#### Human Milk: More than just nutrition for Preterm Infants The Science behind MBM for preterm babies



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ine - Essential mineral for brain and us sustem develo Helps membrane to form around cells and nerve signals to be ransmitted quickly.

Inha-tocopherol. Type of vitamin E, alpha opherol and other vitamin E substances are antioxidants, which means they can help defend the body against a range of membraneraging and degenera conditions

nerals are used in a variety of roles. growth and development and deficiency can lead to illness.

plex array of the mother's immune system to s well as growth factors that stimulate the

- cells that elf-renew to different organs e cells are absorbed t their function n. Stem cells are in research for

(11- 1beta), 11-2, 11-4, 1-6, 11-8, 12-10 - A group of hemical signalling molecules hey are involved in regulation to injection and

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- A hormone that induces feelings of well-being and relaxation in both the child and the mother. Involved in cause The mother's uterus contracts during feeds I for up to to minutes after the

Nucleotides otine monophosphate (5 -Når Guanosine diphosphate (UDP) 5'-(yclic adenosine monophosphate (5'(5'-cyclic AMP) (ytidine diphosphate choline (()P choline) monophosphate (5'-CMP) Wridine diphosphate N-acetylhe Cutidine nosine diphosphat Uridine monophosphate (3'-UMP) <sup>laceno</sup>ne Widine diphosphate hexose (USPH) holine (hloride Beta carotene <sub>Phorine</sub> Folic acid Vitamin B6 Vitamin B8 (Inositol) (obalt Vitamin ( Chromi Niacin a-Tocopherol Phosphorus Nicke

Copper Vitamin B12 Vitamin D Vitamin D Vitamin E Vitamin K Vitamin K Vitamin K Vitamin K Vitamin K Biotin vitamin A Sodium Iodine Riboflavin Manganese Potassium 

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tamins,

ytokines (ytokines Gustrin Motilin Stem cells Parathyroid hormone (PTH) Granulocyte-colony (G-(SF) Macrophage-colony (M-(SF) HGF-a Interferon-gamma Bombesin Epithelial growth factor (EGF)

Insulin-like growth factor-1 (14F-Erythropoietin Neurotensin HMGFI, HMGFII, HMGFI (holecystokinin

Triiodothyronine (T3) FIL Thyroid stimulating hormone (TSH) hyroid releasing hormone (TRH) oxytocin 🗃 Thy Corticosterone GRH Leptin Thrombopoietin Thromboxanes Ghrelin Gonadotropin-releasing hormone (4nRH) Adiponectin PG-EI PG-E2 Eicosanoids PG-F2 Prostaglandins Leukotrienes Hormones

(ortisol

Insulin Thyroxine

- Helps to suppress appetite. Ma , which would prevent children and adults from overeating. Also helps to reduce the amount of body fat.

approximately 16 - 20% of the human body. Amino acidi Valine Aspartate Glutamate Methionine Proline Taurine Glycine Isoleucine Serine

Threonine

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Proteins make up

Phenylalanine Tyrosine Pheni (ystine Histidine Consiting Leucin

esmostero Human Milk Respond Leukocytes Basophils Phagocytes Eosinophils B cells Neutrophils Fibromectin la Macrophages slad IgA2

IgD Lymphocytes-T cells oligosaccha Ign processes IgE Mucosal pathogens (omplement 2: Complement 3: Complement (), complement (), complement 3: complement () (amplement 3: Alpha-lactoghalain complement () (amplement 3: Alpha-lactoghalain (omplement (6, Mucins (omplement (9 , Lactadherin

Amulase polysaccharides Lysozyme Amylase Lipase Histaminase PAF-acetylhydrolase Phosphatase glycosaminoglycans disaccharides Xanthine oxidase Antiproteases a-1-antitrypsin monosaccharides Enzymes & Carbohydrater

i-(0)-i-v-(0)-v

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

> Lysozyme - Found in significant quantities in human milk. It is anti-inflammatory and bactericidal, destroying 1 1.

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orain and nervous system development. Helps to make membranes around and inside the cells and speeds up how quickly nerve signals can be transmitted

104

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).

A fatty acid involved in reducing pain and inflammation. Also thought to play a role in infant brain development

Arachidonic acid (AHA)

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Fats in general have a number of functions

including: energy sto

gliosides (GMI, GMZ, GM3) are critical to normal brain development, help nerves to repair hemselves 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 p Also thought to play a role in infant brain development.

Breastmilk is the most naturally colonised fluid in the body, with over 800 species of bacteria. Milk microbiomics is a hot topic of research, as

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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 icid, 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 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 micororganisms that need iron to survive. It also inhibits infection by Hepatitis B, Hepatitis C, ytomegalovirus, Respiratory Syncytial Virus, Adenovirus (causes the common cold), Poliovirus, Enterovirus

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

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(diarrhoeal virus) and others.

# 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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**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

BodinesRechasIndvances on structure, metabolism, and function 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.

Bioactive Components in Breast Milk	Role in Intestinal Inflammation Regulation or Prevention	Effect	References			
Microbial or microbial m	todulating factors					
Lactobacillus spp,	-Inhibit NF-κB pathway -decrease pro-inflammatory cytokines, TNF-α, IL-6 -reverse intestinal dysbicsis 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	<ul> <li>-regulate commensal bacteria</li> <li>-act as decoy receptors for pathogens</li> <li>-modulate immune signaling</li> <li>pathways, TLR3, TLR5, PAMP</li> </ul>	-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					
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 -inhibit immune response and decrease pro-inflammation -inhibit immune response and					
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-kB 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	-downnegulate 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]			

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

Abbreviations: Nuclear factor kappa B (NF- $\kappa$ B); tumor necrosis factor alpha (TNF- $\alpha$ ); 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 (NLR); transformation growth factor beta (TGF- $\beta$ ); 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- $\gamma$ ); phospholipid (PL);

#### 3.2. Cytokines

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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)- $\gamma$ , and TNF- $\alpha$  (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].

Cytokine Composition in Human Milk and Significance References Human milk IL-1ß attenuates the activation of pro-inflammatory IL-8 and suppresses pro-inflammatory responses of nuclear factor Interleukin (IL)-1 53,64 kappa beta (NF-kB) signaling. Highest in concentration in colostrum and reduced in later stages IL-2 of lactation. [63,65,66] Recruits T cells to stimulate an antigen-specific immune response. Detected in higher levels in term breast milk. Pro-inflammatory properties and is present in the acute phase of infection. IL-6 [63,67,68] Colostrum may contain anti-IL-6 antibodies that cause decreased immunoglobulin A (IgA) production by breast milk leukocytes. Decreased levels of detection in later stages of lactation. Provides chemotactic response of neutrophils. IL-8 [63,69,70] Recombinant IL-8 may improve the viability of intestinal cells when exposed to injury.

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

IL-10	<ul> <li>Maintains anti-inflammatory mechanisms involving limiting the T<sub>h</sub>1 response, inhibiting production of inflammatory cytokines, and promoting immunoglobulin synthesis.</li> </ul>					
	<ul> <li>Detected in decreasing levels with later stages of lactation.</li> </ul>					
IFN-y	<ul> <li>Increases activation of intestinal macrophages and is present in</li> </ul>					
	higher concentrations in the ileum of infants with necrotizing enterocolitis (NEC).	[76-79]				
	<ul> <li>Pro-inflammatory mechanism of action may provide an infant with defense against inflammation and infection.</li> </ul>					
	whit desense against mitaninitation and mitchion.					
	<ul> <li>Detected in decreased levels in colostrum of preterm milk.</li> </ul>					
TNF-a	<ul> <li>Present in breast milk in association with its soluble receptor,</li> </ul>	[63,80,81				
	reducing its pro-inflammatory activity.					

#### **Factors in Human Milk Reported to Enhance Protection to NEC**

<b>Breast Milk Components</b>
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Oral IgA-IgG

PAF acetylhydrolase

Long-chain polyunsaturated fatty acids

Erythropoeitin

Arginine and glutamine supplementation

Epidermal growth factor

Probiotics

Lactoferrin

Oligosaccharides

minesh khashu



**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- $\alpha$ )] 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 <u>LOS</u>, <u>severe ROP</u> 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)

Miller et al snowed 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. 10 10.953101 (2053) 19:57–62. 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.

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Huang, J., Shitta S., 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

## PEDIATRICS

ISTREET Sty: American Academy of Pediatrics

#### 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. Pediatrica 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

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- 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



unique microbiota in their expressed breast milk

BMI

Mothers have



86 mothers of preterm infants490 breast milk samples from weeks 1-8

Microbiota in preterm breast milk changes over time

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.

Delivery mode Antibiotics

3

Maternal factors are related to microbial changes in preterm breast milk during an infant's hospitalization 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,

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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- $\alpha$ ), and interferón gamma (IFN- $\gamma$ ) 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 II-6 on days 15 and 30, in IL-8 on day 30, and in TNF- $\alpha$  and INF- $\gamma$  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

 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

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### 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.



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## 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.



- 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

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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



## **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?

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#### 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<sup>18–23,28–32</sup>



### Summary

"Maternal Breast Milk for preterm babies is an exceptional example of both <u>personalised</u> and <u>precision</u> 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

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# Neonatal Nutrition for Inflammatory Disorders and Necrotizing Enterocolitis

Edited by Misty Good Printed Edition of the Special Issue Published in *Nutrients* 



minesh khashuw.mdpi.com/journal/nutrients

### 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.

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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 Alphalactalbumin

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



# Components that kill cancerous cells

Human milk contains components that kill cancerous cells.



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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.

<sup>8</sup>-@-<sup>8</sup>-<u>4</u>-@-4

oligosaccharides

polysaccharides

Arylsulfatase

Antiproteases

a-1-antitrypsin

Phosphatase glycosaminoglycans

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.

¢

disaccharides a-I-antichymotrypsin

Lysozyme (atal<u>ase</u>

Amylase

Carbohylouet

Enzymes are special

proteins that speed up specific chemical reactions

throughout the body.

Histaminase -

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

Xanthine oxidase

Histaminase

PAF-acetylhydrolase

monosaccharides

Lipase

Enzymes &

human milk

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A fatty acid required for the synthesis of molecules involved in **pain and inflammation**. Also thought to play a role in infant brain development. Human milk contains fats. Fats in general have a number of functions including: energy storage, cell messaging, hormone production and structural roles.

- 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).

Fats

Plasmalogens

2

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

Gangliosides (GMI, 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.

human mil

Tailor-made for tiny humans

GM2 Lauric acid Stigma-and campesterol Desmosterol Globoside (GB4) Methosterol Palmitic acid Glucosylceramide Lysophosphatidylcholine Linoleic acid (concept 5)/ Alpha-Linolenic acid (ALA) (concept 5) Plasmalogens Sphingolipids ....id (pHA) GMI Sphingolipids Lactosylceramide Docosahexaenoic acid (DHA) GMI Lactosylceramide Docosanexaenor Eicosapentaenoic acid (EPN) Globotriaosylceramide (GB3) 7-ketocholesterol Photoberty II Phosphatidylinositol 7-Dehydrocholesterol (holesterol Sphingomyelin Palmitoleic acid pimethylsterol Triacylglycerol (trigl iceride) Lanosterol Heptadecenoic acid Phosphatidylcholine Lathosterol Squalene Sitosterol Lysophosphatidylethanolamine β-Lathosterol (onjugated linoleic acid (Rumenic acid- active omega 6) Stearic acid Sterols GM3 Arachidonic acid (AHA) Phosphatidulethanolamine Arachidonic acid (AHA) Phosphatidylethanolamine

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.

 $\cap$ 

Human

milk

contains

HMO

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.

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

> © Human Milk CIC 2019 www.human-milk.com



 $\alpha$ 

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.

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Cortisol Insulin Corticosterone FIL Prostangelins GRH (holecystokinin Triiodothyronine (T3) Oxytocin Thyrosine Thyroid stimulating hormone (T3H) Thyroid releasing hormone (TRH) Leptin P4-F2 Thrombopoietin Thromboxanes Ghrelin Gonadotropin-releasing hormone (GnRH) Adiponectin P4-E1 PG-E2

Hormones

Eicosanoide Prostaglandins

Leukotrienes

°\_0

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.

human milk Tailor-made for ting humans

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

> 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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Laboratory tests have shown that

lactoferrin inhibits infection by Hepatitis B,

Hepatitis C, Cytomegalovirus (Herpes



# 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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E E E E

(ytidine diphosphate choline ((DP choline) -It appears to play a protective role against hypoxic brain damage, and helps to improve memory and learning.

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 $O_{\rm hmf}$ human milk

Uridine diphosp Vridine monophosphate (3'-VMP) Vridine diphosphoglucuronic acid (VDPGA) Cytidine diphosphate cho Vridine diphosphate-N-acetylhexosamine (VDPAH) (UDP) r'-cutidin

Vridine diphosphate (VDP)

Vridine diphosphate-Nacetylhexosamine (VDPAH) -

Important for the production

of essential sugars required

for normal growth and

development.

 $\stackrel{}{\sim}$ 

Nucleoti

Guanosine diphosphate – r

5'-Adenosine monophosphat

AaBb(c

00

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

 $\cap$ 

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



It causes the milkejection 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.

# 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.



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** 



**#WBW** 

# **#preventNEC**





**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.<sup>15, 82, 115–122</sup> GAG glycosaminoglycans, IL 6 interleukin 6, IgA immunoglobulin A, IL 10 interleukin 10, EGF epidermal growth factor, TNF alpha tumor necrosis factor alpha.

Pediatr Clin North Am. Author manuscript; available in PMC 2014 February 01.

c. t. 1 Exclusive human	Human milk/ unpa Events	Total	TF/pasteuri Events		Weight M	Risk Ratio M-H, Random, 95% CI	Risk Ratio M-H, Random, 95% Cl
Cristofalo 2013	milk vs exclusive pre				100 00	0 70 10 47 1 031	
cristofalo 2013 Subtotal (95% CI)	16	29	19	24	100.0%	0.70 [0.47, 1.03] 0.70 [0.47, 1.03]	
Total events	16		19				
eterogeneity: Not appl			19				
est for overall effect: Z							
	milk vs exclusive pre						
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]	
fanzoni 2013	42	314 546	32	184	83.2%	0.77 [0.50, 1.17]	
ubtotal (95% CI)		240		230	100.0%	0.71 [0.49, 1.05]	-
otal events	50 0.00; Chi <sup>2</sup> = 1.16, df =		40				
est for overall effect. Z		2 (P = 0.56).	1" = 0%				
1.3 Any human milk	vs exclusive preterm	formula; Obs	ervational				
ssad 2016	7	263	0	30	1.4%	1.76 [0.10, 30.10]	
laymore Bier 2002	3	29	0	10		2.57 [0.14, 45.79]	
urman 2003	24	79	10	40	14.4%	1.22 [0.65, 2.29]	
ylander 1998	24	123	29	89	18.4%	0.60 [0.38, 0.96]	
cobi-Pollishook 2016		565	4	46	9.1%	2.83 [1.10, 7.30]	
arker 2012	8	40	13	40	11.8%	0.62 [0.29, 1.32]	
chanler 1999	19	62	22	46	18.0%	0.64 [0.40, 1.04]	
ohr 2006	311	775	91	260		1.15 [0.95, 1.38]	-
ubtotal (95% CI)		1936		561	100.0%	0.95 [0.67, 1.34]	•
otal events	535		169				
eterogeneity: Tau <sup>2</sup> = 0 est for overall effect: Z	0.12; Chi <sup>3</sup> = 17.20, df z = 0.30 (P = 0.76)	= 7 (P = 0.02	); l <sup>2</sup> = 59%				
1.4 Higher vs lower	dose human milk inta	ke; RTs					
orpeleijn 2016	67	183	66	190	43.9%	1.05 [0.80, 1.38]	
Connor 2016	44	181	35	182	21.1%	1.26 [0.85, 1.87]	+
chanler 2005	47	151	33	92	24.9%	0.87 [0.60, 1.25]	
ullivan 2010	34	138	13	69	10.0%	1.31 [0.74, 2.31]	
ubtotal (95% CI)		653		533	100.0%	1.07 [0.89, 1.28]	+
otal events	192		147				
	0.00: Chi <sup>2</sup> = 2.48, df =	3 (P = 0.48);	$1^2 = 0.06$				
Test for overall effect: 2	z = 0.70 (P = 0.49)						
.1.5 Higher vs lower	dose human milk inta	ke; Observati	onal				
Ishaikh 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]	
ishop 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]	
olaizy et al. 2012	17	124	9	47	4.8%	0.72 [0.34, 1.49]	
urman 2003	2	32	13	29	2.2%	0.14 [0.03, 0.57]	
inovart 2016	29	114	19	72	6.4%	0.96 [0.59, 1.59]	
lair 2016	156	819	233	768	8.5%	0.63 [0.53, 0.75]	-
termann 2014	5	162	40	386	3.8%	0.30 [0.12, 0.74]	
lylander 1998	20	58	16	65	6.0%	1.40 [0.81, 2.44]	
acobi-Pollishook 2016		283	103	282	7.5%	0.35 [0.25, 0.49]	
reissl 2017	14	150	35	150	5.8%	0.40 [0.22, 0.71]	
laayan-Metzger 2012	47	188	30	172	7.0%	1.43 [0.95, 2.16]	
ladore 2017	5	56	4	25	2.6%	0.56 [0.16, 1.90]	
Connor 2003	14	141	49	322	5.9%	0.65 [0.37, 1.14]	
isk 2007	18	156	6	46	4.1%	0.88 [0.37, 2.10]	
sk 2017	62	438	15	113	6.2%	1.07 [0.63, 1.80]	
erd 2015 ubtotal (95% CI)	63	148	22	2993	7.3%	1.03 [0.71, 1.49]	
		3328	688	2993	100.0%	0.71 [0.56, 0.90]	
otal events leterogeneity: Tau <sup>2</sup> = 0 est for overall effect: Z	564 0.16; Chi <sup>2</sup> = 65.54, df z = 2.86 (P = 0.004)	= 17 (P < 0.0		4%			
A C Hannahand	s pasteurised human	milk (MOM or	donor); RT				
.1.6 Unpasteurised v.	22	151	31	152	100.0%	0.71 [0.43, 1.18]	
ossev 2013		151		152	100.0%	0.71 [0.43, 1.18]	
ossey 2013 ubtotal (95% CI)			31				
ossey 2013 abtotal (95% CI) otal events	22						
ossey 2013 ubtotal (95% CI) otal events leterogeneity: Not appl	licable						
ossey 2013 ubtotal (95% CI) otal events leterogeneity: Not appl est for overall effect: 2	licable $Z = 1.32 (P = 0.19)$	milk (MOM or	donork Obs	ervationa	4		
ossey 2013 ubtotal (95% CI) otal events leterogeneity: Not appl est for overall effect: 2 .1.7 Unpasteurised v	licable 2 = 1.32 (P = 0.19) s pasteurised human					1 09 10 91 1 311	
cossey 2013 ubtotal (95% CI) otal events leterogeneity: Not appl est for overall effect: 2 .1.7 Unpasteurised v Nicky 2017	licable 2 = 1.32 (P = 0.19) s pasteurised human 246	636	103	290	57.7%	1.09 [0.91, 1.31]	-
Cossey 2013 Jubtotal (95% CI) Total events feterogeneity: Not appli- fest for overall effect: 2 2.1.7 Unpasteurised vi Dicky 2017 Aontjaux-Regis 2011	licable 2 = 1.32 (P = 0.19) s pasteurised human 246 6	636 17	103	290 20	57.7% 3.7%	1.41 [0.52, 3.82]	
Cossey 2013 ubtotal (95% CI) Total events leterogeneity: Not appliest for overall effect: 2 2.1.7 Unpasteurised vi Nicky 2017 Montjaux-Regis 2011 chanler 2005	licable 2 = 1.32 (P = 0.19) s pasteurised human 246 6 17	636 17 70	103 5 30	290 20 81	57.7% 3.7% 13.4%	1.41 [0.52, 3.82] 0.66 [0.40, 1.08]	
ossey 2013 ubtotal (95% CI) otal events leterogeneity: Not appl est for overall effect: 2 .1.7 Unpasteurised v licky 2017 tontjaux-Regis 2011 chanler 2005 isk 2017	licable 2 = 1.32 (P = 0.19) s pasteurised human 246 6 17 46	636 17 70 299	103 5 30 16	290 20 81 139	57.7% 3.7% 13.4% 12.1%	1.41 [0.52, 3.82] 0.66 [0.40, 1.08] 1.34 [0.79, 2.28]	
bossey 2013 ubtotal (95% CI) otal events leterogeneity: Not appl est for overall effect: 2 C.1.7 Unpasteurised v: hicky 2017 tontjaux-Regis 2011 chanler 2005 isk 2017 tock 2015	licable 2 = 1.32 (P = 0.19) s pasteurised human 246 6 17	636 17 70	103 5 30	290 20 81	57.7% 3.7% 13.4% 12.1% 13.0%	1.41 [0.52, 3.82] 0.66 [0.40, 1.08]	
Cossey 2013 Subtotal (95% CI) Fotal events Heterogeneity: Not appl Fest for overall effect: 2	licable 2 = 1.32 (P = 0.19) s pasteurised human 246 6 17 46	636 17 70 299 164	103 5 30 16	290 20 81 139 159	57.7% 3.7% 13.4% 12.1% 13.0%	1.41 [0.52, 3.82] 0.66 [0.40, 1.08] 1.34 [0.79, 2.28] 1.05 [0.63, 1.75]	

S.11 Capalitien 118 months: Any Numan milk vie exclusive preterm formula: Observational become they. Conservational subcorner they. Conservational subcorner they. Conservational subcorner they. Conservational subcorner they are also be associated with the exclusive preterm formula: document of the conservational subcorner they are also be associated with the exclusive preterm formula: document of the conservational subcorner they are also be associated with the exclusive preterm formula: document of the conservational subcorner they are also be associated with the conservational subcorner they are also be associated with the conservational subcorner theorem formula: document of the conservational subco	Study or Subgroup	PTF/pas Mean	steurised SD	Total	Human mil Mean	k/upasteu SD		Weight	Mean Difference IV, Random, 95% CI	Mean Difference IV, Random, 95% CI
Subscription (20% C)										
The for overall effect 2 = 1.31 or = 0.001 Farms 2004 (D) 0.01 0.01 0.01 0.01 0.01 0.01 0.01 0.0	Subtotal (95% CI)	100	12		91	10				
$\begin{aligned} & \text{horman 2006 } (2) & 80.3 & 11.7 & 69 & 80 & 16 & 29 & 16.7 & 8 & 0.50 (-4.60, 7.60) \\ & \text{kateral dives } (16.1) & (27.1) & 14 & 16.0 $		3 (P = 0.0	123							
accol-profilements 7016 (1) 9.4.5 (1:3) 165 9.5 14 46 12.0.% $-0.50[-7.7, 1.7.4]$ thereopenetry. Tur <sup>2</sup> + 0.35 (20 <sup>4</sup> - 1.30, df = 2.0 = 0.14; F = 4.95 (2007 - 1.30,	5.1.2 Cognition 18 to <36 m	onths: Ar	ny huma	n milk v	s exclusive	preterm fo	rmula; o	bservatio	Iso	
$\begin{aligned} & \text{vort} 2006_{10} & \frac{79.9}{10} & 14 & \frac{77.9}{20} & 77.8 & 16 & \frac{200}{20} & \frac{50.08}{100.08} & \frac{4.00}{100.08} & \frac{200}{100.08} & 20$	Furman 2004 (2)	80.5	17.2	69	80	16	29	16.7%	0.50 [-6.60, 7.60]	+
Subtool (95% Cf) 10% Cf) 10.00% 2.01 [-1.35, 5.36] The transmission of the transmission of the transmission of the transmission of the transmission of trans										*
Test to everall effect Z = 1.17 $\theta = 0.2.40$ CMACC 2017 (5) 94 9 26 97 10 29 20.1X 1.00 [-4.02, 6.02] Maders 2017 (7) 94 12 11 14 92.1 11.7 921 30.1X 2.30 [-0.03, 4.00] Maders 2017 (7) 94 12 11 14 92.1 11.7 921 30.1X 2.30 [-0.03, 4.00] Maders 2017 (7) 94 12 11 14 92.1 11.7 921 30.1X 2.30 [-0.03, 4.00] Maders 2017 (7) 94 12 21 12 248 11 12 248 100.0X 2.30 [-0.50, 4.00] Maters 2016 (7) 94 12 248 11 12 248 100.0X 1.00 [-6.10, 4.00] Maters 2016 (7) 94 13 12 14 92.1 11.7 921 30.1X 2.30 [-0.50, 4.00] Maters 2016 (7) 94 13 12 14 192.1 11.7 921 30.1X 2.30 [-0.50, 4.00] Maters 2016 (7) 94 19 10 [-0.50] Maters 2016 (7) 92.0 10.3 151 94.5 10.1 146 100.0X -1.40 [-5.95, 2.75] Maters 2016 (7) 92.0 10.3 151 94.5 10.1 146 100.0X -1.40 [-5.95, 2.75] Maters 2016 (7) 92.0 10.3 151 94.5 10.1 146 100.0X -1.40 [-5.95, 2.75] Maters 2017 (10) 92.0 10.3 151 94.5 10.1 146 100.0X -1.40 [-5.95, 2.75] Maters 2017 (10) 92.0 10.3 151 94.5 10.1 146 100.0X -1.40 [-5.95, 2.75] Maters 2017 (10) 92.0 10.3 151 94.5 10.1 146 100.0X -1.40 [-5.95, 2.75] Maters 2017 (10) 94 154 20 75 15.7 22 7.10X -1.00 [-6.44, 8.44] Maters 2017 (10) 94 154 20 75 15.7 13 2.8 2X -5.00 [-5.14, 1.50] Maters 2017 (10) 94 154 20 75 15.7 13 2.8 2X -5.00 [-5.14, 1.50] Maters 2017 (10) 94 154 20 75 15.7 13 2.8 2X -5.00 [-5.44, 1.50] Maters 2017 (10) 10.9 14.6 10 94.5 11.8 \$100.0X 6.40 [-5.40, 1.50] Maters 2017 (10) 10.9 14.6 10 94.5 11.8 \$100.0X 6.40 [-5.40, 1.60] Maters 2017 (10) 10.9 14.6 10 94.5 11.8 \$100.0X 6.40 [-5.40, 1.60] Maters 2017 (10) 10.9 14.6 10 94.5 11.8 \$100.0X 6.40 [-5.40, 1.60] Maters 2018 (107) 2.7 15.8 563 \$65 16 42 9 14.00 \$10 \$4.00 \$10 \$4.00 \$1.00 \$1.00	Subtotal (95% CI)			1409		16				<b>-</b>
Code c: 2017 (3) 94 9 26 97 10 29 20.1% $1.00 [-0.51, -0.2]$ where a constrained by the second sec				(P = 0.1	(4); $1^2 = 49\%$					
Findman 2000 (6) 94.2 8.4 34 90.5 8.5 31 23.38 3.70 -0.51, 7.91 Sector 2017 (6) 94.2 11, 2 31 92.1 11, 2 32 13.8 4.50 17.06, 1.90 Partial 2007 (6) 92 13 14 91 12 22 13.8 4.50 17.06, 1.90 Partial 2007 (6) 92 13 14 91 12 22 13.8 4.50 17.06, 1.90 Partial 2007 (6) 92 13. 14 91 12 22 13.8 4.50 17.06, 1.90 Partial 2007 (6) 92 19.1 df = 4 0 = 0.03; r <sup>2</sup> = 58 Text to overall effect 2 = 0.39 0 = 0.60 Statustical (6) 92.9 19.3 151 94.5 19.1 148 100.05, -1.60 [-5.9, 2.73] Partial 2007 (6) 92.9 19.3 151 94.5 19.1 148 100.05, -1.60 [-5.9, 2.73] Partial 2007 (6) 92.9 19.3 151 94.5 19.1 148 100.05, -1.60 [-5.9, 2.73] Partial 2007 (6) 92.9 19.3 151 94.5 19.1 148 100.05, -1.60 [-5.9, 2.73] Partial 2007 (6) 92.9 19.3 151 94.5 19.1 148 100.05, -1.60 [-5.9, 2.73] Partial 2007 (6) 92.9 19.3 151 94.5 19.1 148 100.05, -1.60 [-5.9, 2.73] Partial 2007 (6) 92.9 19.3 151 94.5 19.1 148 100.05, -1.60 [-5.9, 2.73] Partial 2007 (1) 84.2 19.0 97 16 27 12.15 100 [-6.4, 8.4] Partial 2007 (1) 84.2 19.0 97 0.03; r <sup>2</sup> = 9.4 Partial 2007 (1) 84.8 13.9 24 94.7 15.1 12 25 8.40 (-0.13.2, 15.2) Partial 2007 (1) 84.8 13.9 24 94.7 15.1 12 25 8.50 (-0.01.3.2, 15.2) Partial 2007 (1) 84.8 13.9 24 94.7 15.1 12 25 8.50 (-0.01.3.2, 15.2) Partial 2007 (1) 84.8 13.9 24 94.7 15.1 18 4.5 20.9 (-0.3) 12.4 12.24 Partial 2007 (1) 84.8 13.9 24 94.7 15.1 18 4.5 100.05 6.40 [-5.40, 15.00] Partial 2007 (1) 84.8 13.9 24 94.7 15.1 18 8.100.05 6.40 [-5.40, 15.00] Partial 2007 (1) 84.8 13.9 24 94.5 11.4 4.100.05 (-2.01 (-5.40, 16.60) Partial 2007 (1) 10.9 10.9 (-0.10) Partial 2007 (1) 10.9 14.6 10 94.5 11.4 8.100.05 (-2.01 (-5.30, 16.60) Partial 2007 (1) 10.9 14.6 10 94.5 11.4 8.100.05 (-2.00 (-5.20, 1-6.51, 1.51) Partial 2007 (1) 200 14.7 12.7 15.4 12.9 10.1 12 12 14.6 14.0 14.0 2.2 10.02 (-2.00 (-5.40, 14.0) Partial 2007 (1) 8.7 11.8 1.1 12 94 18.5 148 100.05 (-2.00 (-5.40, 14.0) Partial 2007 (1) 8.7 11.8 1.2 119 94 18.5 148 100.05 (-2.00 (-5.10, 15.1) Partial 2007 (1) 8.7 11.8 1.2 194 18.5 14.2 10.00 (-2.00 (-5.20, 10.0) Partial 2007 (1) 8.7	5.1.3 Cognition <18 months	: Higher v	s lower	dose hu	man milk; O	bservation	sal			
Mader 2017 (7) 87.6 11.7 33 97.1 11.8 13 12.85 $-5.00 (-1.706 - 1.84)$ 12.28 13.28 $-5.00 (-1.706 - 1.84)$ 12.28 13.00 $-5.00 (-5.00 - 1.84)$ 12.29 13.1 13 14 100.00 $-1.60 (-5.95, 2.73)$ 12.20 14.00 $-5.00 (-5.90 - 1.84)$ 12.20 14.00 $-5.00 (-5.90 - 1.84)$ 12.20 14.00 $-5.00 (-5.84, 8.84)$ 13.1 14 100.00 $-1.60 (-5.95, 2.73)$ 14.8 100.00 $-1.50 (-1.53, 2.15, 2.50)$ 14.8 100.00 $-2.50 (-1.53, 2.15, 2.50)$ 14.8 100.00 $-2.50 (-1.53, 2.50)$ 14.8 100.00 $-2.50 (-1.53, 2.50)$ 14.8 100.00 $-2.50 (-1.53, 2.50)$ 14.8 100.00 $-2.50 (-1.53, 2.50)$ 14.8 100 0.00 $-2.50 (-1.53, 2.50)$ 14.8	Colacci 2017 (5)	98	9	26	97	10	29	20.1%	1.00 [-4.02, 6.02]	+
Decomposition 2003 (a) 94.4 (1).7 (14) 92.1 (1).7 (12) 210.1% (20) (-0.36, 0.90) (20) (-10.36, 0.90) (-10.36,										-
$\begin{aligned} & \text{Partial 2003 rgs}_{CD} & 92 & 15 & \frac{14}{24} & 91 & 12 & 21 & 11.8 & 0.007 (-6.18, 4.03) \\ & \text{Loc}(7-6.18, 4.03) & 0.07 (-6.18, 4.03) \\ & \text{Loc}(7-6.18, 4.03) & 0.03 (-6.18, 6.01, 5.40, 18, 6.01, 5.40, 18, 6.01 \\ & \text{Loc}(7-6.18, 4.03) & 0.03 (-6.18, 6.01, 5.40, 18, 6.01 \\ & \text{Loc}(7-6.18, 4.03) & 0.03 (-6.18, 6.01, 5.40, 18, 6.01, 5.40, 18, 6.01 \\ & \text{Loc}(7-6.18, 4.03) & 0.03 (-6.18, 6.01, 5.40, 18, 6.01 \\ & \text{Loc}(7-6.18, 4.03) & 0.03 (-6.18, 6.01, 5.40, 18, 6.01 \\ & \text{Loc}(7-6.18, 4.03) & 0.03 (-6.18, 6.01, 5.40, 18, 6.01 \\ & \text{Loc}(7-6.18, 6.01, 5.40, 18, 6.01, 5.40, 18, 6.01 \\ & \text{Loc}(7-6.18, 6.01, 5.40, 18, 6.01, 5.40, 18, 6.01 \\ & \text{Loc}(7-6.18, 6.01, 5.40, 18, 6.01, 5.40, 18, 6.01 \\ & \text{Loc}(7-6.18, 6.01, 5.40, 18, 6.01, 5.40, 18, 6.01 \\ & \text{Loc}(7-6.18, 6.01, 5.40, 18, 6.01, 5.40, 18, 6.01 \\ & \text{Loc}(7-6.18, 6.01, 6.01$										
Subtotal (95% CD) 268 ( $p^2 - 38$ ) 416 100.0% 0.67[-2.68,4.03] Histophanetry, Tay 7.95, CD = 9.41, eff = 0 = 0.57; ( $p^2 - 5.88$ ) S.1.4 Cognition 18 0.3 Generations: Higher vs lower dose human milk; RT S.1.4 Cognition 18 0.3 Generations: Higher vs lower dose human milk; RT First for vorsall effect 2 = 0.72 ( $p^2 - 0.47$ ) S.1.5 Cognition 18 0.5 Generations: Higher vs lower dose human milk; observational S.1.5 Cognition 18 0.5 Generations: Higher vs lower dose human milk; observational S.1.5 Cognition 18 0.5 Generations: Higher vs lower dose human milk; observational S.1.5 Cognition 29 ( $p^2 - 0.47$ ) Histophanetry, S.1.5 ( $p^2 - 0.52$ ) Histophanetry, S.1.5 ( $p^2 - 0.54$ ) Histophanetry, S.1.5 ( $p$										
Heterogenetic Tau' = 7.95; Ch <sup>2</sup> = 9.61; df - 4 (P = 0.05); l <sup>2</sup> = 548; Est for varial left Cz = 0.39; C = 0.67] <b>L1.4 Cognition 18 to 36 months: Higher vs lower dose human milk; RT</b> ViChnow 2016 (10) 9.2.9 10.3 151 9.4.5 19.1 144 100.000; -1.60 (-5.95, 2.75] Heterogenetic No applicable Test for varial left Cz = 0.72 (P = 0.47) <b>L1.5 Cognition 18 to 36 months: Higher vs lower dose human milk; observational</b> Codard 2017 (11) 98 14 30 97 16 27 12.156 L00 (-6.84, 8.84] Human 2004 (12) 6113 98 121 28 77 14 22 8.5.0 (-1.3.2, 15.5.2) Madore 2017 (14) 88 13 33 34 9.4.7 15.1 18 8.5.3 -5.90 (-1.3.2, 15.5.2) Madore 2017 (14) 88 13 33 34 9.4.7 15.1 18 8.5.3 -5.90 (-1.3.4, 1.5.4] Human 2004 (12) 10 (9.4.8 13.3) 34 9.4.7 15.1 18 8.5.3 -5.90 (-1.3.4, 1.5.4] Human 2004 (12) 10 (9.4.8 13.3) 34 9.4.7 15.1 18 8.5.3 -5.90 (-1.3.4, 1.5.4] Human 2004 (12) 10 (9.4.8 13.3) 34 9.4.7 15.1 18 4.5.3 -5.90 (-1.3.4, 1.5.4] Human 2004 (12) 10 (9.4.3 1.4.6 10 (9.4.3 1.4.6 4) (-3.9.8 (-3.4.6 (-5.40, 1.5.60)) Human 2004 (12) 10 (9.4.3 1.4.6 10 (9.4.3 1.4.6 4) (-3.9.8 (-5.50, 1.5.60)) Human 2004 (12) 10 (9.4.3 1.4.6 10 (9.4.3 1.4.6 4) (-5.4.6 (-5.4.0, 1.5.60)) Human 2004 (13) 10 (9.4.3 1.4.6 10 (9.4.3 1.4.6 4) (-5.5.80, 18.60) Human 2005 (13) 10 (9.4.3 1.4.6 10 (9.4.3 1.4.6 4) (-5.5.80, 18.60) Human 2006 (17) 22.7 15.8 56 95 14 46 14.6.6 (-5.5.0 (-5.2.5, 1.5.5)) Human 2006 (17) 22.7 15.8 56 95 14 46 14.6.6 (-5.5.0 (-5.2.5, 1.5.5)) Human 2006 (17) 22.7 15.8 56 95 14 46 14.6.6 (-5.2.6.2, 1.5.5)] Human 2006 (17) 22.7 15.8 56 95 14 46 10.0.000 (-0.4.6.3, 1.4.5)] Human 2006 (17) 22.7 15.8 56 95 14 46 10.0.000 (-0.4.6.3, 1.5.5)] Human 2006 (17) 22.7 15.8 56 95 14 46 10.0.000 (-2.2.0 (-5.5.3, 1.5.5)] Human 2006 (17) 22.7 15.8 56 95 14 46 10 (0.000 (-2.2.0 (-6.4.2, 2.0.2)] Human 2006 (19) 15 1.8 17 14 12 (19.6.000 (-2.2.0 (-6.4.2, 2.0.2)] Human 2006 (19) 15 1.8 17 14 12 (19.6.000 (-2.2.0 (-6.4.2, 2.0.2)] Human 2006 (19) 15 1.8 17 14 18 10 (-0.000 (-2.2.0 (-6.4.2, 1.0.2)] Human 2006 (10) 74 15.2 (-7.1.5.2 (-7.1.5.1 14) 114		92	12		31	14				•
L1 4 Capaniton 18 to 456 months: Higher vs lower dose human milt; RT PCCompr 2016 (10) 2.9 19.3 151 94.5 19.1 144 100.0% $-1.60[-5.95, 2.75]$ Hereogeneric, for applicable fear for worked effect 2 = 0.72 $\theta$ = 0.47 L1 5 Capatiton 10 to 56 months: Higher vs lower dose human milt; observational subtraction 2016 (12) 84 15.4 281 95 15.7 281 710% $-1.00[-6.84, 8.64]$ Hereogeneric, for a 30 $\theta$ = 0.37; $\theta$ = 0	Heterogeneity: Tau <sup>3</sup> = 7.95; C				5); i <sup>2</sup> = 5.8%					Ī
$\begin{aligned} & \text{Common 2016 (10)} & 92.9 & 10.3 & 151 & 94.5 & 10.1 & 144 & 100.08 & -1.40 (-5.95, 2.75) \\ & Text for overall effect 2 = 0.72 ($ $ $ $ $ $ $ $ $ $ $ $ $ $ $ $ $ $ $$				lower do	se human n	nilk; RT				
The the comparison is not applicable Test for overall effect $2 = 0.22 \ p = 0.47$ S.1.5 Cognition 18 to 36 months: Higher vs lower dose human milk; observational Colarci 2017 (1) 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% (-0.01.5.2, 15.52) S.1.6 Cognition 18 to 36 months: Higher vs lower dose human milk; observational Colarci 2017 (21) 98 14 12.9 24 79 15.1 13 15.53 - 1.50] Subtoal (15%) 100.9 14.6 10 94.5 11.8 8 100.0% -0.58 (-5.44, 2.24) S.1.6 Cognition - 3 y years; Higher vs lower dose human milk; Observational Teachas 2010 (15) 100.9 14.6 10 94.5 11.8 8 100.0% 6.40 (-5.80, 18.60) Heterogenetic, Tay - 1.17, Ch <sup>2</sup> - 1.30 $p^{2}$ - 0.30) S.1.8 Moort 18 to -34 months: Higher vs lower dose human milk; Observational Teachas 2016 (15) 74.5 17 69 80 16 29 24.6% - 5.50 (-12.57, 1.57) a taobi-following 2016 (16) 74.5 17 69 80 16 29 24.6% - 2.50 (-12.57, 1.57) a taobi-following 2016 (17) 74.5 17 69 80 16 29 24.6% - 2.50 (-12.57, 1.57) a taobi-following 2016 (16) 74.5 17 69 80 16 29 24.6% - 2.50 (-12.57, 1.57) a taobi-following 2016 (17) 92.7 15.8 555 95 14 46 34.6% - 2.50 (-12.57, 1.57) a taobi-following 2016 (17) 92.7 15.8 555 95 14 45 14.8 100.0% -0.40 (-5.56, 19.62) Webbraid 187.0 4 8.4 19 2 (-2.0.0.1); $p^{2} - 77x$ Test for overall effect 2 - 0.30 $p^{2} - 0.70$ ; S.1.9 Moort 18 to -34 months: Higher vs lower dose human milk; Observational Cacco 2017 (20) 84.4 13.7 151 94 18.5 148 100.0% -2.20 (-6.42, 2.02) Webbraid 187x CD = 10.2 $p^{2} - 71x$ Test for overall effect 2 - 1.0 $p^{2} - 0.31$ ; S.1.10 Moort 2016 (17) 9 1.8 18.7 151 94 18.5 148 100.00% -2.20 (-6.42, 2.02) Webbraid 187x CD = 10.2 $p^{2} - 71x$ Test for overall effect 2 - 0.1 $p^{2} - 81x$ Test for overall effect 2 - 0.1 $p^{2} - 81x$ Test for overall effect 2 - 0.1 $p^{2} - 81x$ Test for overall effect 2 - 0.1 $p^{2} - 81x$ Test for overall effect 2 - 0.1 $p^{2} - 81x$ Test for overall effect 2 - 0.1 $p^{2} - 81x$ Test for overall effect 2 - 0.1 $p^{2} - 81x$ Test for overall e	O'Connor 2016 (10)		-	151						
Test for overall effect $2 = 0.72 (P = 0.47)$ S.15 Cognition 18 to 36 months: Higher vs lower dose human milk; observational Colacci 2017 (11) 98 14 30 97 16 27 12.18 1.00 [-6.84, 8.84] Larcobi-Polishook 2016 (13) 94 15.8 283 95 15.7 282 71.00K -1.00 [-3.52, 15.52] Larcobi-Polishook 2016 (13) 94 15.8 283 95 15.7 282 71.00K -0.30 [-5.32, 15.52] Larcobi-Polishook 2016 (13) 94 15.8 283 95 15.7 282 71.00K -0.00 [-5.80, 18.60] Subboal 95% CD 8-8 13.0 df = 3 $P = 0.35$ ; $P^* = 9X$ Test for overall effect 2 = 0.41 $P = 0.68$ S.16 Cognition > 3 years; Higher vs lower dose human milk; Observational Tanaka 2010 (15) 100.9 1.4 10 94.5 11.8 \$ 100.00K 6.40 [-5.80, 18.60] Subboal 95% CD 8-3 727 1.7 8 65 80 14 26 24.40K -2.30 [-5.50, 1-12.57, 1.57] Tanaka 2010 (15) 100.9 1.4 10 97.5 81.3 17 260 4.40K -2.30 [-6.02, 4.42] Volve 2006 (15) 84.6 11 97.75 81.3 17 260 4.40K -2.30 [-6.02, 4.42] Heterogenety, Tau <sup>2</sup> = 15.81 CP <sup>2</sup> = 8.84, df = 2 (P = 0.01); P = 77% Test for overall effect 2 = 0.30 9 .0.70 S.1.9 Moort 18 to -38 months: Higher vs lower dose human milk; RT Volve 2006 (15) 9.1 81.7 151 94 18.5 1448 100.00K -2.20 [-6.40, 4.40] Test for overall effect 2 = 0.30 (P = 0.31) S.1.9 Moort 18 to -38 months: Higher vs lower dose human milk; CD servational Calcol 2017 (20) 87 11 2 6 89 11 29 18.65 -2.00 [-8.40, 4.40] Feetogenety, Not applicable Test for overall effect 2 = 0.30 (P = 0.31); S.1.10 Moort 2.2 0.2 (P = 0.31) S.1.10 Moort 2.2 0.2 (P = 0.31); S.1.10 Moort 2.2 0.2 (P = 0.31); S.1.10 Moort 2.2 0.2 (P = 0.31); S.1.11 Moort 18 to -38 months: Higher vs lower dose human milk; Observational Calcol 2017 (20) 87 113 26 89 11 29 18.65 -2.00 [-8.40, 4.40] Feetogenety, Tau <sup>2</sup> = 17.22; CV <sup>2</sup> = 11.0, df = 4 (P = 0.01); P = 6.95 Test for overall effect 2 = 0.40 (P = 0.33); P = 27 Test for overall effect 2 = 0.40 (P = 0.33); P = 27 Test (P = 0.40, 20); P = 33 1.4.4.3 12 0.5 X 7.40 (-8.31, 33); Heterogenety, Tau <sup>2</sup> = 17.22; CV <sup>2</sup> = 11.0, df = 4 (P = 0.01); P = 27 Test (P = 0.40, 20); P = 3.1 1.4.3 12 0.5 X				151			148	100.0%	-1.00 [-5.95, 2.75]	1
Colact 2017 (11) 98 14 30 97 16 27 12.18 1.00 (-6.84, 8.84) turman 2004 (12) 85 21 28 79 14 25 8.4% 6.00 (-3.52, 15.52) accobi-Polishook 2016 (13) 34 13.8 283 93 13.7 282 71.0% -1.00 (-3.60, 1.80) babbaal 95% CD 37 5 4.7 15.1 347 100.00% -0.39 (-3.41, 2.24) rest for overall effect 2 = 0.310, df = 3 $\theta$ = 0.35); f <sup>2</sup> = 9% rest for overall effect 2 = 0.41 $\theta$ = 0.68 <b>5.16 Cognition &gt; 3 years; Higher vs lower dose human milk; Observational</b> ranka 2010 (15) 100.9 14.6 10 94.5 11.8 8 100.0% 6.40 (-5.80, 18.60) babbaal 95% CD 10.9 3 (-5.90, 11.8) 8 100.0% -5.50 (-1.2.57, 1.57) babbaal 95% CD 10.9 (-1.3.8), 18.60 babbaal 95% CD 20.6 (17) 7.57 (-1.3.8) 555 50 (-1.2.5.7), 15.71 babbaal 95% CD 20.6 (17) 7.57 (-1.3.8) 555 50 (-1.2.5.7), 15.71 babbaal 95% CD 20.6 (17) 7.51 (-1.3.8), 17.2 (-0.4.0.4.8) 13.0 (0.4.5.7, 76] babbaal 95% CD 20.6 (17) 7.51 (-2.7.7) 8 (-3.3.17) 7.56 (-4.0.4.8) 1.9 (-3.60, 18.60) babbaal 95% CD 20.12 (-1.3.8), 13.7 (-2.6.4.6.4.7, 76] babbaal 95% CD 20.12 (-1.3.8), 13.7 (-2.6.4.6.4.7, 76] babbaal 95% CD 20.13 (-1.3.7) 13.8 (-2.6.9, (-4.0.4.4)] babbaal 95% CD 20.13 (-1.3.7) 13.9 (-0.3.1); 13.7 (-2.6.9, -2.00 (-6.4.0, 4.4)] babbaal 95% CD 20.12 (-2.7.9) 65.4 (-1.5.3.9) (-2.5.9, (-3.1.3.7)] babbaal 95% CD 20.12 (-2.7.9) 65.4 (-1.5.9, -2.00 (-6.4.0, 4.4)] babbaal 95% CD 20.12 (-2.7.9) 65.4 (-2.6.9, -0.3.1); 15.7 (-2.7.0) (-6.4.0, 4.4)] babbaal 95% CD 20.12 (-2.7.9) 65.4 (-2.6.9, -0.3.1); 15.7 (-2.7.0) (-6.4.0, 4.4)] babbaal 95% CD 20.12 (-2.7.9) 65.4 (-2.6.9, -0.3.1); 15.7 (-2.7.0) (-6.4.0, 4.4)] babbaal 95% CD 20.6 (-2.7.9) 15.5 (-2.6.9, -0.3.1); 15.7 (-2.7.0) (-6.4.0, 4.4)] babbaal 95% CD 20.6 (-2.7.9) 15.3 (-2.6.		2 (P = 0.4	71							
Lumma 2004 (12)         is         21         28         79         14         25         8.44         6.00 [-3.52, 15.2]           Mador 2017 (14)         8.8         13.9         32         94.7         15.1         137         6.25         71.04         -1.00 [-3.60, 1.600]           Mador 2017 (14)         8.8         13.9         32         94.7         15.1         137         6.25         71.04         -1.00 [-3.60, 1.600]           Station 2017 (14)         8.8         13.9         32         94.7         15.1         137         6.30 [-5.30, 1.600]           Station 2017 (15)         10.9         1.6         10         94.5         11.8         8         100.005         6.40 [-5.80, 18.60]           Station 2017 (15)         10.9         1.0         94.5         11.8         8         100.005         6.40 [-5.80, 18.60]           Station 2017 (15)         10.7         2.7         1.5         5.55         95         1.4         46         34.64         -2.30 [-6.35, 1.55]           Station 2017 (10)         2.7         1.5         5.55         95         1.4         46         34.64         -2.30 [-6.42, 2.02]           Station 2016 (17)         2.7         1.5         8										
lacobi-polishook 2016 (13)       94       15.8       283       95       15.7       282       71.0%       -1.00       1-3.60       1.60         Madore 2017 (14)       85.8       13.9       34.5       94.7       15.1       347       100.0%       -0.59       1-3.43       3.44         Heterogeneity: Tau" = 1.17: CP <sup>2</sup> = 3.30, dt = 3 g = 0.35); t <sup>2</sup> = 9x       -0.53       1.6.6       1.0       94.5       11.8       8       100.0%       6.40       1-3.60, 1.8.60         Test for overall effect: Z = 0.41 (P = 0.68)       10       94.5       11.8       8       100.0%       6.40       1-5.80, 1.8.60         Heterogeneity: Tau" = 1.17: CP <sup>2</sup> = 0.30       5.15       10       94.5       11.8       8       100.0%       6.40       1-5.80, 1.8.60         Heterogeneity: Tau" = 1.03 (P = 0.30)       5.15       16       20       24.64       -5.50 [-12.57, 1.57]       1.8.60         Stabolat (15%)       74.5       17       69       0       16       20       24.64       -5.00 [-6.22, 7.0]       1.5.71       1.51       1.60       1.5.8       1.60       1.5.93       1.60       1.5.93       1.60       1.60       1.6.6       1.60       1.6.6       1.60       1.6.6       1.6.6       1.6.6<										
Maddore 2017 (14) 88.8 13.9 $\frac{34}{24}$ 94.7 15.1 $\frac{13}{24}$ 18.5 $\frac{5.90[-15,34,3.54]}{347}$ 100.05 $\frac{-5.90[-15,34,3.54]}{347}$ 110.05 $\frac{-5.90[-15,34,3.54]}{347}$ 112.05										
Subtatel (95% C) 375 347 100.0% $-0.59$ ( $-3.41$ , $2-2.4$ ) Test for overall effect $2 = 0.41$ , $p = 0.68$ ) S.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) Subtatel (95% C) 100.9 14.6 10 94.5 11.8 8 100.0% 6.40 ( $-5.80$ , 18.60) Heterogeneity: Not applicable Test for overall effect $2 = 1.01$ ( $p = 0.30$ ) S.1.8 Motor 18 to <36 months: Any human milk vs exclusive preterm formula; Observational Furman 2004 (16) ( $17$ 74.5 17 69 80 16 29 24.6% $-5.50$ ( $-12.57$ , $1.57$ ) Yow 2006 (18) Subtatel (95% C) 1409 137 260 40.5% $-2.20$ ( $-6.53$ , $0.580$ , $18.60$ ) S.1.8 Motor 18 to <36 months: Higher vs lower dose human milk; RT O'Connor 2016 (19) 91.5 18.7 151 94 18.7 151 94 18.7 151 94 18.1 00.0% $-2.20$ ( $-6.42$ , $2.02$ ] Heterogeneity: Nat applicable Test for overall effect $2 = 0.31$ ): S.1.9 Motor 18 to <36 months: Higher vs lower dose human milk; CD Servational Kertogeneity: Nat applicable Test for overall effect $2 = 0.31$ ; $p = 0.31$ ; $p = 77\%$ Test for overall effect $2 = 0.31$ ; $p = 0.31$ ; $p = 77\%$ Test for overall effect $2 = 0.31$ ; $p = 0.33$ ; $p = 0$										
Heterogeneity: Tau <sup>2</sup> = 1.17; Ch <sup>2</sup> = 3.30, df = 3 $P = 0.35$ ; t <sup>2</sup> = 9% Test for overall effect Z = 0.41 $P = 0.68$ <b>3.1.6 Cognition</b> > 3 years; Higher vs lower does human milk; Observational Transka 2010 (15) Subtoal (95% CD) 10.0.9 14.6 10 94.5 11.8 \$ 100.0% 6.40 [-5.80, 18.60] Heterogeneity: Not applicable Test for overall effect Z = 1.03 $P = 0.30$ ) <b>3.1.6 nonsh:</b> Any human milk; vs exclusive preterm formula; Observational Turman 2004 (16) 74.5 17 69 80 16 29 24.6% -5.50 [-12.57, 1.57] acobi-folishkok 2016 (17) 92.7 15.8 555 95 14 46 34.6% -3.30 [-6.55, 1.95] Your 2006 (18) 84.6 19 2725 81.3 17 260 46.8% 3.30 [0.44, 5.76] Your 2006 (18) 84.6 19 2725 81.3 17 260 46.8% 3.30 [0.44, 5.76] Your 2006 (18) 84.6 19 2725 81.3 17 260 46.8% 3.30 [0.45, 5.76] Your 2006 (18) 9 1.8 16.7 151 94 125 14.6 100.0% -2.20 [-6.42, 2.02] Heterogeneity: Not applicable Test for overall effect Z = 0.30 $P = 0.76$ ) <b>3.1.9 Motor</b> 18 to <36 months: Higher vs lower dose human milk; <b>Chservational</b> Chance 2017 (20) 87 13 26 89 11 29 18.6% -2.00 [-6.42, 2.02] Heterogeneity: Not applicable Test for overall effect Z = 0.2 $P = 0.31$ ) <b>3.1.10 Motor</b> (18 months: Higher vs lower dose human milk; <b>0</b> Servational Chance 2017 (20) 87 13 26 89 11 29 18.6% -2.00 [-6.42, 2.02] Heterogeneity: Not applicable Test for overall effect Z = 0.13] <b>3.1.10 Motor</b> (18 months: Higher vs lower dose human milk; <b>0</b> Servational Chance 2017 (20) 87 13 26 89 11 49 124 18.7% -2.00 [-6.40, 4.40] Yourma 2003 (21) 85.8 11.5 34 78 11.4 31 20.5% 7.80 (2.2, 3, 13.37] Yourma 2003 (20) 85.3 14.9 141 87.1 14.9 322 26.7% -1.80 (-4.75, 1.15] Yourma 2003 (20) 85.3 14.9 141 87.1 14.9 322 26.7% -1.80 (-4.75, 1.15] Yourma 2003 (20) 85.3 14.9 141 87.1 14.9 322 26.7% -1.80 (-4.75, 1.15] Yourma 2003 (20) 85.3 14.9 141 87.1 14.9 322 26.7% -1.80 (-4.75, 1.15] Yourma 2004 (26) 97 6 15 28 97 17 27 12.0% 1.00 (-6.21, 10.21] Heterogeneity: Tau' = 17.22; Ch'' = 13.10, df = 4 P = 0.01); t <sup>2</sup> = 69% <b>3.1.11 Motor</b> 18 to <36 months: Higher vs lower dose human			13.3		34.1					•
Subtotal (95% Cf) 10 10 8 100.0% 6.40 [-5.80, 18.60] Heterogeneity: Not applicable Test for overall effect Z = 1.03 $\rho = 0.30$ ) 5.1.8 Motor 18 to <36 months: Any human milk vs exclusive preterm formula; Observational forman 2004 (16) 74.5 17 69 80 16 29 24.6% -5.50 [-12.57, 1.57] iacobi-Politohook 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 12 260 40.8% 3.30 [0.6.45, 5.76] iacobi-Politohook 2016 (17) 92.7 15.8 565 95 14 46 34.6% -2.30 [-6.52, 4.42] Heterogeneity: Tau" = 15.81; Che" = 8.84, df = 2 ( $P = 0.01$ ); $P = 77\%$ 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] Heterogeneity: Not applicable Test for overall effect Z = 1.02 ( $P = 0.31$ ) 5.1.19 Motor 18 to <36 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) 85.4 14.4 33 93.1 7.8 13 8.4% -6.70 [-13.19, -0.21] O'Connor 2013 (23) 86.4 14.4 33 93.1 7.8 13 18.4% -6.70 [-13.19, -0.21] O'Connor 2013 (23) 86.4 14.4 33 93.1 7.8 13 18.4% -6.70 [-13.19, -0.21] O'Connor 2013 (23) 86.4 14.4 33 93.1 7.8 13 18.4% -6.70 [-13.19, -0.21] O'Connor 2017 (22) 86.4 14.6 $P = 0.01$ ); $P = 69\%$ Test for overall effect Z = 0.14 $P = 0.49$ 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] 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] Furma 2004 (26) 76 16 28 75 18 25 9.0% 1.00 [-6.81, 8.83] Furma 2004 (26) 76 16 28 75 18 25 9.0% 1.00 [-6.81, 10.21] Furma 2004 (26) 76 16 28 75 18 25 9.0% 1.00 [-6.91, 11.0, 21] Furma 2004 (26) 76 16 28 75 18 25 9.0% 1.00 [-6.91, 11.0, 21] Furma 2004 (26) 76 16 28 75 18 25 9.0% 1.00 [-6.91, 11.0, 21] Furma 2004 (26) 76 16 28 75 18 25 9.0% 1.00 [-6.91, 1.0, 21] Furma 2004 (26	Test for overall effect: Z = 0.4	1 (P = 0.6	58)			rvational				
Heterogeneity: Not applicable Test for overall effect: $2 = 1.03 \ (P = 0.30)$ <b>5.1.8</b> Motor <b>18</b> to <36 months: Any human milk vs exclusive preterm formula: Observational Furman 2004 (16) 74.5 17 69 80 16 29 24.68 -5.50 [-12.57, 1.57] (actob)-Polishoo2 2016 (17) 92.7 15.8 565 95 14 46 34.68 -2.10 (-6.55, 1.95] Subtotal (95% C) 1409 81.3 17 35 100.0% -0.80 [-6.02, 4.42] Heterogeneity: Tau' = 15.81; Chr <sup>2</sup> = 8.84, df = 2 (P = 0.01); t <sup>2</sup> = 77% Test for overall effect: $2 = 0.30 \ (P = 0.76)$ <b>5.1.9</b> Motor <b>18</b> 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] Heterogeneity: Not applicable Test for overall effect: $2 = 1.02 \ (P = 0.31)$ <b>5.1.0</b> Motor <b>18</b> to <36 months: Higher vs lower dose human milk; Observational Colacci 2017 (20) 87 13 26 89 11 29 18.68 -6.70 [-13.19, -0.21] Colacci 2017 (20) 87 13 26 89 11 29 18.69 -6.70 [-13.19, -0.21] Colacci 2017 (20) 87 13 26 89 11 29 18.69 -6.70 [-13.19, -0.21] Colacci 2017 (20) 87 13 26 89 11 29 18.69 -6.70 [-13.19, -0.21] Colacci 2017 (20) 87 13 26 89 11 29 18.69 -6.70 [-13.19, -0.21] Colacci 2017 (20) 87 13 26 89 11 29 18.69 -6.70 [-13.19, -0.21] Colacci 2017 (20) 87 13 26 89 11 29 18.69 -6.70 [-13.19, -0.21] Colacci 2017 (20) 87 13 26 89 11 29 18.69 -6.70 [-13.19, -0.21] Colacci 2017 (20) 87 13 26 89 11 29 18.69 -6.70 [-13.19, -0.21] Colacci 2017 (21) 86.4 14.4 33 93.1 7.8 13 18.4% -6.70 [-13.19, -0.21] Colacci 2017 (22) 86.4 14.4 9 20 2.69 +7 14 21 5.57 1.00 [-4.57, 1.05 [-4.57, 1.05 [-4.57, 1.05 [-4.50, 4.14] Heterogeneity: Tau' = 17.27; Chr <sup>0</sup> = 13.10, df = 4 (P = 0.01); t <sup>2</sup> = 69% Test for overall effect: $2 = 0.14 \ (P = 0.89)$ <b>5.1.11 Motor 18 to 36 months: Higher vs lower dose human milk; observational</b> Colacci 2017 (25) 92 13 30 89 17 27 12.0% 3.00 [-4.52, 10.92] Test for overall effect: $2 = 0.14 \ (P = 0.89)$ <b>5.1.12 61</b> 28 75 18 25 9.0% 1.00 [-4.52, 10.92] Furman 2004 (26) 16 (27) 91 15.1 5 24 92 4 15.4 347 100.0% -1.94 [-4.78, 0.90] Heterogeneity: Tu	Tanaka 2010 (15) Subtotal (95% CD	100.9	14.6		94.5	11.8				
Forman 2004 (16) 74.5 17 69 80 16 29 24.6% -5.50 [-12.57, 1.57] incobi-Pollishook 2016 (17) 92.7 15.8 565 95 14 46 34.6% -2.30 [-6.55, 1.55] Volv 2006 (18) 84.6 19 775 81.3 17 260 40.8% 3.30 [0.84, 5.76] 335 100.0% -0.80 [-6.02, 4.42] Heterogeneity: Tax <sup>2</sup> = 15.81; Ch <sup>2</sup> = 8.84, df = 2 ( $P = 0.013$ ; $P = 77\%$ Test for overall effect: 2 = 0.30 ( $P = 0.36$ ) S1.9 Motor 18 to <36 months: Higher vs lower dose human milk; RT Of Conor 2016 (21) 9.1.8 18.7 151 94 18.5 148 100.0% -2.20 [-6.42, 2.02] Heterogeneity: Not applicable Test for overall effect: 2 = 1.02 ( $P = 0.31$ ) S1.10 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 2013 (23) 85.3 14.9 141 87.1 14.9 122 26.7% -1.80 [-4.75, 1.15] Subto 203 (24) 78 15 34 77 14 21 15.7% 1.00 [-6.83, 8.83] Subto 203 (24) 78 15 34 77 14 21 15.7% 1.00 [-6.83, 8.83] Subto 203 (24) 78 15 34 77 14 21 15.7% 1.00 [-6.83, 8.83] Subto 18 90 x C0 Subto 18 to <36 months: Higher vs lower dose human milk; observational Colacci 2017 (25) 82 13 30 89 17 27 12.0% 3.00 [-4.92, 10.92] Fermina 100 4 (26) 76 16 28 75 18 25 9.0% 1.00 [-6.82, 1.00, 21] Fermina 100 4 (26) 76 16 28 75 18 25 9.0% 1.00 [-6.82, 1.00, 21] Fermina 100 4 (26) 76 16 28 75 18 25 9.0% 1.00 [-6.92, 1.0, 21] Fermina 100 4 (26) 76 16 28 75 18 25 9.0% 1.00 [-6.92, 1.0, 21] Fermina 100 4 (26) 76 16 28 75 18 25 9.0% 1.00 [-6.92, 1.0, 21] Fermina 100 4 (26) 76 16 28 75 18 25 9.0% 2.00 [-6.92, 1.0, 21] Fermina 100 4 (26) 76 16 28 75 18 25 9.0% 2.00 [-6.92, 1.0, 21] Fermina 100 4 (26) 76 16 28 75 18 25 9.0% 2.00 [-6.92, 1.0, 21] Fermina 100 4 (26) 76 16 28 75 18 25 9.0% 2.00 [-6.92, 1.0, 21] Fermina 100 4 (26) 76 16 28 75 18 25 9.0% 2.00 [-6.92, 1.0, 21] Fermina 100 4 (26) 76 16 28 75 18 25 9.0% 2.00 [-6.92, 1.0, 21] Fermina 100 4 (26) 76 16 28 75 18 25 9.0% 2.00 [-6.92, 1.0, 21] Fermina 100 4 (26) 76 16 28 75 18 37 34 24 155 18 8.5% 7.40 [-6.92, 2.1] Fe	Heterogeneity: Not applicable	3 (P = 0.3	(0)				1050			
Forman 2004 (16) 74.5 17 69 80 16 29 24.6% -5.50 [-12.57, 1.57] incobi-Pollishook 2016 (17) 92.7 15.8 565 95 14 46 34.6% -2.30 [-6.55, 1.55] Volv 2006 (18) 84.6 19 775 81.3 17 260 40.8% 3.30 [0.84, 5.76] 335 100.0% -0.80 [-6.02, 4.42] Heterogeneity: Tax <sup>2</sup> = 15.81; Ch <sup>2</sup> = 8.84, df = 2 ( $P = 0.013$ ; $P = 77\%$ Test for overall effect: 2 = 0.30 ( $P = 0.36$ ) S1.9 Motor 18 to <36 months: Higher vs lower dose human milk; RT Of Conor 2016 (21) 9.1.8 18.7 151 94 18.5 148 100.0% -2.20 [-6.42, 2.02] Heterogeneity: Not applicable Test for overall effect: 2 = 1.02 ( $P = 0.31$ ) S1.10 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 2013 (23) 85.3 14.9 141 87.1 14.9 122 26.7% -1.80 [-4.75, 1.15] Subto 203 (24) 78 15 34 77 14 21 15.7% 1.00 [-6.83, 8.83] Subto 203 (24) 78 15 34 77 14 21 15.7% 1.00 [-6.83, 8.83] Subto 203 (24) 78 15 34 77 14 21 15.7% 1.00 [-6.83, 8.83] Subto 18 90 x C0 Subto 18 to <36 months: Higher vs lower dose human milk; observational Colacci 2017 (25) 82 13 30 89 17 27 12.0% 3.00 [-4.92, 10.92] Fermina 100 4 (26) 76 16 28 75 18 25 9.0% 1.00 [-6.82, 1.00, 21] Fermina 100 4 (26) 76 16 28 75 18 25 9.0% 1.00 [-6.82, 1.00, 21] Fermina 100 4 (26) 76 16 28 75 18 25 9.0% 1.00 [-6.92, 1.0, 21] Fermina 100 4 (26) 76 16 28 75 18 25 9.0% 1.00 [-6.92, 1.0, 21] Fermina 100 4 (26) 76 16 28 75 18 25 9.0% 1.00 [-6.92, 1.0, 21] Fermina 100 4 (26) 76 16 28 75 18 25 9.0% 2.00 [-6.92, 1.0, 21] Fermina 100 4 (26) 76 16 28 75 18 25 9.0% 2.00 [-6.92, 1.0, 21] Fermina 100 4 (26) 76 16 28 75 18 25 9.0% 2.00 [-6.92, 1.0, 21] Fermina 100 4 (26) 76 16 28 75 18 25 9.0% 2.00 [-6.92, 1.0, 21] Fermina 100 4 (26) 76 16 28 75 18 25 9.0% 2.00 [-6.92, 1.0, 21] Fermina 100 4 (26) 76 16 28 75 18 25 9.0% 2.00 [-6.92, 1.0, 21] Fermina 100 4 (26) 76 16 28 75 18 25 9.0% 2.00 [-6.92, 1.0, 21] Fermina 100 4 (26) 76 16 28 75 18 37 34 24 155 18 8.5% 7.40 [-6.92, 2.1] Fe	5.1.8 Motor 18 to <36 month	he Any h	uman m	ilk vs.ex	clusive pret	erm formu	la: Obse	evational		
Jacobi-Polishook 2016 (17) 92.7 15.8 565 95 14 46 34.6% -2.30 [-6.55, 1.95] Subtoral 95% CD 440.8% -3.30 [0.84, 5.76] Subtoral 95% CD 54.0 (0.84, 5.76] S1.9 Motor 18 to <36 months: Higher vs lower dose human milk; RT O'Connor 2016 (19) 9.1.8 18.7 151 94 18.5 148 100.0% -2.20 [-6.42, 2.02] Subtoral 95% CD 151 194 18.7 151 94 18.5 148 100.0% -2.20 [-6.42, 2.02] Subtoral 95% CD 151 194 18.7 151 94 18.5 148 100.0% -2.20 [-6.42, 2.02] Subtoral 95% CD 151 194 18.7 151 94 18.5 148 100.0% -2.20 [-6.42, 2.02] Subtoral 95% CD 258 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 18.44 -6.70 [-13.9, -0.21] Phetling 26 30 (20 3) 85.3 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 18.44 -6.70 [-13.9, -0.21] Phetling 26 30 (20 3) 85.3 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 18.44 -6.70 [-13.9, -0.21] Phetling 26 30 (20 3) 85.3 11.5 34 78 11.4 31 20.5% 7.80 [2.23, 13.37] Madore 2017 (22) 86.4 14.4 39 9.1 7.8 13 18.44 -6.70 [-13.9, -0.21] Phetling 26 30 (20 3) 85.3 11.5 34 78 11.4 31 20.5% 7.80 [2.23, 13.37] Madore 2017 (22) 86.4 14.4 39 9.1 7.8 13 18.44 -6.70 [-3.19, -0.21] Phetling 26 30 (20 3) 85.3 11.5 34 78 11.4 31 20.5% 7.80 [2.23, 13.37] Madore 2017 (22) 86.4 14.4 (9 = 0.01); t <sup>2</sup> = 69% S1.11 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] Fermina 2004 (26) 76 16 28 75 18 25 9.0% 1.00 [-6.21, 10.21] Jacobi-Pollishook 2016 (27) 9.15 16.1 283 94 15.5 128 29.0% 2.00 [-6.21, 10.21] Jacobi-Pollishook 2016 (27) 9.15 16.1 283 94 15.5 128 29.0% 2.7.60 (-16.92, 2.10, 92] Madore 2017 (28) 87 CD 375 375 347 190.0% -7.94 [-4.78, 0.90] Heterogeneity: Tau <sup>2</sup> = 1.20; Chu <sup>2</sup> = 3.31, df = 3.0 = 0.35); t <sup>2</sup> = 9% Test for except exter 2.4 1.34 (P = 0.18)									-5.50 (-12.57, 1.57)	
Subtotal (95% CD) 1409 335 100.0% $-0.80$ [-6.02, 4.42] Heterogeneity: Tau <sup>2</sup> = 15.81; Ch <sup>2</sup> = 8.84, df = 2 ( $P = 0.01$ ); $P = 77\%$ Test for overall effect: $Z = 0.30$ ( $P = 0.76$ ) S.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] Heterogeneity: Not applicable Test for overall effect: $Z = 1.02$ ( $P = 0.31$ ) S.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] Colacci 2017 (20) 87 13 26 89 11 29 18.6% $-2.00$ [-8.40, 4.40] Colacci 2017 (20) 87 13 26 89 11 29 18.6% $-2.00$ [-8.40, 4.40] Colacci 2017 (22) 86.4 14.4 31 93.1 7.8 13 18.4% $-6.70$ [-13.19, -0.21] O'Connor 2000 (2) 85.3 14.9 141 87.1 14.9 322 26.7% $-1.80$ [-4.75, 1.15] O'Connor 2000 (2) 85.3 14.9 141 87.1 14.9 322 26.7% $-1.80$ [-4.75, 1.15] O'Connor 2000 (2) 85.3 14.9 141 87.1 14.9 322 26.7% $-1.80$ [-4.75, 1.15] O'Connor 2000 (2) 85.3 14.9 141 87.1 14.9 322 26.7% $-1.80$ [-4.75, 1.15] O'Connor 2000 (2) 85.3 14.9 141 87.1 14.9 322 26.7% $-1.80$ [-4.75, 1.15] O'Connor 2000 (2) 85.3 14.9 141 87.1 14.9 322 26.7% $-1.80$ [-4.75, 1.15] O'Connor 2000 (2) 85.3 14.9 141 87.1 14.9 322 26.7% $-1.80$ [-4.75, 1.15] O'Connor 2000 (2) 85.3 14.9 14.8 87.1 14.9 322 26.7% $-1.80$ [-4.75, 1.15] O'Connor 2000 (2) 85.3 14.9 14.8 87.1 14.9 322 26.7% $-1.80$ [-4.75, 1.15] O'Connor 2000 (2) 85.3 14.9 14.8 87.1 14.9 322 26.7% $-1.80$ [-4.75, 1.15] O'Connor 2000 (2) 85.3 14.9 14.1 87.1 14.9 322 26.7% $-1.80$ [-4.75, 1.15] O'Connor 2000 (2) 85.3 14.9 14.8 87.1 14.9 322 26.7% $-1.80$ [-4.75, 1.15] O'Connor 2000 (2) 85.3 14.9 9.0 0.01; P' = 69% Test for overall effect Z = 0.14 (P = 0.89) S.1.11 Motor 18 to <36 months: Higher vs lower dose human milk; observational Colacci 2017 (28) 85 13.5 34 92.4 15.4 13 8.5% $-7.40$ (-16.92, 10.92] I acobi-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,	Jacobi-Pollishook 2016 (17)									-
Heterogeneiny: $Tau^2 = 15.81$ ; $Ch^2 = 8.84$ , $df = 2$ ( $P = 0.01$ ); $l^2 = 77\%$ Test for overall effect: $Z = 0.30$ ( $P = 0.76$ ) S.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] 148 100.0% -2.20 [-6.42, 2.02] 148 100.0% -2.20 [-6.42, 2.02] 148 100.0% -2.20 [-6.42, 2.02] Heterogeneity: Not applicable Test for overall effect: $Z = 1.02$ ( $P = 0.31$ ) S.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) 8.5 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 14.9 122 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% C0) 268 Subtotal (95% C0) 40 89 17 27 12.0% 3.00 [-4.92, 10.92] S.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] S.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] S.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] S.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] Subtotal (95% C0) 375 34 92.4 15.5 282 70.6% -2.50 [-5.11, 0.11] Madore 2017 (28) 85 13.5 34 92.4 15.5 282 70.6% -2.50 [-5.11, 0.11] Madore 2017 (28) 85 13.5 34 92.4 15.5 282 70.6% -2.50 [-5.11, 0.12] Heterogeneity: Tau <sup>2</sup> = 120; Ch <sup>2</sup> = 3.31, df = 3 (P = 0.35); t <sup>2</sup> = 9% Test:thur events! eXust 2.v 1.34 (P = 0.18)	Vohr 2006 (18)			775				40.8%	3.30 [0.84, 5.76]	
Test for overall effect: $Z = 0.30$ ( $P = 0.76$ ) S.1.9 Motor 18 to <36 months: Higher vs lower dose human milk; RT Of Connor 2016 (19) 91.8 18.7 151 94 18.5 148 100.0% -2.20 [-6.42, 2.02] Subtotal (95% CD) 91.8 18.7 151 94 18.5 148 100.0% -2.20 [-6.42, 2.02] Heterogeneity: Not applicable Test for overall effect: $Z = 1.02$ ( $P = 0.31$ ) S.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 2003 (23) 85.3 14.9 141 87.1 14.9 322 26.7% -1.80 [-4.75, 1.15] Ponell 2003 (24) 78 15 34 77 14 21 15.7% 1.00 [-6.8.8] Subtotal (95% CD) 78 15 268 77 14 21 15.7% 1.00 [-6.4.8, 4.14] Heterogeneity: Tau <sup>2</sup> = 17.22; Ch <sup>2</sup> = 13.10, df = 4 ( $P = 0.01$ ); $P = 69\%$ Test for overall effect: $Z = 0.14$ ( $P = 061$ ); $P = 0.35$ ; $P = 0.35$ ; $P^2 = 9\%$ Test for voerall effect: $Z = 0.14$ ( $P = 061$ ); $P = 0.35$ ; $P^2 = 9\%$ Test (by recruit effect; $Z = 0.14$ ( $P = 055$ ; $P^2 = 9\%$ Test (by recruit effect; $Z = 0.14$ ( $P = 055$ ; $P^2 = 9\%$ Test (by recruit effect; $Z = 0.14$ ( $P = 055$ ; $P^2 = 9\%$ Test (by recruit effect; $Z = 0.14$ ( $P = 055$ ; $P^2 = 9\%$ Test (by recruit effect; $Z = 0.14$ ( $P = 055$ ; $P^2 = 9\%$							335	100.0%	-0.80 [-6.02, 4.42]	•
$\begin{array}{c c c c c c c c c c c c c c c c c c c $				2 (P = 0.	.01); P = 771	6				
Subtotal (95% C) 151 148 100.0% -2.20 [-6.42, 2.02] Heterogeneity: Not applicable Test for overall effect: $Z = 1.02$ ( $P = 0.31$ ) S.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] Pinell 2003 (24) 78 15 34 77 14 21 15.7% 1.00 [-6.83, 8.83] Subtotal (95% C) 268 7 14 21 15.7% 1.00 [-6.83, 8.83] Heterogeneiby: Tau <sup>2</sup> = 17.22; Chi <sup>2</sup> = 13.10, df = 4 ( $P = 0.01$ ); $t^2 = 69\%$ Test for overall effect: $Z = 0.14$ ( $P = 0.89$ ) S.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] Madore 2017 (25) 85 13.5 34 92.4 15.4 13 8.5% -7.40 [-16.92, 2.12] Madore 2017 (26) 85 13.5 34 92.4 15.4 13 8.5% -7.40 [-16.92, 2.12] Madore 2017 (26) 85 13.5 34 92.4 15.4 34 71 100.0% -1.94 [-4.78, 0.90] Heterogeneity: Tau <sup>2</sup> = 1.20; Chi <sup>2</sup> = 3.31, df = 3 ( $P = 0.35$ ); $t^2 = 9\%$ Test for userall effect: $Z = 0.14$ ( $P = 0.18$ )		hs: Higher	r vs lowe			RT				
Heterogeneity: Not applicable Test for overall effect: $Z = 1.02 (P = 0.31)$ S.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] Pinell 2003 (24) 78 15 34 77 14 21 15.7% 1.00 [-6.83, 8.83] Subtotal (95% CI) 268 $+ 12.268 + 1$	O'Connor 2016 (19)	91.8	18.7		94	18.5				
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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 [-13.9, -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 14 77 14 21 15.7% 1.00 [-6.83, 8.83] Subtotal (95% CD) 268 416 100.0% -0.33 [-4.80, 4.14] Heterogeneity: Tau <sup>2</sup> = 17.22; Chi <sup>2</sup> = 13.10, df = 4 ( $P$ = 0.01); $l2 = 69%$ Test for overall effect Z = 0.14 ( $P$ = 0.89) S.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] S.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] S.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] Madore 2017 (28) 85 13.5 34 92.4 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] Madore 2017 (28) 85 13.5 34 92.4 15.4 13 8.5% -7.40 [-16.92, 2.12] Heterogeneity: Thk <sup>2</sup> = 1.20; Chi <sup>2</sup> = 3.31, df = 3 ( $P$ = 0.35); $l2$ = 9% Test fith encode elements: The $l = 0.18$		2 (P = 0.3	112							
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 [-13.9, -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% CD) 268 416 100.0% -0.33 [-4.80, 4.14] Heterogeneity: Tau <sup>2</sup> = 17.22; Chi <sup>2</sup> = 13.10, df = 4 ( $P$ = 0.01); $t2 = 69%$ Test for overall effect: Z = 0.14 ( $P$ = 0.89) S.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] S.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] S.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] Madore 2017 (28) 85 13.5 34 92.4 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] Madore 2017 (28) 85 13.5 34 92.4 15.4 13 8.5% -7.40 [-16.92, 2.12] Heterogeneity: Tau <sup>2</sup> = 1.20; Chi <sup>2</sup> = 3.31, df = 3 ( $P$ = 0.35); $t2$ = 9% Test for one-pit eNarc 2 = 1.34 ( $P$ = 0.18)	5.1.10 Motor<18 months: Hi	gher vs lo	ower dos	e huma	n milk; Obse	ervational				
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, 8.63]         Subtotal (95% CI)       268       416       100.0%       -0.33 [-4.80, 4.14]       4.14]         Heterogeneity: Tau <sup>2</sup> = 17.22; Chi <sup>2</sup> = 13.10, df = 4 (P = 0.01); l <sup>2</sup> = 69%       416       100.0%       -0.33 [-4.80, 4.14]         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]         Madore 2017 (28)       85       13.5       34       92.4       15.5       282       70.6%       -2.50 [-5.11, 0.11]         Madore 2017 (28)       85       13.5       34       92.4       15.5		-					29	18.6%	-2.00 [-8.40, 4.40]	-
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Subtotal (95% C0)       268       416       100.0% $-0.33$ [-4.80, 4.14]         Heterogeneity: Tau <sup>2</sup> = 17.22; Chi <sup>2</sup> = 13.10, df = 4 (P = 0.01); l <sup>2</sup> = 69%       Test for overall effect: Z = 0.14 (P = 0.89)         S1.11 Motor 18 to <36 months: Higher vs lower dose human milk; observational	O'Connor 2003 (23)									
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S.1.11 Motor 18 to <36 months: Higher vs lower dose human milk; observational	Heterogeneity: Tau <sup>2</sup> = 17.22;				0.01); 12 = 65	9%	-10		and Lands and	Ť
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-Poliishook 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       13       8.5%       -7.40 [-16.92, 2.12]         Subtotal (95% C0       375       375       347       100.0%       -1.94 [-4.78, 0.90]       •         Heterogeneity: Tilu <sup>2</sup> = 1.20; Ch <sup>2</sup> = 3.31, df = 3 (P = 0.35); l <sup>2</sup> = 9%       347       100.0%       -1.94 [-4.78, 0.90]       •				the days	human mill	in obvious	ional			
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 [-16.9, 2.12]         Subtotal (95% C0)       85       375       347       100.0%       -1.94 [-4.78, 0.90]       •         Heterogeneity: Tits <sup>2</sup> = 1.20; Chi <sup>2</sup> = 3.31, df = 3 (P = 0.35); l <sup>2</sup> = 9%       347       100.0%       -1.94 [-4.78, 0.90]       •	Colacci 2017 (25)							12.0%	3.00 [-4.92, 10.92]	
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% CB)       375       347       100.0%       -1.94 [-4.78, 0.90]       ●         Heterogeneity: Title <sup>2</sup> = 1.20; Ob <sup>2</sup> = 3.31, df = 3 (P = 0.35); l <sup>2</sup> = 9%       Test (by: coord) eNerce 2 × 1.34 (P = 0.18)       ●	Furman 2004 (26)									
Subtotal (95% C0 375 347 100.0% -1.94 [-4.78, 0.90] Heterogeneity: Tills <sup>2</sup> = 1.20; Chi <sup>2</sup> = 3.31, df = 3 (P = 0.35); l <sup>2</sup> = 9% Test (br: consult eNorce 2 = 1.34 (P = 0.18)	acobi-Pollishook 2016 (27)	91.5	16.1	283	94	15.5	282	70.6%	-2.50 [-5.11, 0.11]	
Heterogeneity: $T_{114}^2 = 5.20$ ; $Ch^2 = 3.31$ , $df = 3$ (P = 0.35); $l^2 = 9\%$ Test (fur except) eNume 2 or 1.34 (P = 0.18)		85	13.5		92.4	15.4			-7.40 [-16.92, 2.12]	
Test (her except) # (Nucc. 2 + 1.34 (P = 0.18)					St. 12		347	100.0%	-1.94 [-4.78, 0.90]	1
				UP = 0.3	37.1. = 306					
-100 -50 0 50			200							
										-100 -50 0 50

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
	Dout	he blinded randoms	ed clinical tr	als	
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 BM supplemented	<37 GA BW <1750 g	197	Control = 11% Experimental = 19%	No significant difference
Innis et al. (2002) [91]	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% Control = 3%	No significant difference No significant
Clandinin et al. (2005) [92]	Formula supplemented with DHA + ARA	<35 GA	361	Experimental = 5%	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 = [0.87-5.22]	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 No significant
Collins et al. (2017) [95]	BM supplemented with 60 mg/kg/day DHA	<29 WCA	1273	Adj. OR = [0.79–1.69]	difference

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

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 minesh khashu

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 "allcause mortality" in preterm infants without adverse effects but authors conclude that the evidence is moderate-to low-quality

Table 1.	Meta-analysis results.	
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	Pooled An	Hete	rogenei ty	Publication Bias				
	RR (95% CI)	p-Value	I <sup>2</sup>	p-Value	Egger's Test		Begg's and Mazdumdar's T	
					Т	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 (breast feeding 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 (breast feeding 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.



#### Nutrients 2018, 10, 707

#### 18 of 35

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### Table 2. Summary of Findings.

Comparison Outcome	EHM vs. EPTF RR or MD (95% CI); N Participants (Studies), <i>I</i> <sup>8</sup> GRADE Certainty of Evidence Interpretation and Absolute effect (95% CI)	Any HM vs. EPTF RR or MD (95% CI); N Participants (Studies), <i>I</i> <sup>2</sup> 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), 1 <sup>2</sup> 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), $l^2 = 0\%$ 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), $l^2 = 7\%$ 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 tesh khashu	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 <sup>2</sup> = 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

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### 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

Authors	Institution(s), Country	Type of Study	Patients in Study (n)	Patients with SIP (n)	Mean GA (wks)	Feeding Regimen Prior to SIP	Comments	
Bachheit [4]	University of Louisville, Uni ted 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 ± Entenal Feeding		
Holland [11]	The Royal Alexandria 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 Un ive rsity Perinatal Center, Japan	R	556	10	26.3	Unknown		
Mass[23]	Tübingen Un iversity Children's Hospital, Gennany	R	77	9	26.7	Enteral feeds were initiated at 20 mL/kg/day of proterm 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	С	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				

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

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