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### Comments

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# Insights into Novel Infant Milk Formula Bioactives

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**Abstract:** Human milk provides optimal infant nutrition for early life development. However, there are situations in which human milk feeding is not the most viable option for infant nutrition. Some factors include maternal illness, lactation insufficiency, storage complications, and time constraints. As a result, modern infant formula has become a substitute or alternative when breastfeeding is not possible or is inadequate for infants. In the optimal design of formula, many factors are considered in creating a product that is safe and provides a nutritional profile either equivalent to human milk or in optimal alignment with infant needs. Human milk composition serves as a reference for infant formula formulation. The purpose of this literature review is to provide a synopsis of recent advances in infant formula research in the past 5 years (2014–2018). Specifically, the breadth of bioactive components of human milk that are not yet available in infant formulas and dose optimization requirements in full-term infants are reviewed. The nutritional value of infant formulas has been improved by adding functional bioactive ingredients such as choline, strains of probiotics, and prebiotic oligosaccharides. There are considerable variations in the composition of available infant formulas between manufacturers and across different countries. Formulas still generally lack important contents such as antibodies and exosomes that are present in human milk. Some of the adverse health outcomes associated with formula feeding as compared with human milk feeding are attributed to the different composition of the foods. This literature review summarizes the most recent research aimed at advancing infant formula composition to narrow the difference in health outcomes between human milk and formula-fed infants.

**Keywords:** nutrition, neonate, pediatrics, infant formula, ganglioside, miRNA

## Introduction

The American Academy of Pediatrics (AAP) and the World Health Organization (WHO) recommend that infants are exclusively breastfed for 6 months after birth for optimal growth and development and that infants should continue to breastfeed up to 2 years along with complementary foods.<sup>1</sup> In the case infants are not breastfed and banked or donor milk may not be available, infant formula is the most adequate food alternative.<sup>2,3</sup> The health benefits of human milk consumption are greater than those of infant formula due to the nutritional and bioactive components in human milk that are absent in infant formula or not present in optimal concentration relative to the infant stage of growth and development.<sup>4</sup> In this context, bioactives are compounds in diet that elicit physiologic effects in the consumer. Important bioactives for newborn health may include whole bacterial or mammalian cells, nanovesicles derived from cells, membrane fragments, or macromolecules. Despite the shortcomings of infant formula, it serves as the most viable substitution for human milk. Therefore, it benefits researchers, practitioners, and industry to

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develop a nutritionally adequate substitute for human milk which can ensure similar acute and chronic health outcomes compared to human milk feeding.

In consideration of adding novel bioactive agents to infant formulas, human milk is used as a model. In general, commercially available infant formula products are nutritionally interchangeable and there is minimal evidence to support that one infant formula is better than another.<sup>5</sup> The composition of infant formula has great importance as it can have a significant impact on long-term health.<sup>6</sup> As a result, when knowledge on optimizing infant nutrition progresses, industry is continuously adapting the composition of infant formula to align growth and development outcomes in formula-consuming infants with those seen in infants that consume human milk. These changes can include alternative sources of bioactive ingredients, consideration of dose optimization, or addition of novel bioactives.

Literature reviews to date have primarily focused on a specific bioactive in extensive detail or on particular health outcomes related to infant milk formula composition. The purpose of this literature review is to provide a comprehensive synopsis of recent advances in infant formula research pertaining to the inclusion, or dose optimization, of novel bioactives. Accordingly, a range of clinical health outcomes are reviewed in relation to the plethora of bioactives present, or being considered for inclusion, in infant formulas.

## Methods

JSTOR, Embase, MedlinePlus, and Pubmed bibliographic databases of published scientific research were accessed for this literature review. The following search terms were used: infant nutrition, neonatal nutrition, baby formula, infant formula, human milk, human breast milk, breastfeeding, and [(human milk OR formula) AND (protein content OR lactalbumin OR lactoferrin OR probiotics OR prebiotics OR oligosaccharide OR essential fat OR polyunsaturated fat OR omega-3 OR omega-6 OR choline OR lutein OR ganglioside OR milk fat globule membrane OR microRNA)]. Peer-reviewed scientific research selected for inclusion in this work was primarily human clinical intervention studies and were published between 2014 and 2018. Other literature published before 2014 was included in this work to provide scope, context, and background to the bioactive agent topic subsections.

## Novel Bioactives

### Protein Content

Bioactive proteins in human milk provide immune protection, assist in digestive processes, contribute towards gut development, and act as carriers for some nutrients.<sup>7</sup> Human milk and infant formula may differ in protein composition and concentration. Human milk provides total protein needs and is rich in essential amino acids.<sup>8,9</sup> Whereas commercially available infant formulas are typically made from bovine milk which has a much higher total protein concentration.<sup>10</sup> Some infant formula products have soy as the primary protein source in order to address allergy to cow's milk. This is less common, as in the United States about 69% of infants consume cow's milk formula and only 12% consume soy formula.<sup>11</sup>

In order for infants to avoid drastically exceeding protein requirements, the quantity and quality of protein in infant formula should match closely to human milk. In infant formula and human milk, higher protein content increases the risk of infants being overweight and obese later in life.<sup>6,12</sup> Rapid early growth during the first 2 years of life is associated with the likelihood of being overweight and obese during childhood.<sup>13</sup> Recent formula products developed contain lower protein concentrations with improved protein quality.<sup>7</sup> Recent clinical evaluations of infant formulas with lowered protein content show that low-protein content formula is safe and promotes adequate growth.<sup>14</sup> Currently, formulas meet protein requirements of 1.8–3.0 g/100 kcal but there is no consensus on the optimal amount of protein in infant formulas.<sup>15</sup> In fact, the European Food Safety Authority (EFSA) had proposed to lower the maximum protein content of infant formula to reduce progressive weight gain in formula-fed infants.<sup>16</sup>

Infant formula products generally contain adequate protein concentrations that provide sufficient quantities of all essential amino acids to meet the needs of developing infants.<sup>17</sup> However, protein concentrations in human milk change over time as protein content is higher at the initial stages of lactation and decrease during the later stages of lactation. In earlier stages of lactation, human milk contains 1.4–1.6 g protein/100 mL of protein. The concentration of protein lowers to 0.8–1.0 g/100 mL after 3 months of lactation and to 0.7–0.8 g/100 mL after 6 months.<sup>1</sup> These changes in human milk protein concentration over the course of lactation necessitate consideration for optimal age-specific products to provide adequate nutrition to

infants at growth and development milestones before being weaned.

## $\alpha$ -Lactalbumin

$\alpha$ -Lactalbumin is a whey protein found in human milk at a concentration of 28% of total protein and in bovine milk at 3% of total protein.<sup>18</sup>  $\alpha$ -Lactalbumin provides essential amino acids and the bovine form is added to infant formulas.<sup>10</sup> Since the introduction of  $\alpha$ -lactalbumin to infant formula, the proportion of  $\alpha$ -lactalbumin has increased in product over time since its structural elucidation reveals it has a high proportion of tryptophan, lysine, and cysteine; and is available and easily obtained from an enriched whey protein fraction.<sup>19</sup>

$\alpha$ -Lactalbumin is bioavailable to the infant. It is digested and absorbed and the oligopeptides and amino acids have been shown to support critical immune functions such as phagocytosis by macrophages and growth of *Bifidobacteria*.<sup>20,21</sup> In addition, the  $\alpha$ -lactalbumin peptides are antimicrobial and have shown activity in the colon against colonization of *Escherichia coli*, *K. pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Streptococci*, and *Candida albicans*.<sup>22</sup> Supplementation of  $\alpha$ -lactalbumin in infant formula containing a protein concentration of 1 g protein/100 mL showed normal growth of infants up to 4 months of age.<sup>23</sup> This study demonstrates that the addition of  $\alpha$ -lactalbumin to infant formula supports a mechanism by which total protein concentration of formula can be lowered, the protein needs of the infant can still be met, and that growth outcomes are still supported. Importantly, it has been shown that  $\alpha$ -lactalbumin does not affect iron absorption from formula in infants. Since iron status can vary greatly depending on weight and time of gestation in infants, research supports the addition of a standard dose of  $\alpha$ -lactalbumin to formula with respect to iron requirement.<sup>24,25</sup>

## Lactoferrin

Lactoferrin is a multifunctional whey protein found in human milk that has antibacterial and antiviral properties; it is involved in immune function, increased iron uptake, and inhibition of carcinogenesis.<sup>26</sup> The concentration is variable in colostrum, but lactoferrin comprises 15–20% of mature human milk protein content.<sup>10,27</sup> The addition of lactoferrin to formula from a bovine source is costly and can exhaust raw product resources from cow milk containing only a very low quantity of the protein. Additionally, it is difficult to preserve during infant formula production due to denaturing at high temperatures.<sup>27,28</sup>

Commercially added lactoferrin has similar bioactive properties as human lactoferrin. When lactoferrin was added to infant formula, it was found that the incidence of respiratory illnesses decreased while hematocrit increased.<sup>27,29</sup> However, enriched lactoferrin did not improve iron absorption since bovine lactoferrin does not bind to human lactoferrin receptors.<sup>30</sup> Lactoferrin is enriched in a small number of commercially available infant formulas and is recognized as a safe ingredient by the Food and Drug Administration.<sup>27,31</sup> Even still, lactoferrin is highly regulated to ensure it is of high quality before its addition in infant formula.<sup>32</sup> Furthermore, there are still gaps in knowledge regarding an optimal dosage of enriched lactoferrin in infant formula products.<sup>31</sup>

## Probiotics

Probiotics are being added to infant formula and have been shown to have multiple beneficial effects (Table 1). Probiotics are microorganisms that can be present in human milk. The most common strains include *Staphylococci*, *Streptococci*, lactic acid bacteria, and *Bifidobacteria*.<sup>33</sup> When consumed, probiotics may influence the gut microbiota composition of the infant which is colonized by over  $10^{14}$  microorganisms.<sup>33</sup> The intestinal microbiota aids in digestion, gut homeostasis, immune function, and in maintaining stool firmness.<sup>33</sup> When probiotics are not consumed in human milk or formula, the rates of negative health outcomes such as necrotizing enterocolitis, infection, and underweight infants increase.<sup>33</sup> During infant development, the gut profile of microbiota is not static and can be influenced by environmental factors like diet.<sup>33</sup> The method of delivery, length of gestation, and antibiotic use can also change the microbial composition of the gut.<sup>33</sup> Thus, there is interest in determining which strains could be consumed to counteract deleterious environmental influences on gut microbiome composition.

Some infant formula manufacturers have started incorporating probiotics into products. Efficacy varies greatly between strains of bacteria used; however, the use has been shown to be safe even if there is no observable effect on health outcomes.<sup>33–36</sup> A study on infants less than 3 months old at enrollment who received *Bifidobacterium infantis* IM1 in a dose of  $10^7$  colony-forming units (cfu) per gram for 12 weeks had less incidence of diarrhea, reduction in *Proteobacteria* in stool samples, and higher stool frequency when compared to the control arm.<sup>35</sup> Additionally, synbiotics, the use of both probiotics and prebiotic, have shown additive beneficial effects. Research on supplementing *Bifidobacterium lactis* and bovine milk-

**Table 1** Human Clinical Research on Health Outcomes Related to Inclusion of Probiotics in Infant Milk Formulae

Author, Year	Region	Participants	Intervention	Outcomes
Escribano et al, 2018 <sup>35</sup>	Spain	Healthy, term newborns born $\geq 37$ weeks of gestation, with normal birth weight (3rd to 97th percentiles for gestational age), normal growth curve, $\leq 3$ months of age at enrollment; N=151 completed.	Double-blinded, randomized, multicenter, controlled clinical trial; infant formula supplemented with $10^7$ cfu/g of <i>B. infantis</i> IM1 (experimental), standard formula (control); 12-week study.	Reduced diarrhea episodes and lowered constipation prevalence in experimental group.
Simeoni et al, 2016 <sup>36</sup>	Switzerland	Full term, exclusively formula-fed babies, mean age of 5 days; N=115.	Randomized, double-blind, control intervention; infant formula supplemented with a bovine milk oligosaccharide ( $5.7 \pm 1.0$ g/100 g of powder formula) and a <i>B. lactis</i> CNCM I-3446 ( $10^7$ cfu/g/l of powder formula; experimental), unsupplemented formula (control); 12-week study.	1) Higher faecal pH and higher diversity of faecal microbiota in control group; 2) <i>B. lactis</i> increased by 100-fold in the stool and was detected in all infants in the experimental group; 3) Increased endogenous <i>Bifidobacteria</i> ( <i>B. longum</i> , <i>B. breve</i> , <i>B. bifidum</i> , <i>B. pseudocatenulatum</i> ) in experimental group.

derived oligosaccharides in formula for infants averaging 5 days old showed a reduction in the number of infants with gastrointestinal infections and diarrhea.<sup>36</sup> The evidence for inclusion of probiotics in infant formula is convincing, and future work should focus on which specific or combination of strains should be included, the dosage, and whether live cultures or lysed fragments are optimal for enhanced infant health outcomes.

## Prebiotics

Prebiotics are non-digestible oligosaccharides that stimulate the growth and function of bacteria in the gastrointestinal tract.<sup>33,37</sup> Prebiotics are found in high concentrations in human milk but are found only in trace amounts in cow's milk. Thus, it is imperative for product manufacturers to fortify infant formulas with prebiotics above and beyond the amount intrinsic in bovine milk in order to elicit positive physiologic effects. Products are most often supplemented with galacto-oligosaccharide, fructo-oligosaccharide or polydextrose.<sup>37</sup> These bioactive prebiotics have been shown to increase the growth of beneficial microorganisms like *Bifidobacteria* in the gut and resilience of the gut microbiome to metabolic stressors.<sup>37</sup> The discrepancies in infection rates, stool pH, and stool consistency between infants consuming human milk and those fed formula are lessened when formula is supplemented with prebiotics.<sup>37</sup> Apart from allergy, possible side effects identified include bloating, abdominal

discomfort, and flatulence in doses of over 20 g/day in infants up to 28 days old.<sup>33</sup>

## Human Milk Oligosaccharides

Human milk oligosaccharides (HMO) are complex, non-digestible carbohydrates that are found in human milk and are not present in bovine milk.<sup>38</sup> HMOs have many potential benefits for developing infants, and the biological roles and functions of HMOs have been studied more extensively only in recent years.<sup>38</sup> More than 200 HMOs have been discovered in human milk, where 2-fucosyllactose is the predominant oligosaccharide as it comprises 20–30% of HMOs.<sup>39</sup> The functions, either additive or synergistic, of all HMOs have not yet been elucidated. In addition, the mechanism of action of most HMOs is unknown, but some may function as prebiotics and stimulate the growth of *Bifidobacteria* which colonize the infant gut and prevent pathogen adhesion to mucosal surfaces to reduce risks of infections.<sup>40</sup> It has been shown that HMOs also support gastrointestinal and immune functions and that there are lower risks of viral infections in infants consuming HMOs from human milk.<sup>41,42</sup>

When evidence for the addition of HMOs to formula was scant, many producers added this bioactive to formulas simply because HMOs are found in human milk.<sup>43</sup> Recently, research on HMO addition to infant formulas revealed evidence showing that prebiotic-enriched formula-fed infants display a profile of gastrointestinal

microbiota that is similar to breastfed infants.<sup>43</sup> HMO-supplemented infants do not have significant differences in length, weight, and head circumference in comparison to breastfed infants and non-prebiotic supplemented infants.<sup>44</sup> Prebiotic-supplemented infants have increased frequencies of stool in comparison to human milk-fed infants and stool pH levels equal to human milk-fed infants.<sup>45</sup>

In a recent clinical study, it was found that infant formula that is enriched with 2'-fucosyllactose and lacto-N-neotetraose is safe and supports adequate infant growth and development. In addition, it was found that there is lower incidence of bronchitis and medication use including antipyretics and antibiotics among infants that were fed HMO-supplemented formula.<sup>46</sup> The addition of 2'-fucosyllactose and lacto-N-neotetraose has contributed towards closing the compositional gap between human milk and infant formula. It is anticipated that more HMOs will be added to infant formula as it is safe for infants and promotes beneficial health outcomes in early life.<sup>47</sup>

## Polyunsaturated Fatty Acids

Polyunsaturated fatty acids (PUFAs) have been added to infant formula products to function as bioactive ingredients (Table 2) that aid in eye and brain development. PUFAs have many biological effects on membrane function and composition, production of inflammatory mediators, and immune responses.<sup>4,48</sup> A meta-analysis published in 2012 showed that PUFA supplementation in infant formulas did not have significant effects on improving early infant cognition.<sup>49</sup> However, intervention studies have shown that PUFA supplementation has positive developmental outcomes that resemble trajectories seen in infants fed human milk with respect to cognitive function, visual acuity, and immune response.<sup>50,51</sup>

Docosahexaenoic acid (DHA) and arachidonic acid (AA) are PUFAs that are found in human milk and have important roles in the development of plasma membrane constituents.<sup>5</sup> DHA and AA are added to formula instead of the respective n-6 and n-3 precursors since the elongation and desaturation processes are slow and inefficient resulting in a very low conversion rate. Infants that are fed formulas supplemented with PUFAs have higher levels of DHA and AA in plasma or red blood cells compared with infants that are breastfed or fed formulas that are not supplemented.<sup>53,54</sup> Infants that are fed formulas that are fortified with DHA and AA have lower incidences of upper respiratory infections and allergies,

bronchitis, nasal congestion, cough, diarrhea that requires medical attention, atopic dermatitis, eczema, and contact dermatitis.<sup>55-57</sup>

Although the concentration of n-6 and n-3 PUFAs in human milk varies highly depending on maternal diet,<sup>52</sup> there is insufficient data that supports the addition of DHA to formula without AA as well.<sup>50</sup> Infant formulas that include AA and DHA have beneficial effects on the immune system of developing infants. The absence of AA in infant formula affects populations of circulating T cells and macrophages and also causes an increase in the presence of B cells expressing CD20+ and CD54+ activation markers compared to infant formula with AA.<sup>58</sup> The proportion of AA and DHA in supplemented infant formula varies. Further research is needed to determine the exact dose and ratio of PUFAs for optimal growth and development of humoral and cell-mediated immunity.<sup>50</sup>

## Choline

Choline is an essential bioactive micronutrient that plays a role in cell signaling, structure of plasma membrane, muscle function, and lipid transport. Choline is found in human milk in a water-soluble form and is essential in fetal cognitive development. The presence of choline alters brain and spinal cord structure and function and can lower risks of neural tube defects.<sup>59</sup>

The European Society for Pediatric Gastroenterology, Hepatology and Nutrition recommends inclusion of no more than 50 mg/100 kcal of choline in infant formula products. Currently, there are no known cases of adverse effects of having high choline intakes. Inadequate choline levels can inhibit vitamin B12 and folic acid metabolism. Current research on choline supplementation in infants has been limited to preterm infants and more studies need to be performed to examine its properties in infant formula.

## Lutein

Lutein is a carotenoid found in human milk and the concentration varies depending on maternal diet, with intake of fruits and vegetables increasing content.<sup>60</sup> It functions as a structural component of the eye, an antioxidant, and as a filter for blue light. Lutein plays a biological and physiological role in the visual development and function in infants;<sup>61</sup> and infants are reliant on breastfeeding to receive adequate quantities.<sup>62</sup> Differences in serum lutein content between breastfed and formula-fed infants are seen as early as 1 month of age.

**Table 2** Human Clinical Research on Health Outcomes Related to Inclusion of Polyunsaturated Fatty Acids in Infant Milk Formulae

Author, Year	Region	Participants	Intervention	Outcomes
Makrides et al, 1995 <sup>53</sup>	Australia	Infants born between 37–42 weeks of gestation; mothers with no history of lipid metabolism disorders, insulin-dependent diabetes, drug or alcohol abuse; babies with appropriate weight for gestation; N=79 completed.	Formula supplemented with fish oil (experimental group), standard unsupplemented formula (control); 30-week study.	1) Visual evoked potential was improved in the experimental group; 2) Erythrocyte DHA was elevated in experimental group; 3) Erythrocyte ARA was decreased in experimental group.
Pastor et al, 2006 <sup>55</sup>	Spain	Healthy infants, born after 36 weeks gestation; N=1342.	Open label clinical trial; 2 groups: formula with 34 mg AA and 17 mg DHA (experimental) vs no added AA/DHA or low amount ranging from 6–13 mg AA and 8–16 mg DHA (control); 12-month study.	1) Similar growth rates among groups; 2) Treatment group had lower incidence of bronchiolitis/bronchitis vs control; 3) Lower occurrence of rhinitis and lower incidence of upper airway infection in the experimental group vs control.
Lapillonne et al, 2014 <sup>56</sup>	France	Healthy, term infants born after 37 weeks of gestation, singleton, less than 60 days of age at enrollment; birth weight, length, and head circumference appropriate for gestational age ( $\geq 10$ th and $\leq 90$ th percentile); N=325.	Multi-center clinical trial; infants consumed formula with 17 mg DHA and 34 mg ARA/100 kcal (experimental) or with no added DHA or ARA (control); duration of study was until 1 year of age	1) Lower incidence of bronchitis, croup, nasal congestion, cough in experimental group; 2) Diarrhea that required medical attention was lower in experimental group; 3) Odds ratio of increased number of incidences of respiratory illness and diarrhea was lower in experimental group.
Foiles et al, 2016 <sup>57</sup>	USA	Healthy infants, exclusively formula-fed, singleton-birth, born between 37–42 weeks of gestation, birth weight between 2490 and 4200 grams, $\leq 9$ days old at time of randomization; N=91.	Four-year follow-up on cohorts that consumed formula supplemented with PUFA (experimental) or without PUFA (control).	1) Lower incidence of allergic diseases and skin allergy in first year of life in experimental group; 2) Longer mean time until first allergic illness in experimental group; 3) Possible reduced risks of allergic disease, wheezing, asthma in experimental group.
Miklavcic et al, 2017 <sup>58</sup>	Canada	Infants less than 21 days of age, singleton birth, healthy, had body weight, length, and head circumference between the 10th and 90th percentile; N=89.	Prospective, double-blind controlled study; formula containing 17 mg DHA and 0, 25, or 34 mg ARA/100 kcal; 10-week study.	1) Plasma ARA level lower in group consuming formula without ARA; 2) Higher proportion of B cells and activation markers in group consuming formula without ARA.

Exclusively breastfed neonates had 6 times the average serum lutein level in comparison to infants consuming formula. Even in infants consuming formula fortified with lutein to a concentration similar to human milk, serum levels were drastically lower than in infants consuming human milk suggesting a difference in bioavailability.<sup>63</sup>

Recent studies demonstrated that lutein-supplemented infant formula is safe and supports the visual and cognitive development of infants.<sup>60–62,64</sup> Presently, there is no research that lutein supplementation influences brain accretion during infancy. A recent study found that the

concentration of lutein is higher in several regions of the brain during infancy than in adulthood which may indicate that lutein may play an important role in early neural development.<sup>60</sup> Currently, there are a number of infant formulas that contain lutein. However, further research on lutein must be conducted to ensure efficacy for optimal growth and development outcomes in infants.<sup>61</sup>

## Ganglioside

Gangliosides are sialylated glycosphingolipids commonly found in the lipid membranes of cells. They play a large



role in neurological development, intracellular trafficking, immune cell signaling, gut integrity, and preventing infections.<sup>65,66</sup> Gangliosides are present in human milk, with disialoganglioside GD3 and monosialodihexosylganglioside (GM3) being the primary molecular species.<sup>67</sup> Total concentration of ganglioside is highest in early stages of lactation and is predominantly comprised of GD3. However, ganglioside GD3 content decreases over period of lactation and GM3 becomes more prominent.<sup>67</sup> In infant milk formulas the content of gangliosides is provided from the bovine milk base, which has lower ganglioside content than human milk.<sup>67</sup> Supplementation of infants with GD3 has shown beneficial effects on neurologic development. Infants aged 2-8 weeks showed increased Hand and Eye Coordination IQ, Performance IQ, and General IQ on the Griffiths scales when formula was supplemented with 2–3 mg of ganglioside/100 g of infant formula.<sup>68</sup> There is a case to be made for the addition of gangliosides to formula; however, very few studies have been performed in human infants despite the observed safety, tolerability, and efficacy of this bioactive.

## MicroRNA

MicroRNA (miRNA) are bioactive components found in human milk. They are small non-coding RNA molecules that regulate the expression of genes involved in the cell cycle, proliferation, differentiation, apoptosis, and the immune response. It was recently discovered that human milk contains various miRNAs that are bioavailable, circulate systemically, and are thought to be involved in infant development.<sup>4,69</sup> In addition to human milk, bovine milk contains miRNA in an exosome vehicle for which bioavailability to human tissue has been demonstrated utilizing an in vitro CaCo-2 cell model, a human colorectal tumor cell line.<sup>70</sup> There is emerging research that miRNAs that are present in human milk can enter phenotypically normal and tumor cells and affect biological functions.<sup>69</sup> Some miRNAs found in bovine milk have similar nucleotide sequences to those from human milk but analogous effects have not been directly demonstrated.<sup>70</sup> Currently, human and bovine milk miRNA are not detected in many infant formulas.<sup>69</sup> This may be due to degradation of miRNA caused by homogenization and heating of milk.<sup>70</sup> Future research in this area could consider whether bovine milk miRNAs are resistant to processing steps in the production of infant

formulas, and whether human milk miRNA elicits physiologic effects different from those of bovine miRNA.

## Conclusion

This literature review summarizes research on the recent advances in research related to the addition of novel bioactive compounds to infant formula (2014–2018). These advances consist of changing the source from which certain ingredients or bioactives are derived, altering the dose of bioactives, such as including specific protein in formula products, and adding novel bioactive components like HMO and miRNA. Health outcomes related to consumption of infant formula do not emulate outcomes in infants that consume human milk exclusively. However, adding bioactive ingredients such as gangliosides, PUFA, and probiotics to infant formulas achieves growth and developmental outcomes in infants that are more in alignment with outcomes observed in infants exclusively consuming human milk. The addition of novel functional bioactive agents that also promote health outcomes beyond growth, namely immunity, cognition, and temperament, is of paramount interest for future work. Currently, no uniform composition of bioactive compounds exists among commercially available infant formulas. The optimization of infant formula composition may be realized with more research in longitudinal cohort studies following acute and long-term health outcomes of children to ensure the efficaciousness of novel bioactives.

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