supplemented with 0.41% ARA + 0.13% DHA	<32 GA BW between 725–1375 g	119	Control = 17.6% Experimental = 2.9%	Significantly decreased
Fewtrell et al. (2002) [90]	Formula supplemented with 0.31% ARA + 0.17% DHA	<37 GA BW <1750 g	197	Control = 11% Experimental = 19%	No significant difference
Innis et al. (2002) [91]	BM supplemented with DHA BM supplemented with ARA + DHA	BW between 846–1560 g	194	Control = 1.6% Experimental = 1.5%	No significant difference
Fewtrell et al. (2004) [87]	Formula supplemented with 0.31% ARA + 0.17% DHA	<35 GA BW ≤2000 g	238	Control = 2% Experimental = 4%	No significant difference
Clandinin et al. (2005) [92]	Formula supplemented with DHA + ARA	<35 GA	361	Control = 3% Experimental = 5%	No significant difference
Henriksen et al. (2008) [93]	BM supplemented with 6.7% ARA + 6.9% DHA	BW <1500 g	141	Control = 3% Experimental = 1.5%	No significant difference
Makrides et al. (2009) [94]	High DHA (1%) Low DHA (0.3%)	<33 GA	657	Adj. OR = [0.87–3.22]	No significant difference
Collins et al. (2016) [95]	Formula supplemented with different doses of DHA	<30 GA	53	Control = 9% Experimental = 9%	No significant difference
Collins et al. (2017) [96]	BM supplemented with 60 mg/kg/day DHA	<29 WGA	1273	Adj. OR = [0.79–1.69]	No significant difference

In the double-blinded randomized clinical trials, the control group was no supplementation feeding. Breast milk (BM); birth weight (BW); weeks of gestational age (GA); the relative risk (RR) or adjusted odd ratio (OR) shown as 95% confidence interval.

We are just beginning to understand the functions & positive impact of various components of MBM

- Lysozyme with a 1000 times higher concentration in human milk than in cow's milk
- Osteopontin which is 10 times more concentrated in human milk than in cow's milk and which plays a role in the immunity of the child
- Bile salt stimulated lipase, present in human milk, which would improve the digestibility of long-chain fatty acids
- α-lactalbumin, which improves the absorption of iron
- Lactoferrin, about 20 times more concentrated in human milk compared to cow's milk, has antimicrobial activity, acts on the absorption of iron and is bifidogenic

Oral lactoferrin supplementation decreases late-onset sepsis, NEC, and "all-cause mortality" in preterm infants without adverse effects but authors conclude that the evidence is moderate-to low-quality

Table 1. Meta-analysis results.

	Pooled Analysis		Heterogeneity		Publication Bias			
					Egger's Test		Begg's and Mazdumdar's Tests	
	RR (95% CI)	*p*-Value	I^2	p-Value	T	*p*-Value	Z	*p*-Value
RCT								
Human milk (breastfeeding and donor) vs preterm formula $k = 6$ [6,27–31]	0.62 (0.42–0.93)	0.02	47.03	0.009	−1.82	0.144	−2.44	0.015
Human milk (breastfeeding and donor) vs preterm formula $k = 4$ * [27–30]	0.57 (0.32–1.01)	0.054	64.01	0.040	−1.64	0.243	−2.04	0.174
Observational studies								
>50° quantile of human milk of total enteral feeding $k = 7$ [48–54]	0.51 (0.31–0.85)	0.001	9.21	0.359	−2.02	0.078	−02.27	0.788
Human milk (breastfeeding and donor) vs preterm formula $k = 18$ [3–5,32–47]	0.45 (0.32–0.62)	<0.001	55.25	0.002	−0.35	0.731	0.11	0.910
Human milk (breastfeeding and donor) vs preterm formula $k = 15$ [32–47]	0.45 (0.30–0.69)	<0.001	56.61	0.004	−0.97	0.35	0.35	0.729
Human milk (breastfeeding and donor) vs mixed feeding $k = 3$ [35–37]	0.74 (0.63–0.91)	0.003	0.00	0.407	0.11	0.925	−0.68	0.497
Mixed feeding vs preterm formula $k = 4$ [37,38].	1.37 (1.13–1.65)	0.001	0.00	0.774	0.23	0.871	0.00	1.00

Legend: RCT: randomized controlled trial; RR: relative risk; CI: confidence interval; k: numbers of primary studies *Excluding paper reporting NEC (necrotizing enterocolitis) incidence >15% of in preterm formula groups.

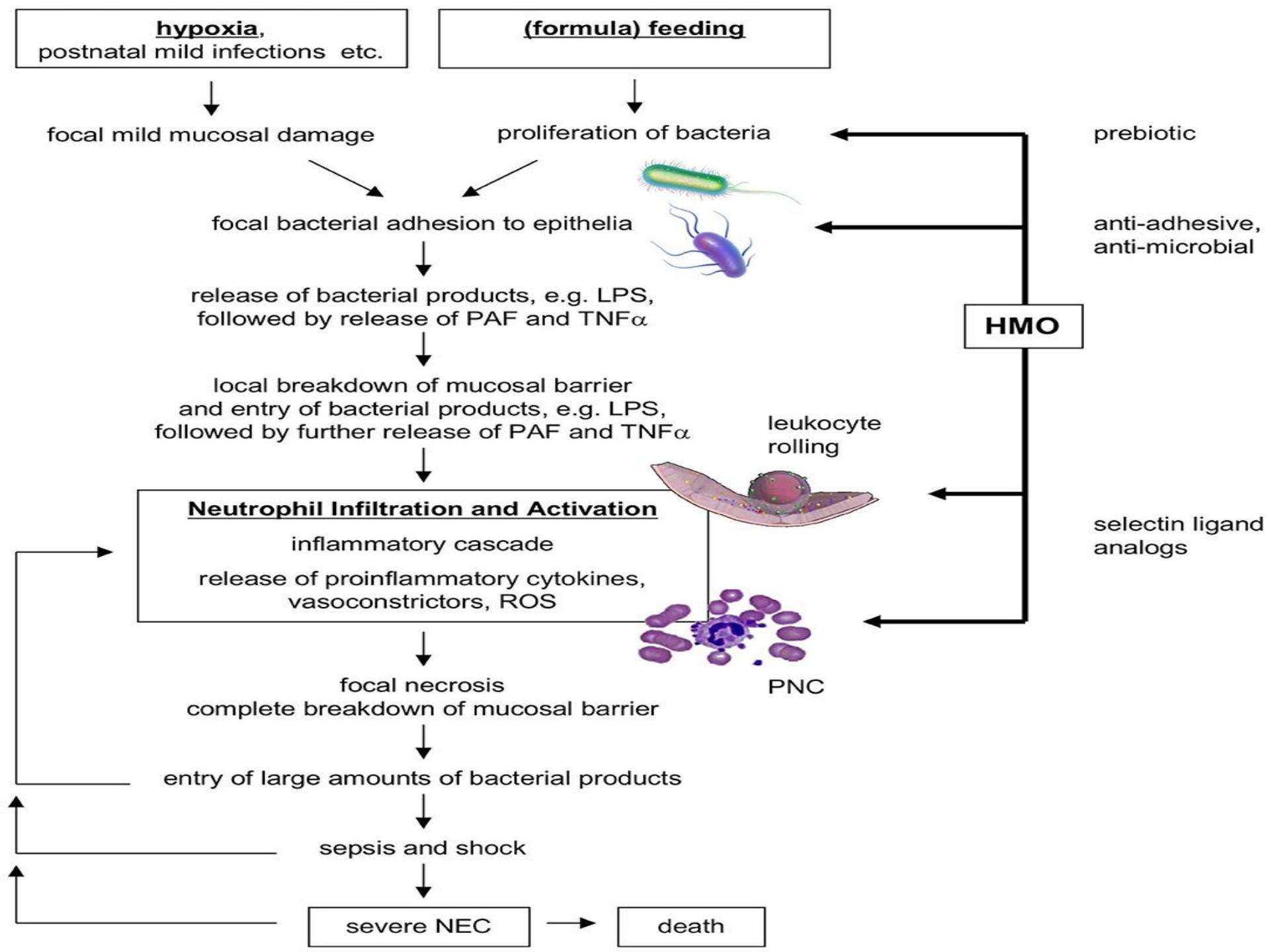

hypoxia,
postnatal mild infections etc.
(formula) feeding
focal mild mucosal damage
proliferation of bacteria
prebiotic
focal bacterial adhesion to epithelia
anti-adhesive,
anti-microbial
release of bacterial products, e.g. LPS,
followed by release of PAF and TNFα
HMO
local breakdown of mucosal barrier
and entry of bacterial products, e.g. LPS,
followed by further release of PAF and TNFα
leukocyte
rolling
Neutrophil Infiltration and Activation
inflammatory cascade
release of proinflammatory cytokines,
vasoconstrictors, ROS
selectin ligand
analogs
PNC
focal necrosis
complete breakdown of mucosal barrier
entry of large amounts of bacterial products
sepsis and shock
severe NEC
death

Table 2. Summary of Findings.

Outcome \ Comparison	EHM vs. EPTF RR or MD (95% CI); N Participants (Studies), I^8 GRADE Certainty of Evidence Interpretation and Absolute effect (95% CI)	Any HM vs. EPTF RR or MD (95% CI); N Participants (Studies), I^2 GRADE Certainty of Evidence Interpretation and Absolute Effect (95% CI)	High vs. Low Dose HM RR or MD (95% CI); N Participants (Studies), I^2 GRADE Certainty of Evidence Interpretation and Absolute Effect (95% CI)	Unpasteurised vs. Pasteurised RR or MD (95% CI); N Participants (Studies), I^2 GRADE Certainty of Evidence Interpretation and Absolute Effect (95% CI)
NEC	RTs RR 0.17 (0.02, 1.32); 53, (1 RT) Certainty: Low Obs RR 0.22 (0.09, 0.54), 933, (3 studies), I^2 = 0% Certainty: Moderate Interpretation Possible reduction in any NEC Absolute risk reduction of 4.3% (from 2.5 to 5 fewer/100)	Obs RR 0.51 (0.35, 0.76); 3783, (9 studies), I^2 = 7% Certainty: Moderate Interpretation Clear reduction in any NEC Absolute reduction of 3.6% (from 1.8 to 4.8 fewer/100)	RTs RR 0.59 (0.39, 0.89) fixed effects; 1116, (4 RTs), I^2 = 50% Certainty: Moderate Obs RR: 0.53 (0.42, 0.67); 8778 (22 studies), I^2 = 28% Certainty: Moderate Interpretation Clear reduction in any NEC Absolute risk reduction between 3.8 and 4.3 % (from 0.2 more to 6.8 fewer/100)	RT RR 1.45 (0.64, 3.30); 303 (1 tRT) Certainty: Low Obs RR 1.28 (0.68, 2.43), 1894 (6 studies), I^2 = 30% Certainty: Very low Interpretation Inconclusive
NEC requiring surgery	RT RR 0.09 (0.01, 1.64); 53, (1 RT) Certainty: Low Obs RR 0.22 (0.03, 1.86), 444, (1 study) Certainty: Very low Interpretation Inconclusive	Obs RR 0.30 (0.05, 1.76); 1420, (3 studies), I^2 = 50% Certainty: Very low Interpretation Inconclusive	RTs RR 0.36 (0.06, 2.04) 580, (2 RTs), I^2 = 66% Certainty: Low Obs RR: 0.51 (0.33, 0.79); 2964 (6 studies), I^2 = 0% Certainty: Moderate Interpretation Possible reduction in severe NEC	RT RR 0.11 (0.01, 2.06); 303 (1 RT) Certainty: Low Obs RR 1.59 (0.14, 17.85), 530 (2 studies), I^2 = 42% Certainty: Very low Interpretation

MBM & Infections

- Breast milk contains many factors that help to protect an infant against infection.
- The protection provided by these factors is uniquely valuable for an infant
- Immunoglobulin, principally sIgA coats the intestinal mucosa and prevents bacteria from entering the cells
- White blood cells which can kill micro-organisms
- Whey proteins (lysozyme and lactoferrin) which can kill bacteria, viruses and fungi
- Oligosacccharides which prevent bacteria from attaching to mucosal surfaces
- sIgA contains antibodies formed in the mother's body against the bacteria in her gut, and against infections that she has encountered, so they protect against bacteria that are particularly likely to be in the baby's environment
- Epidermal growth factor (10) stimulates maturation of the lining of the infant's intestine, so that it is better able to digest and absorb nutrients, and is less easily infected or sensitised to foreign proteins

Human Milk is Protective

- Factors present in human milk play a protective role by reducing inflammation and the subsequent invasion of pathogenic bacterial species in the gastrointestinal tract
  - The local host defenses are enhanced by the addition of secretory IgA, lactoferrin, lysozyme, and cytokines (IL10, IL-11) from human milk
  - Platelet activating factor (PAF) acetylhydrolase, which blunts the immune activation sequence promoted by PAF.
  - Components in human milk, such as epidermal growth factor, nucleotides, and glutamine also stimulate intestinal maturity.
  - Human milk antioxidants, such as vitamin E, carotene, and glutathione, also reduce oxidative stress

Fat & MBM

- High proportion of long-chain polyunsaturated fatty acids (APGI-LC), ω6 (such as arachidonic acid) and ω3 (such as eicosapentaenoic and docosahexaenoic acids [DHA]), which are derived from essential fatty acids: linoleic and α-linolenic acid
- These fatty acids are important for the brain development of the infant
- Compared to cow's milk, breast milk also contains more cholesterol, which is a precursor of hormones and is also involved in brain development
- Milk also contains enzymes including Bile Salt Stimulated Lipase (BSSL), which allows for better lipid digestibility, and better utilization of triglycerides (95% of total lipids), and presumably LC-PUFA, cholesterol, and fat-soluble vitamins

Table 2. Studies with documentation on feeding regimen prior to spontaneous intestinal perforation (SIP) diagnosis.

Authors	Institution(s), Country	Type of Study	Patients in Study (n)	Patients with SIP (n)	Mean GA (wks)	Feeding Regimen Prior to SIP	Comments
Buchheit [4]	University of Louisville, United States	R	42	21	29	Unknown	38% enteral feedings in the SIP, 86% in the NEC group ($p < 0.005$).
Kelleher [21]	Neonatal Research Network, United States	R	15751	652		Total Parenteral Nutrition ± Enteral Feeding	
Holland [11]	The Royal Alexandtia Hospital for Children Australia	R	23	23	27	Enteral Formula Feeds	6 (26%) of the 23 patients received enteral feeds prior to development of SIP
Kawase [22]	Toho University Perinatal Center, Japan	R	556	10	26.3	Unknown	
Maus [23]	Tübingen University Children's Hospital, Germany	R	77	9	26.7	Enteral feeds were initiated at 20 mL/kg/day of preterm formula on day 1.	Rates of NEC were low, whereas that of SIP was rather high at 9.4%.
Meyer [9]	Minneapolis Children's Medical Center, United States	C	250	7		No enteral nutrition	
Shah, J. [3]	The Canadian Neonatal Network, Canada	R	17426	178		Unknown	
Stavel [24]	The Canadian Neonatal Network, Canada	R	4268	129	SIP: 25 All: 34	DOL 0–2	
Varma [25]	Johns Hopkins University School of Medicine, United States	R	111	18			SIP (n = 18) Age at First Feed: 4 d. Mother's Milk: 14 (78%) Donor's Milk: 2 (11%) Cow's Milk: 1 (6%) Hydrolysate: 0 Amino Acid: 0 Unknown: 1 (6%)
	Total:		38504	1047			

R—retrospective chart review, C—case report, wks—weeks, GA—gestational age.

- In the absence of early enteral nutrition there is likely reduced protein synthesis, decreased mucin production, impaired enterocyte phospholipids, inadequate mucosal growth and a predisposition to intestinal injury and subsequent SIP development

- Decreased inflammatory response, stimulates neutrophil recruitment, selectively targets T cells and granulocytes