Supplementary Table 1a. Supplemental Studies on Plausible Mechanisms and Relevance for Pro-Argument on Breastmilk Benefits (Figure 1).

Study	Title	Abstract	Relevance for Deliberation
Vertical and horizontal (environmental) transfer of microbes from maternal diet to gut, mammary tissues, milk, infant			
Gregory et al., 2016 [1]	Influence of maternal breast milk ingestion on acquisition of the intestinal microbiome in preterm infants.	***Background*:** The initial acquisition and early development of the intestinal microbiome during infancy are important to human health across the lifespan. Mode of birth, antibiotic administration, environment of care, and nutrition have all been shown to play a role in the assembly of the intestinal microbiome during early life. For preterm infants, who are disproportionately at risk of inflammatory intestinal disease (i.e., necrotizing enterocolitis), a unique set of clinical factors influence the establishment of the microbiome. The purpose of this study was to establish the influence of nutritional exposures on the intestinal microbiome in a cohort of preterm infants early in life. ***Results*:** Principal component analysis of 199 samples from **30 preterm infants** (<32 weeks) over the first 60 days following birth showed that the intestinal microbiome was influenced by postnatal time ($p < 0.001$, $R^2 = 0.13$), birth weight ($p < 0.001$, $R^2 = 0.08$), and nutrition ($p < 0.001$, $R^2 = 0.21$). Infants who were fed breast milk had a greater initial bacterial diversity and a more gradual acquisition of diversity compared to infants who were fed infant formula. The microbiome of infants fed breast milk were more similar regardless of birth weight ($p = 0.049$), in contrast to the microbiome of infants fed infant formula, which clustered differently based on birth weight ($p < 0.001$). By adjusting for differences in **gut maturity**, an **ordered succession of microbial phylotypes** was observed in breast milk-fed infants, which appeared to be disrupted in those fed infant formula. Supplementation with **pasteurized donor human milk** was **partially successful** in promoting a microbiome more similar to breast milk-fed infants and moderating rapid increases in bacterial diversity.	Microbial ecology of breastmilk promoted intestinal health, feeding and seeding the gut microbiota and protecting pre-term infants against complications of gut immaturity. The dynamic interplay of host and dietary factors (including the microbiota of milks) demonstrated in this study may also be relevant to children and later in life.

		***Conclusions*:** The preterm infant intestinal microbiome is influenced by postnatal time, birth weight, gestational age, and nutrition. Feeding with breast milk appears to mask the influence of birth weight, suggesting a protective effect against gut immaturity in the preterm infant. These findings suggest not only a microbial mechanism underpinning the body of evidence showing that breast milk promotes intestinal health in the preterm infant but also a dynamic interplay of host and dietary factors that facilitate the colonization of and enrichment for specific microbes during establishment of the preterm infant microbiota. https://pubmed.ncbi.nlm.nih.gov/28034306/	
Sawh et al., 2016 [2]	Prevention of necrotizing enterocolitis with probiotics: a systematic review and meta-analysis	***Context*:** Necrotizing enterocolitis (**NEC**) is the **most frequent gastrointestinal emergency** in **neonates**. The microbiome of the preterm gut may regulate the integrity of the intestinal mucosa. Probiotics may positively contribute to mucosal integrity, potentially reducing the risk of NEC in neonates. ***Objective*:** To perform an **updated systematic review and meta-analysis** on the **efficacy** and **safety** of **probiotics** for the **prevention of NEC** in premature infants. ***Data sources*:** Structured searches were performed in: Medline, Embase, and the Cochrane Central Register of Controlled Trials (all via Ovid, from 2013 to January 2015). Clinical trial registries and electronically available conference materials were also searched. An updated search was conducted June 3, 2016. ***Study selection*: Randomized trials** including infants less than 37 weeks gestational age or less than 2,500 g on probiotic vs. standard therapy. ***Data extraction*:** Data extraction of the newly-identified trials with a double check of the previously-identified trials was performed using a standardized data collection tool. ***Results*:** Thirteen additional trials (n = 5,033) were found. The **incidence of severe NEC** (RR 0.53 95% CI [0.42-0.66]) and **all-cause mortality** (RR 0.79 95% CI [0.68-0.93]) were **reduced**. No difference was shown in culture-proven sepsis RR 0.88 95% CI [0.77-1.00]. ***Limitations*:** Heterogeneity of organisms and dosing regimens studied prevent a species-specific treatment recommendation from being made.	A diversity of probiotic bacteria protected pre-term infants against severe NEC and death, consistent with key roles for microbial ecology: protection against infections; strengthening mucosal integrity and immunity; and promoting healthy gut development.

		***Conclusions*: Preterm infants benefit from probiotics** to **prevent severe NEC and death**. https://pubmed.ncbi.nlm.nih.gov/27761306/	
Murphy et al., 2017 [3]	The composition of human milk and infant faecal microbiota over the first three months of life: a pilot study.	Human milk contains a **diverse array of bioactives** and is also a source of bacteria for the developing infant gut. The aim of this study was to **characterize the bacterial communities** in **human milk** and **infant faeces** over the first 3 months of life, in **10 mother-infant pairs**. The presence of viable ***Bifidobacterium*** and ***Lactobacillus*** in human milk was also evaluated. MiSeq sequencing revealed a large diversity of the human milk microbiota, identifying over 207 bacterial genera in milk samples. The **phyla Proteobacteria** and **Firmicutes** and the **genera *Pseudomonas*, *Staphylococcus*** and ***Streptococcus*** were the **predominant** bacterial groups. A core of 12 genera represented 81% of the microbiota relative abundance in milk samples at week 1, 3 and 6, decreasing to 73% at week 12. **Genera shared** between infant **faeces** and human **milk** samples accounted for **70-88%** of the total relative abundance in infant faecal samples, **supporting** the **hypothesis** of **vertical transfer of bacteria** from milk to the infant gut. In addition, **identical strains** of ***Bifidobacterium breve*** and ***Lactobacillus plantarum*** were **isolated from the milk and faeces** of one mother-infant pair. **Vertical transfer of bacteria via breastfeeding** may contribute to the initial establishment of the microbiota in the developing infant intestine. https://pubmed.ncbi.nlm.nih.gov/28094284/	Findings supported vertical transmission of microbes from breastmilk to infant gut, reinforcing the roles of breastmilk in seeding and feeding infants.
Toscano et al., 2017 [4]	Impact of delivery mode on the colostrum microbiota composition.	***Background*:** Breast milk is a rich nutrient with a temporally dynamic nature. In particular, numerous alterations in the **nutritional**, **immunological** and **microbiological** content occur during the **transition** from **colostrum** to **mature milk**. The objective of our study was to evaluate the potential impact of **delivery mode** on the microbiota of colostrum, at both the quantitative and qualitative levels (bacterial abundance and microbiota network). ***Methods*: Twenty-nine Italian mothers** (15 vaginal deliveries vs 14 Cesarean sections) were enrolled in the study. The microbiota of colostrum samples was analyzed by next generation sequencing (Ion Torrent Personal Genome Machine). The colostrum microbiota network associated with Cesarean section and vaginal delivery was evaluated by means of the Auto Contractive Map (AutoCM), a mathematical methodology based on Artificial Neural Network (ANN) architecture.	The networks of microbes in colostrum were influenced by birth method and included anaerobic bacterial genera, relevant to seeding and feeding the infant gut.

		Results*:** Numerous differences between Cesarean section and vaginal delivery colostrum were observed. **Vaginal delivery colostrum** had a **significant lower abundance** of ***Pseudomonas spp., ***Staphylococcus*** spp. and ***Prevotella*** spp. when compared to Cesarean section colostrum samples. Furthermore, the mode of delivery had a strong influence on the **microbiota network**, as Cesarean section colostrum showed **a higher number of bacterial hubs** if compared to vaginal delivery, sharing only 5 hubs. Interestingly, the colostrum of mothers who had a Cesarean section was richer in environmental bacteria than mothers who underwent vaginal delivery. Finally, both Cesarean section and vaginal delivery colostrum contained a greater number of **anaerobic bacteria genera**. ***Conclusions*:** The mode of delivery had a large impact on the microbiota composition of colostrum. Further studies are needed to better define the meaning of the differences we observed between Cesarean section and vaginal delivery colostrum microbiota. https://pubmed.ncbi.nlm.nih.gov/28946864/	
De Andres et al., 2018 [5]	Physiological translocation of lactic acid bacteria during pregnancy contributes to the composition of the milk microbiota in mice.	The human milk microbiota is a **complex and diverse ecosystem** that seems to play a relevant role in the mother-to-infant **transmission** of microorganisms during early life. Bacteria present in human milk may arise from **different sources**, and recent studies suggest that at least some of them may be originally present in the **maternal digestive tract** and may reach the mammary gland through an **endogenous route** during pregnancy and lactation. The objective of this work was to elucidate whether some lactic acid bacteria are able to **translocate** and **colonize the mammary gland and milk**. For this purpose, two lactic acid bacteria strains (***Lactococcus lactis*** MG1614 and ***Lactobacillus salivarius*** PS2) were **transformed** with a plasmid containing the *lux* genes; subsequently, the transformed strains were **orally administered to pregnant mice**. The murine model allowed the **visualization**, **isolation**, and Polymerase Chain Reaction (**PCR**)-**detection** of the **transformed bacteria in different body locations**, including **mammary tissue** and **milk**, reinforcing the hypothesis that **physiological translocation** of maternal bacteria during pregnancy and lactation may contribute to the **composition of the mammary and milk microbiota**. https://pubmed.ncbi.nlm.nih.gov/29295502/	Transformed lactococci and lactobacilli orally administered to pregnant mice translocated to different murine tissues, including mammary glands and milk microbiota, demonstrating enteromammary route in mice.

Ojo-Okunola et al., 2018 [6]	Human breast milk bacteriome in health and disease.	It is well-known that, beyond nutritional components, **human breast milk (HBM**) contains a wide variety of **non-nutritive bio-factors** perfectly suited for the growing infant. In the **pre-2000 era**, **HBM was considered sterile** and devoid of micro-organisms. Though HBM was **not included** as part of the **human microbiome project launched in 2007**, great strides have been made in studying the bacterial diversity of HBM in both a **healthy state** and **diseased state**, and in understanding their role in infant health. HBM provides a vast array of beneficial micro-organisms that play a key role in **colonizing the infant's mucosal system**, including that of the gut. They also have a role in **priming the infant's immune system** and **supporting its maturation**. In this review, we provide an in-depth and updated insight into the **immunomodulatory, metabolic, and anti-infective** role of HBM bacteriome (bacterial community) and its effect on infant health. We also provide key information from the literature by exploring the **possible origin of microbial communities** in HBM, the bacterial diversity in this niche and the determinants influencing the HBM bacteriome. Lastly, we investigate the role of the HBM bacteriome in **maternal infectious disease** (human immunodeficiency virus (HIV) and mastitis)), and **cancer**. Key gaps in HBM bacterial research are also identified. https://pubmed.ncbi.nlm.nih.gov/30400268/	Plausible functions of the raw milk microbiota include those that influence **benefits** (**increasing resistance to pathogens** by the innate and adaptive immune systems of offspring and decreasing the likelihood and/or severity of disease). 1. Vertical transmission and gut seeding 2. Protection from maternal and infant infections by direct means (e.g., antibiotics, bacteriocins, competitive exclusion, colonization resistance) and indirect means (e.g., lowering pH by organic acid production, stimulating host to produce defensins) 3. Immunomodulatory activity that promotes homeostasis and increases host resistance (balances tolerance of non-pathogens and clearance of pathogens, activates host immune cells, modifies cross talk of microbiota with immune cells via cytokines and chemokines) 4. Protection against allergy by downregulation of Th2 cytokines by inducing Th1 response and optimized development, maturation, and regulation of infant immune systems 5. Metabolic functions for digestion and absorption (including organic acid production that favors infant and disfavors survival and growth of pathogens)

			6. Protection against cancers (e.g., breast, colon) relevant to maternal and infant health Protection against breast and colon cancer was based on *in vitro* studies. Some key functional roles for the milk microbiota included colonizing mucosal systems and priming immune system and supporting its maturation. Evidence was introduced supporting the enteromammary origin of the milk microbiota and protective effects of the milk microbiome for maternal and infant health.
Moossavi & Azad 2019 [7]	Origins of human milk microbiota: new evidence and arising questions.	Human milk contains a diverse community of bacteria. The growing appreciation of commensal microbes and increasing availability of high-throughput technology has set the stage for a **theory-driven approach** to the study of milk microbiota, and **translation of this knowledge** to **improve maternal and child health**. We recently profiled the milk microbiota of **healthy Canadian mothers** and applied **theory-driven causal modeling**, finding that mode of breast milk feeding (nursing **directly at the breast** vs. **pumping and feeding breast milk from a bottle**) was **significantly associated with milk microbiota composition**. This observation could reflect an increased exposure to pumps and/or a decreased exposure to the infant mouth. Either way, it provides evidence for the **retrograde mechanism of milk inoculation**. Here, we discuss the implications of this research and related controversies, and raise new questions about the origins and function of milk bacteria. https://pubmed.ncbi.nlm.nih.gov/31684806/	Pumping versus nursing at the breast influenced the composition of the milk microbiota, pointing to the influence of retrograde mechanisms of milk inoculation (between mother and nursing infant).
Van Daele et al., 2019 [8]	Microbial transmission from mother to child: improving infant intestinal microbiota development by	Industrialisation has introduced several lifestyle changes and medical advancements but their impact on intestinal microbiota acquisition is often overlooked. Even though these consequential changes in the microbiota could contribute to the disease burden that accompanies industrialisation, such as obesity and atopic disease. A healthy intestinal microbiota is acquired early in life but its exact origin is not fully elucidated. The maternal microbiota is a	Data on mother-infant pairs was emphasized. Some identical stains shared in 3 relevant ecosystems, maternal gut, breastmilk, and infant gut, can persist in infant gut for months. Formula feeding was discussed as an obstacle to development of healthy gut

	identifying the obstacles.	likely source because the infant and mother intestinal microbiota share identical strains. Successfully transmitting microbes from mother to child requires microbes in the maternal donor, contact between the maternal source and the infant, and an acquiring infant recipient. Transmission can be altered by changes to any of those three transmission determinants: (1) maternal microbiota sources are shaped by the mother's genotype, diet, health status and perturbing antimicrobial exposure; (2) maternal contact is reduced through C-section and formula feeding and (3) engraftment in the infant recipient is determined by host habitat filtering, the established microbes and antibiotic disruptions. This review gives an overview of the possible maternal transmission routes, the disruptions thereof, and the missing links that should be addressed in future research to investigate the maternal transmissions that are crucial for obtaining a healthy infant microbiota.	microbiota. Dose-dependent effects on gut microbiota can be attributed to formula feeding, linked to lack of or reduced intakes of human milk oligosaccharides (HMOs), growth - promotors, xanthine oxidase activity, microbes, and microbial modulators. Three hypotheses with supporting data were described for the possible origins of milk microbes.
Wang et al., 2020 [9]	Maternal vertical transmission affecting early-life microbiota development	The association of the human microbiome with health outcomes has attracted much interest toward its **therapeutic manipulation**. The **likelihood of modulating** the **human microbiome** in **early life** is high and offers great potential to **exert profound effects on human development** since the early microbiota shows **more flexibility compared to that of adults**. The human microbiota, being similar to human genetics, can be transmitted from mother to infant, providing insights into early **microbiota acquisition**, **subsequent development**, and **potential opportunities for intervention**. Here, we review adaptations of the maternal microbiota during pregnancy, birth, and infancy, the **acquisition and succession of early-life microbiota**, and highlight recent efforts to elucidate **mother-to-infant microbiota transmission**. We further discuss how the mother-to-infant microbial transmission is shaped; and finally we address potential directions for future studies to promote our understanding within this field.	Advancing knowledge of the raw milk microbiota and studies of its origins, including enteromammary transmission, can contribute to strategies for modulating the human microbiome therapeutically.
Microbial ecology			
Arroyo et al., 2010 [10]	Treatment of infectious mastitis during lactation: antibiotics versus oral	***Background***: Mastitis is a common infectious disease during lactation, and the main etiological agents are **staphylococci**, **streptococci**, and/or **corynebacteria**. The efficacy of oral administration of *Lactobacillus fermentum* CECT5716 or *Lactobacillus salivarius* CECT5713, two lactobacilli strains isolated from breast	Clearance and prevention of infections of breast tissue (mastitis) by oral treatments with lactobacilli in specific probiotic products suggest that the natural microbiota of breast milk may

	administration of *Lactobacilli* isolated from breast milk.	milk, to treat lactational mastitis was evaluated and was compared with the efficacy of antibiotic therapy. ***Methods***: In this study, **352 women with infectious mastitis** were **randomly assigned** to 3 groups. Women in groups A (n = 124) and B (n = 127]) ingested daily 9 log(10) colony-forming units (CFU) of *L. fermentum* CECT5716 or *L. salivarius* CECT5713, respectively, for 3 weeks, whereas those in group C (n =101) received the antibiotic therapy prescribed in their respective primary care centers. ***Results***. On day 0, the mean bacterial counts in milk samples of the 3 groups were similar (4.35-4.47 log(10) CFU/mL), and lactobacilli could not be detected. On day 21, the mean bacterial counts in the probiotic groups (2.61 and 2.33 log(10) CFU/mL) were lower than that of the control group (3.28 log(10) CFU/mL). *L. fermentum* CECT5716 and *L. salivarius* CECT5713 were isolated from the milk samples of women in the probiotic groups A and B, respectively. Women assigned to the **probiotic groups improved more** and had **lower recurrence of mastitis** than those assigned to the antibiotic group. ***Conclusions***. The use of ***L. fermentum*** CECT5716 or ***L. salivarius*** CECT5713 appears to be an **efficient alternative** to the use of commonly prescribed **antibiotics** for the treatment of infectious mastitis during lactation. ClinicalTrials.gov identifier. https://pubmed.ncbi.nlm.nih.gov/20455694/	similarly clear and prevent infections in the infant gut.
Fernandez et al., 2016 [11]	Prevention of infectious mastitis by oral administration of *Lactobacillus salivarius* PS2 during late pregnancy.	***Background*:** Previous studies have shown that oral administration of lactobacilli can be an efficient approach to treat lactational infectious mastitis. In this trial, we have evaluated the potential of *Lactobacillus salivarius* PS2 to prevent this condition when orally administered during late pregnancy to women who had experienced infectious mastitis after previous pregnancies. ***Methods*:** In this study, **108 pregnant women** were randomly assigned to one of 2 groups. Those in the probiotic group (n = 55) ingested daily 9 log10 colony-forming units of *L. salivarius* PS2 from approximately week 30 of pregnancy until delivery, whereas those in the placebo group (n = 53) received a placebo. The occurrence of mastitis was evaluated during the first 3 months after delivery. ***Results*:** Globally, **44** of 108 women (**41**%) developed **mastitis**; however, the percentage of women with mastitis in the **probiotic group** (25% [n = 14]) was **significantly lower** than in the control	Clearance and prevention of infections of breast tissue (mastitis) by oral treatments with lactobacilli in specific probiotic products suggest that the natural microbiota of breast milk may similarly clear and prevent infections in the infant gut.

		group (57% [n = 30]). When mastitis occurred, the milk **bacterial counts** in the probiotic group were **significantly lower** than those obtained in the placebo group. ***Conclusions*:** Oral administration of *L. salivarius* PS2 during late pregnancy appears to be an efficient method to prevent infectious mastitis in a susceptible population. https://pubmed.ncbi.nlm.nih.gov/26611780/	
Cacho et al., 2017 [12]	Personalization of the microbiota of donor human milk with mother's own milk.	The American Academy of Pediatrics recommends that extremely preterm infants receive **mother's own milk (MOM**) when available or **pasteurized donor breast milk (DBM**) when MOM is unavailable. The goal of this study was to determine whether DBM could be inoculated with MOM from mothers of preterm infants to restore the live microbiota (RM). Culture dependent and culture independent methods were used to analyze the fluctuations in the overall population and microbiome, respectively, of DBM, MOM, and RM samples over time. Using MOM at time = 0 (T0) as the **target for the restoration** process, this level was reached in the 10% (RM-10) and 30% (RM-30) mixtures after 4 h of incubation at 37°C, whereas, the larger dilutions of 1% (RM-1) and 5% (RM-5) after 8 h. The **diversity indexes** were **similar** between MOM and DBM samples, however, **different genera were prevalent** in each group. Interestingly, 40% of the bacterial families were able to expand in DBM after 4 h of incubation indicating that a large percentage of the bacterial load present in MOM can grow when transferred to DBM, however, no core microbiome was identified. In summary, the microbiome analyses indicated that each mother has a unique microbiota and that live microbial reestablishment of DBM may provide these microbes to individual mothers' infants. The agreement between the results obtained from the viable bacterial counts and the microbiome analyses indicate that DBM incubated with 10-30% v/v of the MOM for 4 h is a reasonable restoration strategy. https://pubmed.ncbi.nlm.nih.gov/28824595/	The protective benefits of raw breastmilk appear transferable to pasteurized donor breastmilk. Future studies are needed to determine if raw donor breastmilk would restore health more quickly than pasteurized donor breastmilk inoculated with raw breastmilk.

Microbiome:Immune system cross-talk, indirect colonization resistance, recent reviews			
Ward et al., 2013 [13]	Human milk metagenome: a functional capacity analysis.	***Background***: Human milk contains a diverse population of bacteria that likely influences colonization of the infant gastrointestinal tract. Recent studies, however, have been limited to characterization of this microbial community by 16S rRNA analysis. In the present study, a metagenomic approach using Illumina sequencing of a pooled milk sample (ten donors) was employed to determine the genera of bacteria and the types of bacterial open reading frames in human milk that may influence bacterial establishment and stability in this primal food matrix. The **human milk metagenome** was also compared to that of **breast-fed** and **formula-fed infants' feces** (n = 5, each) and mothers' feces (n = 3) at the phylum level and at a functional level using open reading frame abundance. Additionally, **immune-modulatory bacterial-DNA motifs** were also searched for within human milk. ***Results***: The bacterial community in human milk contained over 360 prokaryotic genera, with sequences aligning **predominantly** to the **phyla** of **Proteobacteria** (65%) and **Firmicutes** (34%), and the **genera** of **Pseudomonas** (61.1%), **Staphylococcus** (33.4%) and **Streptococcus** (0.5%). From assembled human milk-derived contigs, 30,128 open reading frames were annotated and assigned to functional categories. When compared to the metagenome of infants' and mothers' feces, the human milk metagenome was less diverse at the phylum level, and contained more open reading frames associated with **nitrogen metabolism**, **membrane transport** and **stress response** ($P < 0.05$). The human milk metagenome also contained a similar occurrence of **immune-modulatory DNA motifs** to that of infants' and mothers' fecal metagenomes. ***Conclusions***: Our results further expand the **complexity** of the **human milk metagenome** and enforce the **benefits** of human milk ingestion on the **microbial colonization of the infant gut** and **immunity**. Discovery of immune-modulatory motifs in the metagenome of human milk indicates more exhaustive analyses of the functionality of the human milk metagenome are warranted. https://pubmed.ncbi.nlm.nih.gov/23705844/	The human milk metagenome was enriched in genes for nitrogen metabolism, membrane transport, and stress response, observations that are consistent with defense functions in the proteomes of human and bovine milks cited above. Deeper understanding of the functionality of the microbiota of milks could catalyze tremendous breakthroughs in therapeutic and preventative medicine, to nurture healthy microbiota or restore dysbiotic microbiota.

Chong et al., 2018 [14]	Factors affecting gastrointestinal microbiome development in neonates	The **gut microbiome** is established in the newborn period and is recognised to interact with the host to **influence metabolism**. Different environmental factors that are encountered during this critical period may influence the gut microbial composition, **potentially impacting upon later disease risk**, such as **asthma**, **metabolic disorder**, and **inflammatory bowel disease**. The **sterility dogma of the foetus in utero** is **challenged** by studies that identified bacteria, bacterial DNA, or bacterial products in **meconium, amniotic fluid, and the placenta**; indicating the initiation of maternal-to-offspring **microbial colonisation in utero**. This narrative review aims to provide a better understanding of factors that affect the development of the gastrointestinal (GI) microbiome during **prenatal**, **perinatal** to **postnatal** life, and their reciprocal relationship with GI tract development in neonates. https://pubmed.ncbi.nlm.nih.gov/29495552/	Advancing mechanistic knowledge of maternal-to-offspring transfer of microbes *in utero* extends understanding about the importance of microbes in prenatal, perinatal, and postnatal development of neonates.
Dietert 2018 [15]	A focus on microbiome completeness and optimized colonization resistance in neonatology.	The human microbiome contributes a majority of genes and significant metabolic capacity to the newborn. The infant's bacteria, archaea, viruses, and fungi are also critical for **immune maturation** and n**eurologic developmen**t. Because a microbiota is **highly malleable**, it is an **ideal target** for **improving infant health**. Yet, management of this major biological resource to reduce health risk for the infant has been **comparatively neglected to date**. This review discusses the opportunities for a **more holistic, ecological approach** to infant health with an **emphasis on the microbiome**, which includes 1) **the benefits of microbiome completeness** (microbial **seeding and feeding**), as well as 2) **optimized colonization resistance**. The latter can **better protect against infectious** as well as **noncommunicable diseases** by **shifting pathogen load requirements** for **producing disease**, **protecting mucosal barriers**, and **optimizing immune homeostasis**. https://neoreviews.aappublications.org/content/19/2/e78	Holistic, ecological approaches to health and development via raw milk were described, including: microbial seeding and feeding; and optimization of colonization resistance that protects against infectious and noncommunicable disease, strengthens mucosal barriers, and optimizes immune homeostasis.

van den Elsen et al., 2019 [16]	Shaping the gut microbiota by breastfeeding: the gateway to allergy prevention?	Evidence is accumulating that demonstrates the importance of the gut microbiota in health and diseases such as allergy. Recent studies emphasize the importance of the "**window of opportunity**" in **early life**, during which **interventions altering the gut microbiota** induce **long-term effects**. The neonate's gut **microbiota composition** and **metabolism c**ould therefore play an essential role in **allergic disease risk**. Breastfeeding shapes the gut microbiota in early life, both **directly** by exposure of the neonate to the milk microbiota and **indirectl**y, via maternal milk factors that affect bacterial growth and metabolism such as human milk oligosaccharides, secretory IgA, and anti-microbial factors. The potential of breastmilk to **modulate the offspring's early gut microbiota** is a **promising tool for allergy prevention**. Here, we will review the existing evidence demonstrating the impact of breastfeeding on shaping the neonate's gut microbiota and highlight the potential of this strategy for allergy prevention.	Evidence on direct seeding of the infant gut by the raw milk microbiota and subsequent direct and indirect modulation of infant immune systems is providing clues to future strategies for allergy prevention and health.

Supplementary Table 1b. Supplemental Studies on Plausible Mechanisms and Relevance for Contra-Argument on Breastmilk Risks (Figure 1).

Study	Title	Abstract	Relevance for Deliberation
Pathogen susceptibility to innate defenses including microbiota			
Cacho & Lawrence 2017 [17]	Innate immunity and breast milk.	Human milk is a **dynamic source of nutrients and bioactive factors**; unique in providing for the human infant's optimal growth and development. The growing infant's immune system has a number of developmental immune deficiencies placing the infant at increased risk of infection. This review focuses on how **human milk directly contributes** to the **infant's innate immunity**. Remarkable new findings clarify the **multifunctional nature of human milk bioactive components**. New research techniques have expanded our understanding of the potential for **human milk's effect** on the infant that **will never be possible with milk formulas**. Human milk microbiome **directly shapes** the infant's **intestinal microbiome**, while the human milk **oligosaccharides drive** the **growth of these microbes within the gut**. New techniques such as **genomics, metabolomics, proteomics, and glycomics** are being used to describe this **symbiotic relationship**. An expanded role for **antimicrobial proteins/peptides within human milk** in **innate immune protection** is described. The **unique milieu** of **enhanced immune protection** with **diminished inflammation** results from a **complex interaction** of **anti-inflammatory and antioxidative factors** provided by human milk to the intestine. New data support the concept of **mucosal-associated lymphoid tissue** and its **contribution** to the **cellular content of human milk**. **Human milk stem cells (hMSCs**) have recently been discovered. Their direct role in the infant for **repair and regeneration** is being investigated. The existence of these hMSCs could prove to be an easily harvested source of multilineage stem cells for the study of cancer and tissue regeneration. As the infant's	The symbiotic associations between microbes in raw milk and in the infant gut influenced innate immunity through mucosal-associated lymphoid tissue and the cellular content of raw milk. Raw milk was unique in providing enhanced immune protection with diminished inflammation, balanced by anti-inflammatory and anti-oxidative factors in raw milk.

		gastrointestinal tract and immune system develop, there is a comparable transition in human milk over time to provide fewer immune factors and more calories and nutrients for growth. Each of these new findings opens the door to future studies of human milk and its effect on the innate immune system and the developing infant. https://pubmed.ncbi.nlm.nih.gov/28611768/	
Dietert 2018 [15]	A focus on microbiome completeness and optimized colonization resistance in neonatology.	The human microbiome contributes a majority of genes and significant metabolic capacity to the newborn. The infant's bacteria, archaea, viruses, and fungi are also critical for **immune maturation** and n**eurologic developmen**t. Because a microbiota is **highly malleable**, it is an **ideal target** for **improving infant health**. Yet, management of this major biological resource to reduce health risk for the infant has been **comparatively neglected to date**. This review discusses the opportunities for a **more holistic, ecological approach** to infant health with an **emphasis on the microbiome**, which includes 1) **the benefits of microbiome completeness** (microbial **seeding and feeding**), as well as 2) **optimized colonization resistance**. The latter can **better protect against infectious** as well as **noncommunicable diseases** by **shifting pathogen load requirements** for **producing disease**, **protecting mucosal barriers**, and **optimizing immune homeostasis**. https://neoreviews.aappublications.org/content/19/2/e78	Primary superorganism-based factors determine susceptibility to infections: microbiota-conditioned immune system status; integrity of barrier-protected mucosal tissues; and direct and indirect pathways of colonization resistance. The primary infectious disease prevention strategy proposed was increasing pathogen load requirements by strengthening colonization resistance (increasing pathogen thresholds before infant defenses were overwhelmed, producing symptomatic disease). Depleted microbiomes (e.g., via antibiotic treatment) rendered infants biologically and functionally incomplete (suggested birth defect), increased risks of diseases for infants, and increased susceptibility to lower doses of potential pathogens. Tipping points for infectious disease now recognized as dependent on microbial ecology of barriers and immune-microbe interactions.
Le Doare et al., 2018 [18]	Mother's milk: A purposeful contribution to the development of the infant microbiota and immunity.	Breast milk is the **perfect nutrition for infants**, a result of millions of years of evolution. In addition to providing a source of nutrition, breast milk contains a **diverse array of microbiota** and **myriad biologically active components** that are thought to **guide the infant's developing mucosal immune system**. It is believed that bacteria from the **mother's intestine** may **translocate to breast milk** and	The diverse array of microbiota and myriad biologically active components (HMOs, EVs, exosomes) of raw milk contributed to protection against many respiratory, diarrheal, and systemic illnesses. The raw milk microbiota can contribute to development of healthy gut and immune systems and protect

		dynamically transfer to the infant. Such **interplay** between mother and her infant is a key to establishing a **healthy infant intestinal microbiome**. These **intestinal bacteria protect against many respiratory and diarrheal illnesses**, but are subject to environmental stresses such as antibiotic use. Orchestrating the development of the microbiota are the **human milk oligosaccharides (HMOs**), the synthesis of which are partially determined by the maternal genotype. HMOs are thought to play a role in **preventing pathogenic bacterial adhesion though multiple mechanisms**, while also **providing nutrition for the microbiome**. **Extracellular vesicles (EVs**), including **exosomes**, carry a diverse cargo, including mRNA, miRNA, and cytosolic and membrane-bound proteins, and are readily detectable in human breast milk. Strongly implicated in **cell-cell signaling**, EVs could therefore may play a further role in the development of the infant microbiome. This review considers the emerging role of breast milk microbiota, bioactive HMOs, and EVs in the establishment of the neonatal microbiome and the **consequent potential** for **modulation of neonatal immune system development**. https://pubmed.ncbi.nlm.nih.gov/29599768/	against environmental stresses, including antibiotic-induced dysbiosis of the gut, that increase susceptibility to pathogens.
Ojo-Okunola et al., 2018 [6]	Human breast milk bacteriome in health and disease.	It is well-known that, beyond nutritional components, **human breast milk (HBM**) contains a wide variety of **non-nutritive bio-factors** perfectly suited for the growing infant. In the **pre-2000 era**, **HBM was considered sterile** and devoid of micro-organisms. Though HBM was **not included** as part of the **human microbiome project launched in 2007**, great strides have been made in studying the bacterial diversity of HBM in both a **healthy state** and **diseased state**, and in understanding their role in infant health. HBM provides a vast array of beneficial micro-organisms that play a key role in **colonizing the infant's mucosal system**, including that of the gut. They also have a role in **priming the infant's immune system** and **supporting its maturation**. In this review, we provide an in-depth and updated insight into the **immunomodulatory, metabolic,**	Plausible functions of the raw milk microbiota that may influence **risks** (**increasing susceptibility of pathogens** to the innate and adaptive immune systems and decreasing the likelihood and/or severity of disease). 1. Promotes healthy gut function (seeding gut microbiota), normally protecting against dysbiosis unless GI system is disturbed by antibiotics (or other pharmaceuticals and chemical toxicants) 2. Promotes homeostasis, healthy balance of Th1 and Th2 immune system function, including tolerance of commensals (non-pathogens) and activation against pathogens that enhances resistance (e.g.,

		and anti-infective role of HBM bacteriome (bacterial community) and its effect on infant health. We also provide key information from the literature by exploring the **possible origin of microbial communities** in HBM, the bacterial diversity in this niche and the determinants influencing the HBM bacteriome. Lastly, we investigate the role of the HBM bacteriome in **maternal infectious disease** (human immunodeficiency virus (HIV) and mastitis)), and **cancer**. Key gaps in HBM bacterial research are also identified. https://pubmed.ncbi.nlm.nih.gov/30400268/	minimizing inflammation and pathogen growth and maximizing pathogen clearance) 3. Directly compete with ingested pathogens in gut lumen and GI mucosal surfaces (e.g., competitive exclusion, production of antimicrobial compounds), unless at high pathogen doses that overwhelm immature immune system 4. Promote mucin production and improve intestinal barrier function, minimizing pathogen adherence or invasion of host cells 5. Activate immune cells (e.g., Tregs, natural killer cells, mononuclear cells) to decrease exaggerated inflammatory response of Th2 immune responses by inducing Th1 responses.

References Cited Herein

1. Gregory, K.E. Influence of Maternal Breast Milk Ingestion on Acquisition of the Intestinal Microbiome in Preterm Infants. *Microbiome* **2016**, *4*, 1–15.
2. Sawh, S.C. Prevention of Necrotizing Enterocolitis with Probiotics: A Systematic Review and Meta-Analysis. *Peer J* **2016**, *4*, e2429.
3. Murphy, K. The Composition of Human Milk and Infant Faecal Microbiota Over the First Three Months of Life: A Pilot Study. *Scientific Reports* **2017**, *7*, 1–10.
4. Toscano, M.; De Grandi, R.; Peroni, D.G.; Grossi, E.; Facchin, V.; Comberiati, P.; Drago, L. Impact of Delivery Mode on the Colostrum Microbiota Composition. *BMC Microbiology* **2017**, *17*, 205.
5. De Andrés, J. Physiological Translocation of Lactic Acid Bacteria during Pregnancy Contributes to the Composition of the Milk Microbiota in Mice. *Nutrients* **2018**, *10*, 14.
6. Ojo-Okunola, A.; Nicol, M.; Toit, E. Human Breast Milk Bacteriome in Health and Disease. *Nutrients* **2018**, *10*.
7. Moossavi, S. Origins of Human Milk Microbiota: New Evidence and Arising Questions. *Gut Microbes* **2020**, *12.1*, 1667722.
8. Van Daele, E.; Knol, J.; Belzer, C. Microbial Transmission from Mother to Child: Improving Infant Intestinal Microbiota Development by Identifying the Obstacles. *Critical Reviews in Microbiology* **2019**, *45*, 613–648.
9. Wang, S.; Ryan, C.A.; Boyaval, P.; Dempsey, E.M.; Ross, R.P.; Stanton, C. Maternal Vertical Transmission Affecting Early-Life Microbiota Development. *Trends in Microbiology* **2020**, *28*, 28–45.
10. Arroyo, R. Treatment of Infectious Mastitis during Lactation: Antibiotics versus Oral Administration of Lactobacilli Isolated from Breast Milk. *Clinical Infectious Diseases* **2010**, *50*, 1551–1558.
11. Fernández, L. Prevention of Infectious Mastitis by Oral Administration of Lactobacillus Salivarius PS2 During Late Pregnancy. *Clinical Infectious Diseases* **2016**, *62*, 568–573.
12. Cacho, N.T. Personalization of the Microbiota of Donor Human Milk with Mother's Own Milk. *Frontiers in Microbiology* **2017**, *8*, 1470.
13. Ward, T.L. Human Milk Metagenome: A Functional Capacity Analysis. *BMC Microbiology* **2013**, *13*, 1–12.
14. Chong, C.Y.L. Factors Affecting Gastrointestinal Microbiome Development in Neonates. *Nutrients* **2018**, *10.3*, 274.
15. Dietert, R.R. A focus on microbiome completeness and optimized colonization resistance in neonatology. *NeoReviews* **2018**, *19*, 78–88.
16. van den Elsen, L.W.J. Shaping the Gut Microbiota by Breastfeeding: The Gateway to Allergy Prevention? *Frontiers in Pediatrics* **2019**, *7*, 47.
17. Cacho, N.T.; Lawrence, R.M. Innate Immunity and Breast Milk. *Frontiers in Immunology* **2017**, *8*, 584.
18. Le Doare, K. Mother's Milk: A Purposeful Contribution to the Development of the Infant Microbiota and Immunity. *Frontiers in Immunology* **2018**, *9*, 361.