Title

Evolutionary bridges: how factors present in amniotic fluid and human milk help mature the gut.

Journal

Journal of Perinatology, 44(11)

Authors

Chaaban, Hala

Burge, Kathryn

McElroy, Steven

Publication Date

2024-11-01

DOI

10.1038/s41372-024-02026-x

Peer reviewed

HHS Public Access

Author manuscript

J Perinatol. Author manuscript; available in PMC 2024 December 01.

Published in final edited form as:

J Perinatol. 2024 November ; 44(11): 1552–1559. doi:10.1038/s41372-024-02026-x.

Evolutionary bridges: how factors present in amniotic fluid and human milk help mature the gut

Hala Chaaban[1,✉], **Kathryn Burge**[1], **Steven J. McElroy**[2]

[1]Department of Pediatrics, Division of Neonatal-Perinatal Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA.

[2]Department of Pediatrics, Division of Neonatology, University of California, Davis, Sacramento, CA, USA.

Abstract

Necrotizing enterocolitis (NEC) continues to be a leading cause of morbidity and mortality in preterm infants. As modern medicine significantly improves the survival of extremely premature infants, the persistence of NEC underscores our limited understanding of its pathogenesis. Due to early delivery, a preterm infant's exposure to amniotic fluid (AF) is abruptly truncated. Replete with bioactive molecules, AF plays an important role in fetal intestinal maturation and preparation for contact with the environment, thus its absence during development of the intestine may contribute to increased susceptibility to NEC. Human milk (HM), particularly during the initial phases of lactation, is a cornerstone of neonatal intestinal defense. The concentrations and activities of several bioactive factors in HM parallel those of AF, suggesting continuity of protection. In this review, we discuss the predominant overlapping bioactive components of HM and AF, with an emphasis on those associated with intestinal growth or reduction of NEC.

INTRODUCTION

Necrotizing enterocolitis (NEC) continues to be one of the most devastating gastrointestinal pathologies in the neonatal period [1]. It is estimated that 4–7% of preterm infants develop NEC with a 30–50% mortality [2]. Infants who survive NEC are also at risk of significant morbidities, such as neurodevelopmental impairment, bronchopulmonary dysplasia, and short gut syndrome. Identifying modifiable factors that contribute to development of NEC is currently a priority.

The pathophysiology of NEC appears to be multifaceted, involving a complex interplay of risk factors including prematurity, dysbiosis, and feeding [1, 3]. Vulnerability of the premature gut is largely attributed to its underdevelopment [3, 4], with research suggesting key intestinal cellular components, such as Paneth cells defending against

✉ **Correspondence** and requests for materials should be addressed to Hala Chaaban. Hala-chaaban@ouhsc.edu.

AUTHOR CONTRIBUTIONS

HC wrote the manuscript. KYB and SJM edited and prepared the final version of the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

pathogens and supporting stem cells, are found in fewer numbers in preterm infants [5]. Additionally, the production of mucins by goblet cells and the expression and localization of tight junction (TJ) proteins, essential for barrier integrity, are diminished, increasing the risk of pathogen invasion [6]. Overexpression of toll-like receptor 4 (TLR4) in the immature intestinal epithelium further compounds this risk [7]. While TLR4 is critical for intestinal development in utero, its activation post-birth in a preterm gut can instigate exaggerated inflammatory responses and negatively impact cellular processes such as stem cell proliferation, leading to increased apoptosis and upregulated autophagy pathways [8]. Collectively, these factors lead to a compromised intestinal barrier prone to bacterial translocation, inflammation, and mucosal necrosis via immune cell interactions with bacterial antigens [9].

Since the intestinal epithelium is a single-cell barrier facing the lumen, examining substances swallowed by the fetus or infant and transiting through the gastrointestinal tract (GIT) is a reasonable place to expect maturation factors. Central to this discussion is amniotic fluid (AF), a unique liquid serving as a reservoir of nutrients, cytokines, growth factors, and essential bioactive molecules indispensable for developmental maturation of the fetus [10]. The fetal gut prepares for postnatal complex digestion and microbial colonization via continuous AF exposure through swallowing (Fig. 1) [10, 11]. Interruption of in utero maturation due to premature birth may lead to a deficit in protective AF bioactive exposures, leaving the neonate vulnerable to pathologies such as NEC [11]. Hirai et al. compared the trophic effects of AF or recombinant growth factors on a human fetal small intestinal cell line [11]. While individual and combined recombinant growth factors stimulated cell growth, the impact was less pronounced than that of comprehensive AF. These findings support the notion that AF functions as a primary source of trophic factors essential for fetal intestinal development and maturation. Disruption of this crucial exposure may emerge as a significant risk factor for NEC in preterm infants, potentially explaining why infants born at lower gestations (with reduced accumulated AF exposure) might have proportionately higher rates of NEC.

Fortunately, human milk (HM) contain a diverse array of bioactive substances mirroring those in AF [10, 12]. HM, particularly colostrum and transitional milk, is highly concentrated with immune and growth factors, such as epidermal growth factor (EGF), transforming growth factor beta (TGF-β), oligosaccharides, and lactoferrin [13]. Moreover, the bioactive composition of preterm milk is distinct from term milk, suggesting an evolutionary adaptation tailored to the unique needs of infants born prematurely with underdeveloped GITs [14, 15]. Importantly, NEC onset typically occurs 2–3 weeks after birth [16], offering a pivotal opportunity for early prevention. During this crucial window, provision of mother's own milk (MOM), especially the bioactive-rich colostrum and transitional milk, becomes indispensable, given the rich overlap with bioactive components found in AF.

This review emphasizes the critical role of maternal milk (MOM) in early preterm life as a key strategy to enhance intestinal maturation beyond the levels achieved through AF, showcasing the evolutionary architecture of HM as a dynamic bridge from prenatal to postnatal life, tailored to the specific developmental lactational stage and reflecting varying

neonatal needs. Further, the roles of common nutrients, cytokines and growth factors within AF and HM (Table 1), and changes in their composition over lactation and birth maturity (Table 2), are discussed, with a focus on intestinal development and NEC prevention.

GROWTH FACTORS AND CYTOKINE CONCENTRATIONS ACROSS LACTATION AND BIRTH MATURITY

Cytokines and growth factors are abundant in both AF and HM and are particularly critical in shaping neonatal immune function. The composition of AF and HM bioactive factors is influenced by gestational age, perinatal infections, and maternal physical activity. Further, the cytokine profile in HM changes across lactation stages, and the implications of specific cytokine concentrations as they relate to neonatal health remain a topic of ongoing research and discussion [17].

- EGF plays a critical role in maturation and repair of the intestinal mucosa [18]. EGF is found in significant quantities in AF and increases with increased gestation [10, 11]. HM EGF levels are almost 500 times higher than any other HM growth factor, with levels exceeding those in maternal serum. EGF is particularly pronounced in the milk of mothers who have delivered extremely preterm infants, between 23 and 27 weeks gestation, where EGF levels are about 60–80% higher than in the milk of mothers of older preterm or full-term infants [11]. Resistant to degradation by stomach acid and digestive enzymes, EGF reaches the small intestine intact without loss of functional activity.

Previous studies have shown EGF plays an important role in intestinal development and maturation. For example, EGF administration in mouse pups increased villous height and crypt depth, promoted proliferation and differentiation of enterocytes into goblet cells, and played a role in regulating TJ protein expression [18]. Moreover, in preclinical NEC models, enteral administration of EGF demonstrates protective efficacy, attributable in part to the upregulation of TJ proteins and mitigation of apoptotic pathway effects [19, 20]. In a small study, infants who developed NEC had diminished levels of EGF in their saliva and serum compared with age-matched controls, suggesting EGF deficiency in NEC susceptibility [21]. Collectively, these findings suggest a potential therapeutic potential of EGF supplementation as a targeted intervention to mitigate the risk of NEC.

- Insulin-Like Growth Factor-1 (IGF-1) is a potent growth-promoting peptide that plays a role in fetal and postnatal development. Its presence in high concentrations in AF underscores the likely significance to fetal development [22]. Premature birth leads to an immediate drop in IGF-1 levels, with recovery reliant on the eventual increase in endogenous production from the neonatal liver [23]. Importantly, reduced postnatal IGF-1 in infant serum is correlated with restricted growth, retinopathy of prematurity, and NEC [24]. In HM, IGF-1 concentration is relatively high in colostrum and steadily decreases over the first six months of lactation [25]. Studies have documented either congruent or elevated concentrations of IGF-1 levels in preterm versus term milk [22, 26]. When bound to IGF binding protein-2, IGF-1 remains stable in the stomach and reaches the small intestine intact [26]. Functionally, IGF-1 exhibits protective effects predominantly by inhibiting cell apoptosis, increasing the proliferation of intestinal stem cells, and supporting

the survival of cells in the crypt during injuries inflicted by radiation or oxidative stress [27]. Enteric administration of IGF-1 in rats had protective effects against NEC by enhancing intestinal barrier function and attenuating the inflammatory response [28]. Moreover, in murine models, exogenous IGF-1 increased endothelial cell proliferation, microvascular development, and was associated with increased expression of mucin and secretory IgA (sIgA) while protecting against oxidative injury [27, 29].

Given the potential benefits, the use of IGF-1 in clinical studies has been of interest. In a randomized, controlled trial with very low birthweight neonates, supplementing formula with IGF-1 improved gut barrier function. However, the benefits were transient with no obvious effects on feeding tolerance or weight gain. Moreover, while intravenous administration of recombinant IGF-1 was well-tolerated in Phase 2 clinical trials, there has been no discernable effect on NEC, underscoring the need for more extensive studies [25, 30].

– Vascular Endothelial Growth Factor (VEGF): The presence of VEGF in early HM and its interplay with the inhibitory factor sFlt-1 (soluble fms-like tyrosine kinase 1, also known as sVEGFR-1) is a subject of considerable interest. VEGF is found at concentrations approximately 300 times higher in early HM compared to adult serum. Simultaneously, the concentration of sFlt-1 in HM is about ten times greater than that in the serum of healthy pregnant women [31]. As lactation matures, a marked decline is noted, particularly in sFlt-1. The variations in VEGF levels between term and preterm HM, as well as the notably lower levels in the milk of mothers with small for gestational age infants, suggest a potential role in perinatal development [31]. Within the neonatal gut, VEGF serves as a critical angiogenic agent, stimulating the development of new blood vessels that expand mucosal surface area, facilitating efficient nutrient uptake, and contributing to cellular growth and differentiation [32]. Furthermore, experimental rat models of NEC, incorporating the risk factors of hypoxia and lipopolysaccharide, have shown VEGF administration can offer protection against intestinal damage [33]. Despite the optimistic implications of these findings, direct benefits of HM VEGF for the prevention of NEC in preterm infants have yet to be conclusively established.

– Erythropoietin (EPO), a glycoprotein originating primarily from the liver and kidneys, stimulate red blood cell production, primarily in response to cellular hypoxia. EPO receptors are functional during fetal and neonatal life and are present on intestinal epithelial cells. Both HM and AF contain EPO, underlining its potential significance in both prenatal and postnatal intestinal development [34, 35].

Postnatally, enteral and systemic administration of recombinant human EPO increases small intestinal length and villus surface area in rat pups. Further evidence of EPO's growth-promoting effects comes from observations that treatment of dams with intraperitoneal EPO for 1–2 weeks increases rat pup small intestinal length by 9% and villus surface area by 15% [36]. The protective effects of EPO in injury models are linked to promotion of epithelial cell migration and anti-apoptotic effects, particularly against TNF-induced apoptosis. Additionally, EPO strengthens the intestinal barrier in experimental NEC by maintaining TJ integrity and modulating both autophagy and apoptosis [36, 37]. In clinical

settings, EPO utilization in preterm infants has shown some promise [38, 39]. A meta-analysis incorporating 22 randomized, controlled trials with over 5,000 infants revealed that early administration of EPO can reduce the risk of confirmed NEC by 23%. Evidence from this analysis was moderate-to-low in quality, however [40]. Additionally, a recent large trial with extremely preterm infants focusing on the impact of high-dose EPO on neurodevelopmental outcomes revealed no significant difference in NEC incidence [41]. These studies underscore the need for further high-quality, double-blinded randomized controlled trials to substantiate these findings.

- Granulocyte colony-stimulating factor (G-CSF) is a glycoprotein found in significant quantities in AF [42] and colostrum [43]. Additionally, HM from mothers with intraamniotic infection had higher levels of G-CSF, indicating a possible immune response to the infection [43]. Both natural and synthetic forms of G-CSF maintain their stability against acidity in the stomach when administered within the pH-buffering matrix of HM, but break down when administered with infant formula [44]. When ingested, G-CSF binds to receptors on intestinal villi, promoting growth and maturation of the fetal gut in situ [45].

Studies in animal models have presented mixed results: while G-CSF reduced gut damage in a rat model of hypoxiareoxygenation [46], it exacerbated inflammation in a mouse model of NEC when administered subcutaneously, suggesting that the benefits of G-CSF might be context-dependent and potentially associated with neutrophil activity.

In a pilot study, infants with early-stage NEC showed improvement and a reduction in disease progression when given G-CSF enterally [47]. These infants also demonstrated more rapid clinical and radiological recovery, resulting in shorter hospital stays. Another trial echoed these benefits, with preterm infants displaying better feeding tolerance and a lower incidence of NEC following enteral G-CSF administration, without a rise in serum G-CSF levels [48]. These findings indicate that G-CSF has potential as a therapeutic agent when given enterally, but the protective mechanism is not yet clear.

– TGF-β is an extracellular peptide with multiple roles in cellular growth, differentiation, motility, and apoptosis [49]. Among its three isoforms, TGF-β2 is found naturally in AF and HM [50, 51]. Similar to concentrations of other growth factors, those of TGF-β2 peak in colostrum and notably diminish by 4–6 weeks postpartum. TGF-β2 is renowned for its broad immunomodulatory functions, promoting intestinal maturity and defense mechanisms, facilitating the IgM to IgA class switch in B lymphocytes, enhancing immunoglobulin synthesis in both the mammary gland and the neonatal GIT, contributing to intestinal mucosal repair, and fostering oral tolerance [51–53]. Notably, in preterm infants, lower levels of TGF-β have been observed prior to NEC onset, suggesting a deficiency or compromised activity of this factor is associated with an increased risk of NEC [54]. The importance of TGF-β in intestinal health is further highlighted in experimental models of NEC, where disruption of TGF-β signaling worsened inflammatory injury. Additionally, enteral supplementation of recombinant TGF-β2 in formula attenuated intestinal mucosal injury in NEC models [54].

– Interleukin (IL)-1β is a potent pro-inflammatory cytokine that plays a central role in immune responses, especially during the early stages of infection or injury. In HM, IL-1β is found in higher concentrations in colostrum compared to mature milk [53]. This elevated level in colostrum is believed to prime the neonatal immune system [11]. IL-1β assists in recruiting immune cells to sites of infection or injury and promotes the maturation of B cells. Studies have indicated IL-1β concentrations are generally higher in the milk of mothers who delivered preterm. This increased level might offer additional protection to premature infants who are at a greater risk of infections due to underdeveloped immune systems.

– IL-6 is a multifunctional cytokine with roles in inflammation, modulation of immune response, and hematopoiesis. Like those of IL-1β, concentrations of IL-6 are significantly elevated in colostrum, gradually decreasing as lactation progresses [53]. While IL-6 has pro-inflammatory properties, it also plays a role in transitioning from inflammation to anti-inflammation during the resolution phase of an immune response. Moreover, IL-6 aids in the differentiation of B cells into antibody-producing plasma cells, bolstering the adaptive immune response. Preterm HM generally exhibits higher levels of IL-6 than term HM [11].

– IL-8 has gained attention for its potential role in neonatal gut development, given its significant concentrations in AF and HM [10, 55]. Fluctuations in concentrations of IL-8 and its receptors, chemokine receptor 1 and 2 (CXCR-1 and CXCR-2), throughout different lactation stages has been reported previously, with findings revealing IL-8 concentrations are notably higher in colostrum compared to mature milk, and decline by 15 days postpartum in both term and preterm deliveries [56]. Notably, introducing recombinant human IL-8 to human fetal and adult intestinal cells leads to enhanced cell migration, proliferation, and differentiation, hinting at broader roles for IL-8 beyond the established function as a neutrophil attractant.

IMMUNOGLOBULINS

Immunoglobulins (Igs) are integral to passive immunity in infants, and detectable in both AF and HM. The composition and concentration of Igs is influenced by various factors, including maternal infectious history, vaccination status, genetic predispositions, environmental exposures, and overall health [57]. In HM, secretory IgA (sIgA) dominates, accounting for 90–95% of Ig content, with levels highest in colostrum while demonstrating both interindividual variability and variability across lactation periods [58]. This variability and heterogeneity in sIgA levels is also notable when evaluating differences in term and preterm HM [59, 60]. Studies show HM-derived sIgA plays a significant role in establishment of the host-microbiota symbiosis in preterm infants, with a decrease in IgA-bound bacteria correlating with NEC onset [61]. It is important to note that the primary source of significant sIgA during the critical first weeks of life is MOM, underscoring its importance in reducing NEC [62, 63].

Despite the potential benefits of enteral Ig supplementation suggested by early studies [64], a Cochrane review analyzing three trials with 2,095 neonates concluded that enteral IgG or IgG/IgA combinations do not significantly affect the incidence of NEC, the need for NEC

surgery, or death from NEC [65]. The effectiveness of sIgA sourced from HM has yet to be evaluated.

LACTOFERRIN

Lactoferrin is an abundant glycoprotein found within AF and HM with potent antimicrobial properties [66, 67]. Though a role for AF lactoferrin in fetal development or immunity has not yet been identified, postnatally, oral intake of lactoferrin is associated with enhanced immunity, improved GIT function, and possibly reduced incidence of neonatal infections. Notably, colostrum from mothers delivering prematurely contains elevated lactoferrin concentrations compared to that of mothers delivering at term [68]. As the lactational phase transitions from early to mature milk, lactoferrin concentrations decrease [69]. Lactoferrin is resistant to proteolytic breakdown in the neonatal GIT and binds specific receptors on the enterocyte brush border, facilitating intestinal cell proliferation and differentiation [67]. As a transferrin family member, lactoferrin binds iron, providing antioxidant and bacteriostatic effects for the host by favoring bacteria with minimal iron needs, such as *Lactobacillus* and *Bifidobacterium* species. In addition, lactoferrin is adept at neutralizing endotoxin-mediated reactions, thereby inhibiting the activation of mononuclear cells and associated pro-inflammatory cytokine release [70]. Concurrently, lactoferrin enhances the intestinal epithelial barrier by modulating TJ protein expression [71].

Given its bioactive properties, lactoferrin has been investigated as a therapeutic option for preterm infants. Pilot studies have confirmed the protective effects of bovine lactoferrin supplementation in reducing incidence of both late-onset sepsis and NEC. However the largest, most recent randomized, controlled trial involving 2,203 infants has shown lactoferrin supplementation, alone, does not significantly reduce incidence of NEC or infections [72]. This underscores the complexity of NEC etiology and could imply that the protective effects against NEC are due to synergistic effects from multiple HM components rather than the individual influences of isolated factors.

HM OLIGOSACCHARIDES (HMOS)

HMOs are unconjugated, lactose-based carbohydrate structures discovered in HM, and most recently revealed and quantified in AF during early pregnancy [73]. These studies have identified the presence of 3’-sialyllactose and 2’-fucosyllactose in AF, and a significant positive correlation between gestational age and the concentrations of both individual and total HMOs in AF. This suggests that various fetal tissues, including the gut, may be exposed to HMOs throughout development, although the full implications of these exposures are yet to be understood.

HMOs exhibit a more diverse profile in HM than in the milk of other mammals, influenced by genetic factors like secretor and Lewis genes, and environmental elements such as maternal diet and metabolic status [74]. The concentration of sialylated HMOs is initially high in postpartum HM but decreases throughout lactation. Notably, HM from mothers of preterm infants has higher oligosaccharide levels, demonstrating the adaptability of HMO production and hinting at significant impacts on preterm infants [75].

HMOs also act as prebiotics, fostering growth and diversity of beneficial gut bacteria, including *Bifidobacterium*. In addition, HMOs enhance gut barrier function by reducing host intestinal permeability and promoting mucin expression [75].

Studies, both in animals and humans, have provided insights into the potential contributions of HMOs to decreased incidence of NEC. In a mouse model of NEC, pups fed formula supplemented with HMOs exhibited elevated mucin expression and reduced intestinal permeability [76]. Similarly, in a rat model of NEC, pups receiving HMO-supplemented formula showed improved survival rates, with the HMO disialyllacto-N-tetraose identified as driving much of this protection. Additionally, formulas enriched with 2'-fucosyllactose have demonstrated associations with decreased NEC rates in rodent models.

Furthermore, studies have shown that low levels of disialyllacto-N-tetraose in HM are associated with increased rates of NEC. Additionally, a reduced diversity of HMOs, particularly of lacto-N-difucohexaose I in the first month postpartum, is linked to a greater risk of NEC in preterm infants. This underscores the critical role of HMOs in neonatal health, particularly among preterm infants [76].

HYALURONIC ACID (HA)

HA is a key molecule in human tissues, contributing to various processes like cell signaling, wound healing, and tissue regeneration. During embryonic development, HA concentrations peak in AF around gestational weeks 16 to 20, particularly vital for GIT differentiation [77]. Postnatally, interaction of HA with CD44 and TLR4 is crucial for GIT development, as evidenced by studies indicating mice lacking these receptors have shortened GIT despite normal general growth [78].

HM is a critically important source of exogenous HA during infancy, aiding GIT development and protection against neonatal intestinal pathogens [79, 80]. HM HA concentrations vary during lactation, with the highest concentrations (755 ng/mL) during the first week postpartum and then decreasing to static levels after 60 days of lactation [81].

Studies have focused on oral administration of low molecular weight HA, specifically HA35 (35 kDa HA) administration, and its impact on intestinal maturation and inflammation [80, 82]. Notably, HA35 treatment has been associated with increased ileal villus length, deeper crypts, heightened proliferation of intestinal epithelial cells, and an increase in the numbers of goblet and Paneth cells within the murine small intestine [80]. Furthermore, pre-treatment with HA35 resulted in a dose-dependent improvement in survival, intestinal injury, and bacterial translocation in murine NEC models [82, 83]. These data collectively suggest that both HA derived from both AF and HM may play a crucial role in accelerating intestinal development, enhancing the integrity of the intestinal epithelium, and potentially protecting against NEC.

NUTRIENTS IN AF AND HM

AF and HM are both rich sources of nutrients for fetal and neonatal development, including a comprehensive array of amino acids (AAs) [84]. Both fluids contain protein-bound AAs

and free amino acids (FAAs), with FAAs crucial for rapid absorption and direct benefits on neonatal growth. FAA levels, particularly of glutamine, glutamate, and arginine, increase significantly during the first three months of lactation. This is believed to support infant growth by aiding intestinal function, immune development, and influencing gut microbiota composition [84, 85].

– L-arginine, a precursor to nitric oxide (NO), is crucial for vascular regulation in the endothelium and serves as a powerful vasodilator in the neonatal intestine. Research on neonatal piglets has shown decreased arginine levels are a precursor to NEC, and supplementation with L-arginine can reduce intestinal damage, likely through enhanced NO synthase activity and NO production [86, 87]. In preterm infants, a correlation has been established between low levels of L-arginine and an increased risk of developing NEC. Although animal studies and some randomized controlled trials suggest L-arginine supplementation may prevent NEC, a 2017 Cochrane review offers a more detailed analysis, noting that while supplementation significantly lowers the risk of NEC at Bell's Stage 1, it does not provide the same protection for the more severe Stages 2 and 3 [86, 87].

– Glutamine (Gln) is the most abundant essential FAA in HM, especially in the first three months of lactation [86]. Similar to L-arginine, low Gln levels have been linked to NEC at days 7 and 14 post-birth. In preclinical studies, Gln supplementation was associated with increased intestinal epithelial cell proliferation and regulation of TJ protein expression. Despite these benefits, a study involving 1,433 infants showed no difference in NEC incidence between infants receiving glutamine and controls. This finding was echoed by a Cochrane review of 12 trials with 2,877 subjects, indicating no clear evidence that glutamine supplementation benefits preterm infants in terms of mortality, NEC, or invasive infections.

CONCLUSIONS

NEC remains a challenge in neonatal medicine, with a significant impact on the survival and long-term health of preterm infants. Here, we highlight the complex network of factors whose aberrant concentrations potentially contribute to NEC pathogenesis, emphasizing the critical role played by the abrupt termination of AF exposure due to premature birth. AF, a rich reservoir of nutrients, cytokines, growth factors, and bioactive molecules, serves as a catalyst for fetal intestinal development and primes the gut for postnatal challenges. The similarity between bioactive components in AF and HM highlights the importance of providing MOM during the critical early stages of preterm infant life, especially colostrum and transitional milk. These bioactive substances form a continuum from prenatal to postnatal life, potentially providing essential protection against NEC. Furthermore, although donor milk (DM) offers improved protection over infant formula, the degree of protection does not match that of MOM, likely due to the dynamic changes and bioactive richness associated with early MOM lactational stages. These differences are exacerbated by the processing, including pasteurization, and term milk origins required of DM, which significantly reduce concentrations and activities of bioactive HM components. Therefore, as the field of neonatal medicine progresses, clinical initiatives should focus on enhancing the availability of MOM for preterm infants, particularly during the vulnerable postnatal period, to mitigate the risk of NEC.

FUNDING

HC is supported by an NIH NICHD R01HD109784 and R43HD114348. KYB is supported by an NIH NICHD R21HD112659, NIH NIGMS P20GM134973, and Harold Hamm Diabetes Center seed grant. SJM is supported by an NIH NIDDK R01DK125415.

REFERENCES

1. Hackam D, Caplan M. Necrotizing enterocolitis: Pathophysiology from a historical context. Semin Pediatr Surg. 2018;27:11–8. [PubMed: 29275810]
2. Neu J, Walker WA. Necrotizing enterocolitis. N Engl J Med. 2011;364:255–64. [PubMed: 21247316]
3. Pammi M, Cope J, Tarr PI, Warner BB, Morrow AL, Mai V, et al. Intestinal dysbiosis in preterm infants preceding necrotizing enterocolitis: a systematic review and meta-analysis. Microbiome. 2017;5:31. [PubMed: 28274256]
4. Battersby AJ, Gibbons DL. The gut mucosal immune system in the neonatal period. Pediatr Allergy Immunol. 2013;24:414–21. [PubMed: 23682966]
5. Lueschow SR, McElroy SJ. The Paneth Cell: The Curator and Defender of the Immature Small Intestine. Front Immunol. 2020;11:587. [PubMed: 32308658]
6. Chaaban H, Patel MM, Burge K, Eckert JV, Lupu C, Keshari RS, et al. Early Antibiotic Exposure Alters Intestinal Development and Increases Susceptibility to Necrotizing Enterocolitis: A Mechanistic Study. Microorganisms. 2022;10:519. [PubMed: 35336095]
7. Nanthakumar N, Meng D, Goldstein AM, Zhu W, Lu L, Uauy R, et al. The mechanism of excessive intestinal inflammation in necrotizing enterocolitis: an immature innate immune response. PloS One. 2011;6:e17776. [PubMed: 21445298]
8. Neal MD, Sodhi CP, Dyer M, Craig BT, Good M, Jia H, et al. A critical role for TLR4 induction of autophagy in the regulation of enterocyte migration and the pathogenesis of necrotizing enterocolitis. J Immunol. 2013;190:3541–51. [PubMed: 23455503]
9. Lu P, Sodhi CP, Hackam DJ. Toll-like receptor regulation of intestinal development and inflammation in the pathogenesis of necrotizing enterocolitis. Pathophysiology. 2014;21:81–93. [PubMed: 24365655]
10. Dasgupta S, Arya S, Choudhary S, Jain SK. Amniotic fluid: Source of trophic factors for the developing intestine. World J Gastrointest Pathophysiol. 2016;7:38–47. [PubMed: 26909227]
11. Hirai C, Ichiba H, Saito M, Shintaku H, Yamano T, Kusuda S. Trophic effect of multiple growth factors in amniotic fluid or human milk on cultured human fetal small intestinal cells. J Pediatr Gastroenterol Nutr. 2002;34:524–8. [PubMed: 12050579]
12. Carr LE, Virmani MD, Rosa F, Munblit D, Matazel KS, Elolimy AA, et al. Role of Human Milk Bioactives on Infants' Gut and Immune Health. Front Immunol. 2021;12:604080. [PubMed: 33643310]
13. Lyons KE, Ryan CA, Dempsey EM, Ross RP, Stanton C. Breast Milk, a Source of Beneficial Microbes and Associated Benefits for Infant Health. Nutrients. 2020;12:1039. [PubMed: 32283875]
14. Guo J, Tan M, Zhu J, Tian Y, Liu H, Luo F, et al. Proteomic Analysis of Human Milk Reveals Nutritional and Immune Benefits in the Colostrum from Mothers with COVID-19. Nutrients. 2022;14:2513. [PubMed: 35745243]
15. Sari RN, Pan J, Zhang W, Li Y, Zhu H, Pang X, et al. Comparative Proteomics of Human Milk From Eight Cities in China During Six Months of Lactation in the Chinese Human Milk Project Study. Front Nutr. 2021;8:682429. [PubMed: 34458300]
16. Caplan MS, Underwood MA, Modi N, Patel R, Gordon PV, Sylvester KG, et al. Necrotizing Enterocolitis: Using Regulatory Science and Drug Development to Improve Outcomes. J Pediatrics. 2019;212:208–15.e1.
17. Kobata R, Tsukahara H, Ohshima Y, Ohta N, Tokuriki S, Tamura S, et al. High levels of growth factors in human breast milk. Early Hum Dev. 2008;84:67–9. [PubMed: 17716837]

18. Tang X, Liu H, Yang S, Li Z, Zhong J, Fang R. Epidermal Growth Factor and Intestinal Barrier Function. Mediators Inflamm. 2016;2016:1927348. [PubMed: 27524860]
19. Clark JA, Lane RH, Maclennan NK, Holubec H, Dvorakova K, Halpern MD, et al. Epidermal growth factor reduces intestinal apoptosis in an experimental model of necrotizing enterocolitis. Am J Physiol Gastrointest Liver Physiol. 2005;288:G755–62. [PubMed: 15528252]
20. Nolan LS, Parks OB, Good M. A Review of the Immunomodulating Components of Maternal Breast Milk and Protection Against Necrotizing Enterocolitis. Nutrients. 2019;12:14. [PubMed: 31861718]
21. Warner BB, Ryan AL, Seeger K, Leonard AC, Erwin CR, Warner BW. Ontogeny of salivary epidermal growth factor and necrotizing enterocolitis. J Pediatrics. 2007;150:358–63.
22. York DJ, Smazal AL, Robinson DT, De Plaen IG. Human Milk Growth Factors and Their Role in NEC Prevention: A Narrative Review. Nutrients. 2021;13:3751. [PubMed: 34836007]
23. Hellström A, Ley D, Hansen-Pupp I, Hallberg B, Löfqvist C, van Marter L, et al. Insulin-like growth factor 1 has multisystem effects on foetal and preterm infant development. Acta Paediatr. 2016;105:576–86. [PubMed: 26833743]
24. Giapros VI, Schiza V, Challa AS, Pantou C, Theocharis PD, Andronikou SK. Serum insulin-like growth factor I (IGF-I), IGF-binding proteins-1 and -3, and postnatal growth of late preterm infants. Horm Metab Res. 2012;44:845–50. [PubMed: 22791601]
25. Milsom SR, Blum WF, Gunn AJ. Temporal changes in insulin-like growth factors I and II and in insulin-like growth factor binding proteins 1, 2, and 3 in human milk. Horm Res. 2008;69:307–11. [PubMed: 18259111]
26. Elmlinger MW, Hochhaus F, Loui A, Frommer KW, Obladen M, Ranke MB. Insulin-like growth factors and binding proteins in early milk from mothers of preterm and term infants. Horm Res. 2007;68:124–31. [PubMed: 17341887]
27. Baregamian N, Song J, Jeschke MG, Evers BM, Chung DH. IGF-1 protects intestinal epithelial cells from oxidative stress-induced apoptosis. J Surg Res. 2006;136:31–7. [PubMed: 16999977]
28. Tian F, Liu GR, Li N, Yuan G. Insulin-like growth factor I reduces the occurrence of necrotizing enterocolitis by reducing inflammatory response and protecting intestinal mucosal barrier in neonatal rats model. Eur Rev Med Pharm Sci. 2017;21:4711–9.
29. Ozen S, Akisu M, Baka M, Yalaz M, Sozmen EY, Berdeli A, et al. Insulin-like growth factor attenuates apoptosis and mucosal damage in hypoxia/reoxygenation-induced intestinal injury. Biol Neonate 2005;87:91–6. [PubMed: 15528875]
30. Ley D, Hallberg B, Hansen-Pupp I, Dani C, Ramenghi LA, Marlow N, et al. rhIGF-1/rhIGFBP-3 in Preterm Infants: A Phase 2 Randomized Controlled Trial. J Pediatr. 2019;206:56–65.e8. [PubMed: 30471715]
31. Loui A, Eilers E, Strauss E, Pohl-Schickinger A, Obladen M, Koehne P. Vascular Endothelial Growth Factor (VEGF) and soluble VEGF receptor 1 (sFlt-1) levels in early and mature human milk from mothers of preterm versus term infants. J Hum Lact. 2012;28:522–8. [PubMed: 22729710]
32. Gregory KE, Walker WA. Immunologic Factors in Human Milk and Disease Prevention in the Preterm Infant. Curr Pediatr Rep. 2013;1:222–8.
33. Yang HB, Kim HY, Kim SH, Kim SY. Suppressive role of vascular endothelial growth factor on intestinal apoptosis in induced necrotizing enterocolitis in rats. Ann Surg Treat Res. 2023;105:157–64. [PubMed: 37693290]
34. Seikku L, Stefanovic V, Rahkonen P, Teramo K, Paavonen J, Tikkanen M, et al. Amniotic fluid and umbilical cord serum erythropoietin in term and prolonged pregnancies. Eur J Obstet Gynecol Reprod Biol. 2019;233:1–5. [PubMed: 30529256]
35. Juul SE, Joyce AE, Zhao Y, Ledbetter DJ. Why is erythropoietin present in human milk? Studies of erythropoietin receptors on enterocytes of human and rat neonates. Pediatr Res. 1999;46:263–8. [PubMed: 10473039]
36. Juul SE, Ledbetter DJ, Joyce AE, Dame C, Christensen RD, Zhao Y, et al. Erythropoietin acts as a trophic factor in neonatal rat intestine. Gut. 2001;49:182–9. [PubMed: 11454792]

37. Yu Y, Shiou SR, Guo Y, Lu L, Westerhoff M, Sun J, et al. Erythropoietin protects epithelial cells from excessive autophagy and apoptosis in experimental neonatal necrotizing enterocolitis. PLoS One. 2013;8:e69620. [PubMed: 23936061]
38. Hosseini M, Azampour H, Raeisi S, Behtari M, Valizadeh H, Saboohi R. The effects of enteral artificial amniotic fluid-containing erythropoietin on short term outcomes of preterm infants. Turk J Pediatr. 2019;61:392–8. [PubMed: 31916717]
39. Wang Y, Song J, Sun H, Xu F, Li K, Nie C, et al. Erythropoietin prevents necrotizing enterocolitis in very preterm infants: a randomized controlled trial. J Transl Med. 2020;18:308. [PubMed: 32771013]
40. Ananthan A, Balasubramanian H, Mohan D, Rao S, Patole S. Early erythropoietin for preventing necrotizing enterocolitis in preterm neonates - an updated meta-analysis. Eur J Pediatr. 2022;181:1821–33. [PubMed: 35122138]
41. Juul SE, Comstock BA, Wadhawan R, Mayock DE, Courtney SE, Robinson T, et al. A Randomized Trial of Erythropoietin for Neuroprotection in Preterm Infants. N. Engl J Med. 2020;382:233–43. [PubMed: 31940698]
42. Raynor BD, Clark P, Duff P. Granulocyte colony-stimulating factor in amniotic fluid. Infect Dis Obstet Gynecol. 1995;3:140–4. [PubMed: 18476037]
43. Calhoun DA, Lunoe M, Du Y, Christensen RD. Granulocyte colony-stimulating factor is present in human milk and its receptor is present in human fetal intestine. Pediatrics. 2000;105:e7. [PubMed: 10617744]
44. Calhoun DA, Lunøe M, Du Y, Staba SL, Christensen RD. Concentrations of granulocyte colony-stimulating factor in human milk after in vitro simulations of digestion. Pediatr Res. 1999;46:767–71. [PubMed: 10590037]
45. Gersting JA, Christensen RD, Calhoun DA. Effects of enterally administering granulocyte colony-stimulating factor to suckling mice. Pediatr Res. 2004;55:802–6. [PubMed: 14764911]
46. Canpolat FE, Yurdakök M, Ozsoy S, Haziroğlu R, Korkmaz A. Protective effects of recombinant human granulocyte colony stimulating factor in a rat model of necrotizing enterocolitis. Pediatr Surg Int. 2006;22:719–23. [PubMed: 16871399]
47. Canpolat FE, Yurdakök M, Korkmaz A, Yiğit S, Tekinalp G. Enteral granulocyte colony-stimulating factor for the treatment of mild (stage I) necrotizing enterocolitis: a placebo-controlled pilot study. J Pediatr Surg. 2006;41:1134–8. [PubMed: 16769348]
48. El-Ganzoury MM, Awad HA, El-Farrash RA, El-Gammasy TM, Ismail EA, Mohamed HE, et al. Enteral granulocyte-colony stimulating factor and erythropoietin early in life improves feeding tolerance in preterm infants: a randomized controlled trial. J Pediatr. 2014;165:1140–5.e1. [PubMed: 25155966]
49. Kulkarni AB, Huh CG, Becker D, Geiser A, Lyght M, Flanders KC, et al. Transforming growth factor beta 1 null mutation in mice causes excessive inflammatory response and early death. Proc Natl Acad Sci USA. 1993;90:770–4. [PubMed: 8421714]
50. Frost BL, Jilling T, Lapin B, Maheshwari A, Caplan MS. Maternal breast milk transforming growth factor-beta and feeding intolerance in preterm infants. Pediatr Res. 2014;76:386–93. [PubMed: 24995914]
51. Morita Y, Campos-Alberto E, Yamaide F, Nakano T, Ohnisi H, Kawamoto M, et al. TGF-β Concentration in Breast Milk is Associated With the Development of Eczema in Infants. Front Pediatrics. 2018;6:162.
52. Penttila IA, van Spriel AB, Zhang MF, Xian CJ, Steeb CB, Cummins AG, et al. Transforming growth factor-beta levels in maternal milk and expression in postnatal rat duodenum and ileum. Pediatr Res. 1998;44:524–31. [PubMed: 9773841]
53. Hawkes JS, Bryan DL, James MJ, Gibson RA. Cytokines (IL-1beta, IL-6, TNF-alpha, TGF-beta1, and TGF-beta2) and prostaglandin E2 in human milk during the first three months postpartum. Pediatr Res. 1999;46:194–9. [PubMed: 10447115]
54. Maheshwari A, Kelly DR, Nicola T, Ambalavanan N, Jain SK, Murphy-Ullrich J, et al. TGF-beta2 suppresses macrophage cytokine production and mucosal inflammatory responses in the developing intestine. Gastroenterology. 2011;140:242–53. [PubMed: 20875417]

55. Maheshwari A, Lu W, Lacson A, Barleycorn AA, Nolan S, Christensen RD, et al. Effects of interleukin-8 on the developing human intestine. Cytokine. 2002;20:256–67. [PubMed: 12633567]
56. Polat A, Tunc T, Erdem G, Yerebasmaz N, Tas A, Beken S, et al. Interleukin-8 and Its Receptors in Human Milk from Mothers of Full-Term and Premature Infants. Breastfeed Med. 2016;11:247–51. [PubMed: 27105439]
57. Cederqvist LL, Ewool LC, Bonsnes RW, Litwin SD. Detectability and pattern of immunoglobulins in normal amniotic fluid throughout gestation. Am J Obstet Gynecol. 1978;130:220–4. [PubMed: 619662]
58. Atyeo C, Alter G. The multifaceted roles of breast milk antibodies. Cell. 2021;184:1486–99. [PubMed: 33740451]
59. Rio-Aige K, Azagra-Boronat I, Castell M, Selma-Royo M, Collado MC, Rodríguez-Lagunas MJ, et al. The Breast Milk Immunoglobulinome. Nutrients. 2021;13:1810. [PubMed: 34073540]
60. Mehta R, Petrova A. Biologically active breast milk proteins in association with very preterm delivery and stage of lactation. J Perinatol. 2011;31:58–62. [PubMed: 20523299]
61. Donald K, Petersen C, Turvey SE, Finlay BB, Azad MB. Secretory IgA: Linking microbes, maternal health, and infant health through human milk. Cell Host Microbe. 2022;30:650–9. [PubMed: 35550668]
62. Gopalakrishna KP, Macadangdang BR, Rogers MB, Tometich JT, Firek BA, Baker R, et al. Maternal IgA protects against the development of necrotizing enterocolitis in preterm infants. Nat Med. 2019;25:1110–5. [PubMed: 31209335]
63. Johnson-Hence CB, Gopalakrishna KP, Bodkin D, Coffey KE, Burr AHP, Rahman S, et al. Stability and heterogeneity in the anti-microbiota reactivity of human milk-derived Immunoglobulin A. J Exp Med. 2023;220:e20220839. [PubMed: 37462916]
64. Rubaltelli FF, Benini F, Sala M. Prevention of Necrotizing Enterocolitis in Neonates at Risk by Oral Administration of Monomeric IgG. Dev Pharmacol Therapeutics. 2017;17:138–43.
65. Foster JP, Seth R, Cole MJ. Oral immunoglobulin for preventing necrotizing enterocolitis in preterm and low birth weight neonates. Cochrane Database Syst Rev. 2016;4:CD001816. [PubMed: 27040323]
66. Niemelä A, Kulomaa M, Vija P, Tuohimaa P, Saarikoski S. Lactoferrin in human amniotic fluid. Hum Reprod. 1989;4:99–101.
67. Buccigrossi V, de Marco G, Bruzzese E, Ombrato L, Bracale I, Polito G, et al. Lactoferrin induces concentration-dependent functional modulation of intestinal proliferation and differentiation. Pediatr Res. 2007;61:410–4. [PubMed: 17515863]
68. Albenzio M, Santillo A, Stolfi I, Manzoni P, Iliceto A, Rinaldi M, et al. Lactoferrin Levels in Human Milk after Preterm and Term Delivery. Am J Perinatol. 2016;33:1085–9. [PubMed: 27603541]
69. Mastromarino P, Capobianco D, Campagna G, Laforgia N, Drimaco P, Dileone A, et al. Correlation between lactoferrin and beneficial microbiota in breast milk and infant's feces. Biometals. 2014;27:1077–86. [PubMed: 24970346]
70. Liu Y, Perego M, Xiao Q, He Y, Fu S, He J, et al. Lactoferrin-induced myeloid-derived suppressor cell therapy attenuates pathologic inflammatory conditions in newborn mice. J Clin Investig. 2019;129:4261–75. [PubMed: 31483289]
71. Reniker LN, Frazer LC, Good M. Key biologically active components of breast milk and their beneficial effects. Semin Pediatr Surg. 2023;32:151306. [PubMed: 37276783]
72. ELFIN trial investigators group. Enteral lactoferrin supplementation for very preterm infants: a randomised placebo-controlled trial. Lancet. 2019;393:423–33. [PubMed: 30635141]
73. Jantscher-Krenn E, von Schirnding L, Trötzmüller M, Köfeler H, Kurtovic U, Fluhr H, et al. Human Milk Oligosaccharides Are Present in Amniotic Fluid and Show Specific Patterns Dependent on Gestational Age. Nutrients. 2022;14:2065. [PubMed: 35631205]
74. Johnson PH, Watkins WM. Purification of the Lewis blood-group gene associated alpha-3/4-fucosyltransferase from human milk: an enzyme transferring fucose primarily to type 1 and lactose-based oligosaccharide chains. Glycoconj J. 1992;9:241–9. [PubMed: 1490103]
75. Bode L Human milk oligosaccharides: every baby needs a sugar mama. Glycobiology. 2012;22:1147–62. [PubMed: 22513036]

76. Bode L Human Milk Oligosaccharides in the Prevention of Necrotizing Enterocolitis: A Journey From in vitro and in vivo Models to Mother-Infant Cohort Studies. Front Pediatrics. 2018;6:385.
77. Dahl LB, Dahl IM, Børresen AL. The molecular weight of sodium hyaluronate in amniotic fluid. Biochem Med Metab Biol. 1986;35:219–26. [PubMed: 3707753]
78. Riehl TE, Santhanam S, Foster L, Ciorba M, Stenson WF. CD44 and TLR4 mediate hyaluronic acid regulation of Lgr5+ stem cell proliferation, crypt fission, and intestinal growth in postnatal and adult mice. Am J Physiol Gastrointest Liver Physiol. 2015;309:G874–87. [PubMed: 26505972]
79. Burge K, Bergner E, Gunasekaran A, Eckert J, Chaaban H. The Role of Glycosaminoglycans in Protection from Neonatal Necrotizing Enterocolitis: A Narrative Review. Nutrients. 2020;12:546. [PubMed: 32093194]
80. Chaaban H, Burge K, Eckert J, Trammell M, Dyer D, Keshari RS, et al. Acceleration of Small Intestine Development and Remodeling of the Microbiome Following Hyaluronan 35 kDa Treatment in Neonatal Mice. Nutrients. 2021;13:2030. [PubMed: 34204790]
81. Hill DR, Rho HK, Kessler SP, Amin R, Homer CR, McDonald C, et al. Human milk hyaluronan enhances innate defense of the intestinal epithelium. J Biol Chem. 2013;288:29090–104. [PubMed: 23950179]
82. Gunasekaran A, Eckert J, Burge K, Zheng W, Yu Z, Kessler S, et al. Hyaluronan 35 kDa enhances epithelial barrier function and protects against the development of murine necrotizing enterocolitis. Pediatric Res. 2020;87:1177–84.
83. Burge K, Eckert J, Wilson A, Trammell M, Lueschow SR, McElroy SJ, et al. Hyaluronic Acid 35 kDa Protects against a Hyperosmotic, Formula Feeding Model of Necrotizing Enterocolitis. Nutrients. 2022;14:1779. [PubMed: 35565748]
84. Sano M, Nagura H, Ueno S, Nakashima A. Amino Acid Composition of Amniotic Fluid during the Perinatal Period Reflects Mother's Fat and Carbohydrate Intake. Nutrients. 2021;13:2136. [PubMed: 34206490]
85. Mesavage WC, Suchy SF, Weiner DL, Nance CS, Flannery DB, Wolf B. Amino acids in amniotic fluid in the second trimester of gestation. Pediatr Res. 1985;19:1021–4. [PubMed: 4058974]
86. Sami AS, Frazer LC, Miller CM, Singh DK, Clodfelter LG, Orgel KA, et al. The role of human milk nutrients in preventing necrotizing enterocolitis. Front Pediatrics. 2023;11:1188050.
87. Richir MC, Siroen MP, van Elburg RM, Fetter WP, Quik F, Nijveldt RJ, et al. Low plasma concentrations of arginine and asymmetric dimethylarginine in premature infants with necrotizing enterocolitis. Br J Nutr. 2007;97:906–11. [PubMed: 17381965]
88. Vuorela P, Helske S, Hornig C, Alitalo K, Weich H, Halmesmäki E. Amniotic fluid-soluble vascular endothelial growth factor receptor-1 in preeclampsia. Obstet Gynecol. 2000;95:353–7. [PubMed: 10711543]
89. Ozgurtas T, Aydin I, Turan O, Koc E, Hirfanoglu IM, Acikel CH, et al. Vascular endothelial growth factor, basic fibroblast growth factor, insulin-like growth factor-I and platelet-derived growth factor levels in human milk of mothers with term and preterm neonates. Cytokine. 2010;50:192–4. [PubMed: 20202860]
90. Hardin J, Kroeker K, Chung B, Gall DG. Effect of proinflammatory interleukins on jejunal nutrient transport. Gut. 2000;47:184–91. [PubMed: 10896908]
91. Otsuki K, Yoda A, Saito H, Mitsuhashi Y, Toma Y, Shimizu Y, et al. Amniotic fluid lactoferrin in intrauterine infection. Placenta. 1999;20:175–9. [PubMed: 10195738]
92. Wise A, Robertson B, Choudhury B, Rautava S, Isolauri E, Salminen S, et al. Infants Are Exposed to Human Milk Oligosaccharides Already in utero. Front Pediatrics. 2018;6:270.
93. Dahl LB, Kimpton WG, Cahill RN, Brown TJ, Fraser RE. The origin and fate of hyaluronan in amniotic fluid. J Dev Physiol. 1989;12:209–18. [PubMed: 2634062]
94. Rogier EW, Frantz AL, Bruno ME, Wedlund L, Cohen DA, Stromberg AJ, et al. Secretory antibodies in breast milk promote long-term intestinal homeostasis by regulating the gut microbiota and host gene expression. Proc Natl Acad Sci USA. 2014;111:3074–9. [PubMed: 24569806]

95. Gulbiniene V, Balciuniene G, Petroniene J, Viliene R, Dumalakiene I, Pilypiene I, et al. The Significance of Epidermal Growth Factor in Noninvasively Obtained Amniotic Fluid Predicting Respiratory Outcomes of Preterm Neonates. Int J Mol Sci. 2022;23:2978. [PubMed: 35328399]

96. Cummins AG, Thompson FM. Effect of breast milk and weaning on epithelial growth of the small intestine in humans. Gut. 2002;51:748–54. [PubMed: 12377819]

97. Dvorak B, Fituch CC, Williams CS, Hurst NM, Schanler RJ. Increased epidermal growth factor levels in human milk of mothers with extremely premature infants. Pediatr Res. 2003;54:15–9. [PubMed: 12646719]

98. Wathen NC, Wang HS, Cass PL, Campbell DJ, Chard T. Insulin-like growth factor-1 and insulin-like growth factor binding protein-1 in early human pregnancy. Early Hum Dev. 1992;28:105–10. [PubMed: 1375140]

99. Scott GM, Chow SS, Craig ME, Pang CN, Hall B, Wilkins MR, et al. Cytomegalovirus infection during pregnancy with maternofetal transmission induces a proinflammatory cytokine bias in placenta and amniotic fluid. J Infect Dis. 2012;205:1305–10. [PubMed: 22383678]

100. Vuorela P, Andersson S, Carpén O, Ylikorkala O, Halmesmäki E. Unbound vascular endothelial growth factor and its receptors in breast, human milk, and newborn intestine. Am J Clin Nutr. 2000;72:1196–201. [PubMed: 11063449]

101. Siafakas CG, Anatolitou F, Fusunyan RD, Walker WA, Sanderson IR. Vascular endothelial growth factor (VEGF) is present in human breast milk and its receptor is present on intestinal epithelial cells. Pediatr Res. 1999;45:652–7. [PubMed: 10231859]

102. Teramo KA, Widness JA. Increased fetal plasma and amniotic fluid erythropoietin concentrations: markers of intrauterine hypoxia. Neonatology. 2009;95:105–16. [PubMed: 18776724]

103. Kling PJ, Sullivan TM, Roberts RA, Philipps AF, Koldovský O. Human milk as a potential enteral source of erythropoietin. Pediatr Res. 1998;43:216–21. [PubMed: 9475287]

104. Artym J, Zimecki M. Antimicrobial and Prebiotic Activity of Lactoferrin in the Female Reproductive Tract: A Comprehensive Review. Biomedicines. 2021;9:1940. [PubMed: 34944756]

105. Yang Z, Jiang R, Chen Q, Wang J, Duan Y, Pang X, et al. Concentration of Lactoferrin in Human Milk and Its Variation during Lactation in Different Chinese Populations. Nutrients. 2018;10:1235. [PubMed: 30189612]

106. Gabrielli O, Zampini L, Galeazzi T, Padella L, Santoro L, Peila C, et al. Preterm milk oligosaccharides during the first month of lactation. Pediatrics. 2011;128:e1520–31. [PubMed: 22123889]

107. Cauchi MN, Lim D. Secretory IgA levels in the amniotic fluid. J Obstet Gynaecol. 1981;1:213–5.

108. Granger CL, Lamb CA, Embleton ND, Beck LC, Masi AC, Palmer JM, et al. Secretory immunoglobulin A in preterm infants: determination of normal values in breast milk and stool. Pediatr Res. 2022;92:979–86. [PubMed: 34952939]

109. Zhang Z, Adelman AS, Rai D, Boettcher J, Lőnnerdal B. Amino acid profiles in term and preterm human milk through lactation: a systematic review. Nutrients. 2013;5:4800–21. [PubMed: 24288022]

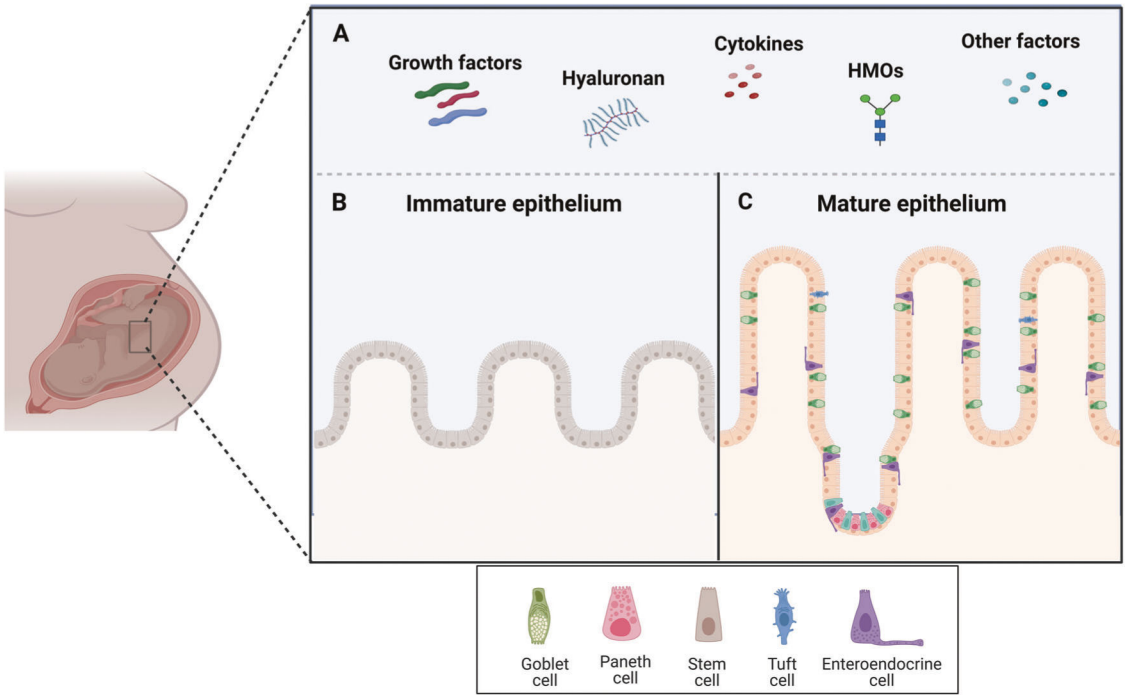

Fig. 1. Schematic representation of the role of amniotic fluid (AF) in fetal gut development.
This figure illustrates how AF, enriched with cytokines, growth factors, and bioactive molecules (**A**), contributes to developmental maturation of the fetal gut (**B**) and the intestinal epithelium after birth (**C**).

Table 1.

Intestinal epithelial effects of bioactive factors found in both amniotic fluid and human milk.

Bioactive factors	Intestinal epithelial effects
Epidermal growth factor	Stimulates cell mitosis, differentiation, and epithelial cell proliferation; enhances barrier function and cell migration; attenuates toll-like receptor 4-mediated inflammatory response [10, 11]
Insulin-like growth factor 1	Mediates perinatal growth; promotes healing post-injury [22, 25]
Vascular endothelial growth factor	Promotes development and vascularization of infant intestine; barrier integrity; promotes mucosal healing [17, 31, 88, 89]
Erythropoietin	Increases villus height/area, crypt depth, and epithelial cell proliferation; protects against necrotizing enterocolitis injury [34, 35, 38]
Granulocyte colony-stimulating factor	Advances healing post-necrotizing enterocolitis; epithelial cell maintenance [42, 46]
Transforming growth factor beta	Intestinal mucosal repair; growth, differentiation, and modulation of inflammation [11, 50]
Interleukin family	Enhances epithelial cell restitution, proliferation, and integrity of barrier tight junctions; nutrient uptake [55, 90]
Lactoferrin	Epithelial cell proliferation, differentiation, and migration; enhances barrier function via tight junction expression; inhibits apoptosis; acts as prebiotic; protects against experimental necrotizing enterocolitis [66, 69, 91]
Human milk oligosaccharides	Provides prebiotic, antimicrobial, and antiadhesive effects to shape postnatal microbiome; epithelial cell proliferation and differentiation; tight junction expression and localization; protection against necrotizing enterocolitis [73, 92]
Hyaluronan	Epithelial cell proliferation, differentiation, tight junction expression; protects against murine necrotizing enterocolitis [81, 93]
sIgA	Neutralizes bacteria and bacterial toxins; influences composition and activity of gut microbiome [61, 94]
Glutamine	Energy substrate; anti-oxidative properties; supports epithelial proliferation and differentiation; promotes intestinal barrier function by upregulating tight junction proteins [86]
L- arginine	Enhances nitric oxide synthase activity and nitric oxide production in the intestine; increases intestinal stem cell function [86]

Table 2.

Bioactive concentrations across lactation and birth maturity.

Bioactive factor	Average concentration			
	Amniotic fluid	**Colostrum**	**Preterm milk**	**Term milk**
EGF	41.07–133.38 pg/mL (22–34 weeks) [95]	25–40 ng/mL [96]	16–17 μg/100 mL [97]	10–11 μg/100 mL [97]
IGF-1	203–413 μg/L [98]	10 ng/mL [96]	0.419 μg/100 mL [89]	0.031 μg/100 mL [89]
VEGF	0.3–63.3 pg/mL [99]	57–95 μg/L [100]	3 μg/100 mL [101]	8 μg/100 mL [101]
EPO	2–19 mU/mL [102]	0.16 to 0.79 mU/mL [103]	11.7 mU/mL [103]	11.7 mU/mL [103]
G-CSF	2.7–1051.1 pg/mL [99]	unknown	0.008 μg/100 mL [43]	0.0156 μg/100 mL [43]
Lactoferrin	5–15 μg/mL (32–40 weeks) [104]	3.16 g/L [105]	1.03 g/L [105]	0.9 g/L [105]
HMOs	3.435–7.58 nmol/mL [73]	20–25 g/L [75]	10–15 g/L [106]	5–15 g/L [75]
Hyaluronan	1–5 mg/L [93]	755 ng/ml [81]	unknown	215 ng/ml [81]
sIgA	5 to 20 μg/ml [107]	1.5– 83.7 g/L [61]	0.74–1.57 g/L[108]	1.6–2 g/L[61]
Arginine	3.0–1910 μmol/L [84, 85, 109]	94.3 μmol/L [109]	56.7 μmol/L [109]	30.2 –36.7 μmol/L [109]
Glutamine	0.0–1340.0 μmol/L [84, 85]	1089.9–1201.2 μmol/L [109]	1168.1 [109]	1175.0 μmol/L [109]

EGF epidermal growth factor, *IGF-1* insulin-like growth factor 1, *VEGF* vascular endothelial growth factor, *EPO* erythropoietin, *G-CSF* granulocyte colony-stimulating factor, *HMOs* human milk oligosaccharides, *sIgA* secretory immunoglobulin A.