Chapter

Breastfeeding and the Influence of the Breast Milk Microbiota on Infant Health

Fatima Chegdani, Badreddine Nouadi and Faiza Bennis

Abstract

Nutrition is an essential condition for physical, mental, and psycho-emotional growth for both children and adults. It is a major determinant of health and a key factor for the development of a country. Breastfeeding is a natural biological process, essential for the development of the life of the newborn at least during the first six months by ensuring a nutritional contribution adapted to the needs of the latter. Thus, breast milk is the physiological and natural food best suited to the nutrition of the newborn. It contains several various components, which are biologically optimized for the infant. Cells are not a negligible component of breast milk. Breast milk is also a continuous source of commensal and beneficial bacteria, including lactic acid bacteria and bifidobacteria. It plays an important role in the initiation, development, and composition of the newborn's gut microbiota, thanks to its pre- and probiotic components. Current knowledge highlights the interdependent links between the components of breast milk, the ontogeny of intestinal functions, the development of the mucus intestinal immune system, colonization by the intestinal microbiota, and protection against pathogens. The quality of these interactions influences the health of the newborn in the short and long term.

Keywords: breastfeeding, probiotic, microbiota, newborn, prematurity

1. Introduction

Nutrition is an essential condition for physical, mental, and psycho-emotional growth for both children and adults. It is a major determinant of health and a key factor for the development of a country. Nutritional disorders hamper economic growth and perpetuate poverty through three factors: direct losses in productivity linked to poor physical condition, losses resulting from increased health care costs and indirect losses due to poor cognitive function and school failures (Global Strategy 2003 WHO / UNICEF, Moroccan Strategy 2011–2019 / UNICEF) [1, 2]. Furthermore, nutritional disorders are not only the result of food insecurity since children living in good conditions are subject to deficiency syndromes, such as anemia, being under or overweight or even stunted growth (Report global malnutrition UNICEF, 2018) [3].

The global food strategy for infants and young children focuses on promoting appropriate infants and young children. Breastfeeding is the best way of providing ideal food for the growth and development of healthy infants.

Poor feeding practices would be particularly due to lack of information on the benefits of breastfeeding and complementary feeding practices. Therefore, breast milk is the most suitable physiological and natural food for the nutrition of the newborn [4]. Breastfeeding is therefore a real global public health problem. Indeed, the World Health Organization (WHO) recommends exclusive breastfeeding for at least 6 months, giving the infant a good start in life [2]. Breast milk plays an important role in the initiation, development and composition of the gut microbiota of the newborn, thanks to its pre- and probiotic components [5–7].

Breast milk is particularly important in cases of prematurity and pronounced low birth weight at term, as the child is at increased risk of infection, long-term health problems and death due to the immaturity and dysfunction of virtually all components of innate immunity [8]. At birth, newborns inherit part of their mother's microbiota, but there is growing evidence that breastfeeding forges this microbiota in the infant's gut [9, 10]. The establishment of the microbiota in the newborn is a critical period that has an impact on the overall state of his future health. The mode of colonization and the composition of the intestinal microbiota of the newborn vary depending on the lifestyle of the mother, the delivery route and environmental factors [11–13]. Children born prematurely have an intestinal dysbiosis that diminishes over time. It does not seem to be related to environmental factors but could be correlated to the length of gestation [14].

The aim of this chapter is to highlight the relationship between breastfeeding, the gut microbiota and the health of the newborn.

2. Human milk: composition and evolution

2.1 Human milk composition

Breastfeeding is a natural biological process, essential for the development of the life of the newborn at least during the first six months. Breastmilk provides all the calories and nutrients a child needs in the first few months of life and continues to provide half or more of the nutritional requirements in the second half of life, and up to one third in the second year. It promotes sensory and cognitive development and protects infants against infectious and chronic diseases [4, 15].

Human breast milk is a complex fluid made up of a number of diverse components, which are biologically optimized for the infant and which change dynamically between women (**Figure 1**) [16–19].

The beneficial health effects of human milk have been linked to the abundance of bioactive molecules present, including secretory antibodies, immune cells, antimicrobial proteins like lactoferrin, CD14 and lysozyme, regulatory cytokines and oligosaccharides [17]. Proteins and lipids beyond their role in the nutritional supply and essential source of energy for the newborn, have antimicrobial and immunomodulatory activities. Nucleotides are also beneficial for the development and maturation of the gastrointestinal tract, as well as for microbiota development and immune function. The immunoglobulins present in milk in the form of secretory IgA and secretory IgG provide initial immunity to the immature immune system of the newborn [17]. The indigestible oligosaccharides are not directly intended to feed the baby, but to nourish his intestinal bacteria [20]. Breast milk also includes short chain fatty acids (SCFAs) from the fermentation of indigestible foods which are a source of prebiotics for the breastfed infant [21].

In addition to the major components of water, carbohydrates, fats, proteins and micronutrients, breast milk is also a continuous source of non-bacterial cells and bacterial cells of maternal origin, which are beneficial to the newborn. Cells are not

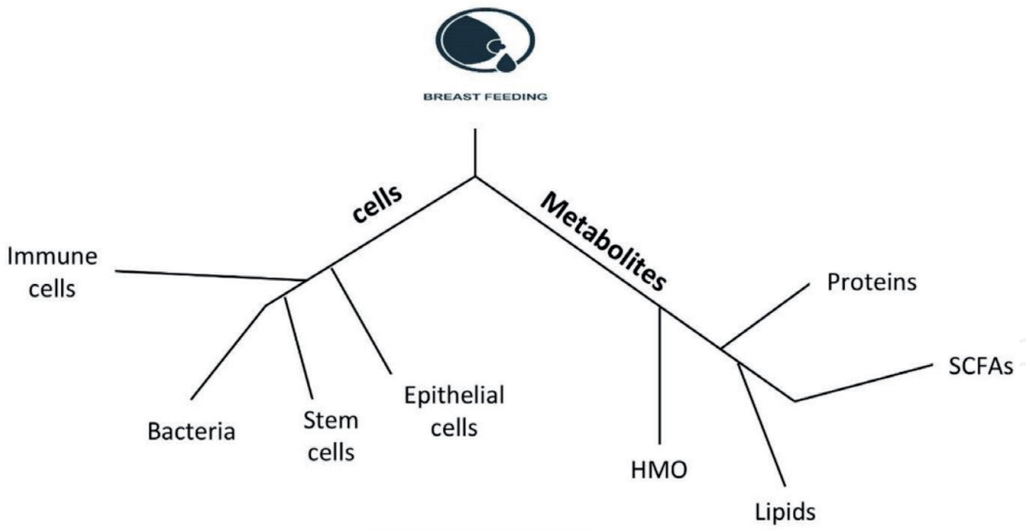

Figure 1.
Schematic illustration of the different components of human breast milk.

a negligible component of breast milk because of their impact on the health of the newborn [22]. Epithelial cells and cellular debris of glandular origin and immune cells of blood origin (macrophages, lymphocytes and polymorphonuclear cells) are present in breast milk. Recent breakthroughs have shown that breast milk is much more heterogeneous and that it also contains stem cells [22]. Ten years ago, the microbiome of breast milk was characterized in which bacteria form an ecosystem of probiotics beneficial to the newborn [23]. These probiotics are involved in the digestion of nutrients including the metabolism of oligosaccharides, although their most likely role is the modulation of immunity [24].

The predominant bifidobacteria and lactobacilli constitute the natural microbiota, originating from the gastrointestinal tract of the mother via an enteromammary cycle [5, 7, 25–30]. High throughput sequencing, by pushing back the limits imposed by the passage through microbial cultures, has allowed a more complete and faster characterization of the microbiota. In fact, metagenomics, a revolutionary approach to the genetic study of a complex sample, has made it possible to capture 90% of the diversity of the microbiota in breast milk. *Bacteroides, Blautia, Faecalibacterium, Ruminococcus, Roseburia, Subdoligranulum, Enterococcus and Escherichia-Shigella*, have been repeatedly detected in breast milk in different studies using molecular approaches based on the 16S rRNA gene [11, 29–32].

Breast milk is a natural source of nutrients, rich in various bioactive molecules. The Cells, both eukaryotic and prokaryotic, are an important component and also play a crucial role in the well-being of newborns.

2.2 Breast milk evolution

The major components of breast milk, namely water, micronutrients and macronutrients, change during lactation to meet the needs of the newborn, particularly in relation to the establishment of the newborn's initial immunity and the development of the intestinal epithelium and central nervous system [33–36]. This composition of breast milk is required to constantly change depending on the needs and age of the baby, the time of breastfeeding or the beginning and end of breastfeeding and the period of breastfeeding [13, 37]. Indeed, between the 3rd and 5th day, colostrum is produced, then transitional milk is produced over the following 15 days and finally, mature milk is produced approximately 3 weeks to 1 month

after the start of breastfeeding [38, 39]. This mature mother's milk from the first month, rich in prebiotics and probiotics, ensures intestinal homeostasis. In addition, breastfeeding is an interactive process where the baby's behavior can to some extent determine the composition of his food. The composition profile of breast milk is relatively stable around the world and varies only slightly depending on the lifestyle and diet of the mother [40]. SCFAs in breast milk, secondary metabolites whose role is crucial in modulating the immune system, the development of the intestinal epithelium and the intestinal barrier function of the newborn, evolve like the other components. Dai et al. [21], have shown that the concentrations of SCFAs are higher in mature milk as well as in milk from mothers of full-term infants than from mothers of preterm infants.

3. The newborn's gut microbiota

3.1 First colonization

The newborn, considered sterile at birth, presents a beginning of colonization of the gut by the bacteria in the amniotic fluid, placenta, cord blood and meconium [14, 41, 42]. The bacterial DNA detected in these different matrices comes mainly from commensal bacteria belonging to the phyla Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes and Fusobacteria [43, 44].

Colonization at birth of the baby's digestive tract by bacteria allows an incessant dialog to be established between microorganisms and all the newborn's defenses. The immune system thus learns to recognize and differentiate between pathogenic and non-pathogenic bacteria and beneficial food proteins. However, it also learns to scale up its reactions. It is through this intensive training that a good balance between the different immune responses is achieved [45].

It is highly likely that babies are born with an initial bacterial composition from the maternal womb. This initial culture may change during pregnancy, which explains the difference in microbial diversity in newborns at birth [44]. This initial (fetal) colonization is likely to reorganize the intestinal epithelium to prepare the environment to receive the new colonizing bacteria at birth.

At birth, the genus *Streptococcus*, and particularly the species *Streptococcus thermophilus*, is among the first GIT colonizers as it has been detected in the stools and breast milk of infants [28, 46, 47]. *Streptococcus thermophilus* is able to induce differentiation of intestinal epithelial cells [48]. Indeed, work carried out on axenic rats inoculated with *Streptococcus thermophilus* has shown that this bacterial species undergoes a progressive adaptation in the gut to stabilize after 30 days. The main impact of *Streptococcus thermophilus* is to massively stimulate the glycolysis pathway, leading to lactate production in the cecum. In addition, expression studies showed overexpression of SLC16A1 and SLC5A8, monocarboxylic acid transporters, as well as p27 (kip1) a protein involved in cell cycle arrest. These results suggest that lactate could be a signal produced by *Streptococcus thermophilus* and modulating the colon epithelium development [48, 49].

3.2 Factors impacting the microbiota diversity

The establishment of the gut microbiota in newborns is a critical period that has an impact on the overall state of their future health. The microbiota at birth will be impacted by various environmental factors, the most important of which are mode of delivery, type of infant feeding, gestational age, infant hospitalization and infant antibiotic use. Infants born at term vaginally, at home and exclusively breastfed

appear to have healthy gut microbiota, with the highest levels of Bifidobacteria and the lowest levels of *Clostridium difficile* and *Escherichia coli* [11, 50].

Interdependencies have been demonstrated between breast milk components, ontogeny of gut function, development of the mucosal intestinal immune system, colonization by the gut microbiota and protection against pathogens [15]. Williams et al. have suggested complex microbial interactions between breastfeeding mothers and their infants and support the hypothesis that variation in the milk microbiome may influence the infant GI microbiome. In fact, the result of this work provides that infant oral, maternal oral, and milk microbial communities were predominated by Firmicutes; maternal feces by Firmicutes and Bacteroidetes; and infant feces was characterized by a relatively even distribution of Firmicutes, Bacteroidetes, and Proteobacteria. Regarding bacterial genera, the most abundant in milk and maternal and infant oral samples was *Streptococcus*, whereas *Bacteroides* were predominant in maternal and infant fecal microbiomes [51]. Moreover, the ontological prediction study carried out by *Nouadi et al.*, revealed an interconnection between the breast milk microbiota and the microbiota of the full-term newborn. The exploitation of the bioinformatics tool in this study, helped to provide a new representation and interpretation of the interactions between genes differentially expressed in the host intestine induced by the microbiota. Subsequently, giving an overview of the global patterns of gene expression in the epithelial cells of term infants. This study found that IL1β, RELA, INS, IRS1 and NFKBIA (Core Network Proteins) are the major regulators of four important biological processes. These processes induced in the first few months of a newborns' life have concerned intestinal development, effect of nutrition, and impact of other environmental exposures on the intestinal microbiota colonization. This study indicates the importance of these interactions in health homeostasis [52].

3.3 Role of SCFAs in intestinal development

Short-chain fatty acids, produced by fermenting anaerobic bacteria are secondary metabolites, found on the surface of the intestinal lumen; they act as signaling molecules transferring information between the microbiota and the immune system [53].

These organic acids have an aliphatic chain of 1 to 6 carbons. The most abundant SCFAs in humans are acetic acid (C2: 0), propionic acid (C3: 0) and butyric acid (C4: 0). SCFAs are the precursors of long chain fatty acids. The production of SCFAs in the gastrointestinal tract is particularly dependent on the diet and the type of host microbiota [54]. SCFAs found in breast milk are an important source of energy as well as being essential for maturing the gastrointestinal tract [55, 56].

Acetic acid represents 50–60% of SCFAs, it is produced by the genera *Bifidobacteria* and *Lactobacilli*. Acetic acid plays many roles including the synthesis of cholesterol and fatty acids, lipogenesis, insulin sensitivity and pH regulation of the intestinal lumen [57–60]. *Bacteroides*, *Firmicutes* and *Lachnospiraceae* are the main intestinal bacteria producing propionic acid. Depending on the substrate, these bacteria can also produce butyric acid. Propionic acid is involved in several biological processes, including lipid metabolism, anti-inflammatory activity, and anti-cancer activity. Butyric acid is produced by the genera *Faecalibacterium*, *Eubacterium rectale* and *Roseburia spp.* [61]. The latter is the least abundant, but exhibits significant beneficial effects on energy metabolism and intestinal homeostasis [62, 63]. Moreover, butyric acid plays a key role in the down regulation of pro-inflammatory effectors of lamina propria macrophages and determines the expression of cytokines in T cells [64, 65]. Effectively, it is involved in the activation and differentiation of a memory phenotype of CD8 + T lymphocytes [66].

4. Prematurity and microbiota

4.1 Causes of prematurity

Prematurity is a syndrome that includes all the situations associated with the occurrence of a birth before 37 weeks of amenorrhea. It is classified according to gestational age, extremely premature (less than 28 weeks), very premature (between 28 and less than 32 weeks) and moderate premature (between 32 and less than 37 full weeks of gestation) [67, 68]. Preterm birth can result in adverse effects and even short-term neonatal mortality [69, 70]. The long-term health consequences of prematurity throughout adulthood of infants is an increased risk of type 2 diabetes, obesity, hypertension, asthma, anxiety, spectrum disorders autistic, cerebral palsy, epilepsy and cognitive impairment, heart disease, chronic renal failure, lung function abnormalities and neurocognitive disorders [71–78]. These effects can have a negative impact on health care costs, education as well as quality of life [79, 80].

During pregnancy, along with hormonal, metabolic and immune changes, the different ecosystems of the mother's microbiome (vaginal, uterine, etc.) change. Indeed, the vaginal microbiota is less diverse and more stable, with an abundance of bacteria of the genus *Lactobacillus* [81]. Changes in the gut microbiota also appearing in late pregnancy may help support the increased energy requirements needed for fetal development [82]. All these observations thus suggest a real role of the microbiota in the physiology of pregnancy. The occurrence of premature rupture and preterm of membranes was preceded in one-third of cases by depletion of *Lactobacillus*, protective bacteria in the vaginal flora [83]. Dysbiosis could therefore play a role as a biomarker associated with pathology, but also be directly involved in pathophysiological processes. Indeed, vaginal dysbiosis could initiate an inflammatory cascade leading to prematurity. A modification of the vaginal microbiota, with a decrease in protective *Lactobacillus*, allows pathogens such as *Gardnerella vaginalis* or *Mycoplasma hominis* to proliferate [84] (**Figure 2**). These pathogens could then pass through the cervix and contaminate the chorio-decidual space. In the face of this aggression, an increased production of inflammatory cytokines, as a result of the immune response, would stimulate the recruitment of neutrophils, the production of prostaglandins and the synthesis of metalloproteinases [85]. This cascade of events would promote uterine contractility, shortening of the cervix, weakening of the fetal membranes and could lead to premature delivery [86]. The sequelae due to the exposure of the preterm newborn to an intrauterine inflammatory environment will accumulate with those of its developmental immaturity, which may result in early and severe neonatal disorders, such as necrotizing enterocolitis (NEC), cystic periventricular leukomalacia or bronchopulmonary dysplasia [87]. Maternal dysbiosis will influence the initial fetal culture and consequently the colonization at birth of the gut microbiota in the preterm infant.

Also, when a newborn is born prematurely, it is necessarily separated from its mother for care reasons. This maternal separation and prematurity, responsible for dysbiosis, would increase the risk of developing pathologies [88].

Environmental factors, maternal microbes and breast milk shape healthy colonization of the infant gut. The colonizing microbes influence the development of the gut and also participate in several biological processes related to growth and survival. Several factors lead to dysbiosis in the gut microbiota of newborns, increasing the risk of neonatal conditions such as necrotizing enterocolitis and long-term health problems such as asthma, hypertension and obesity.

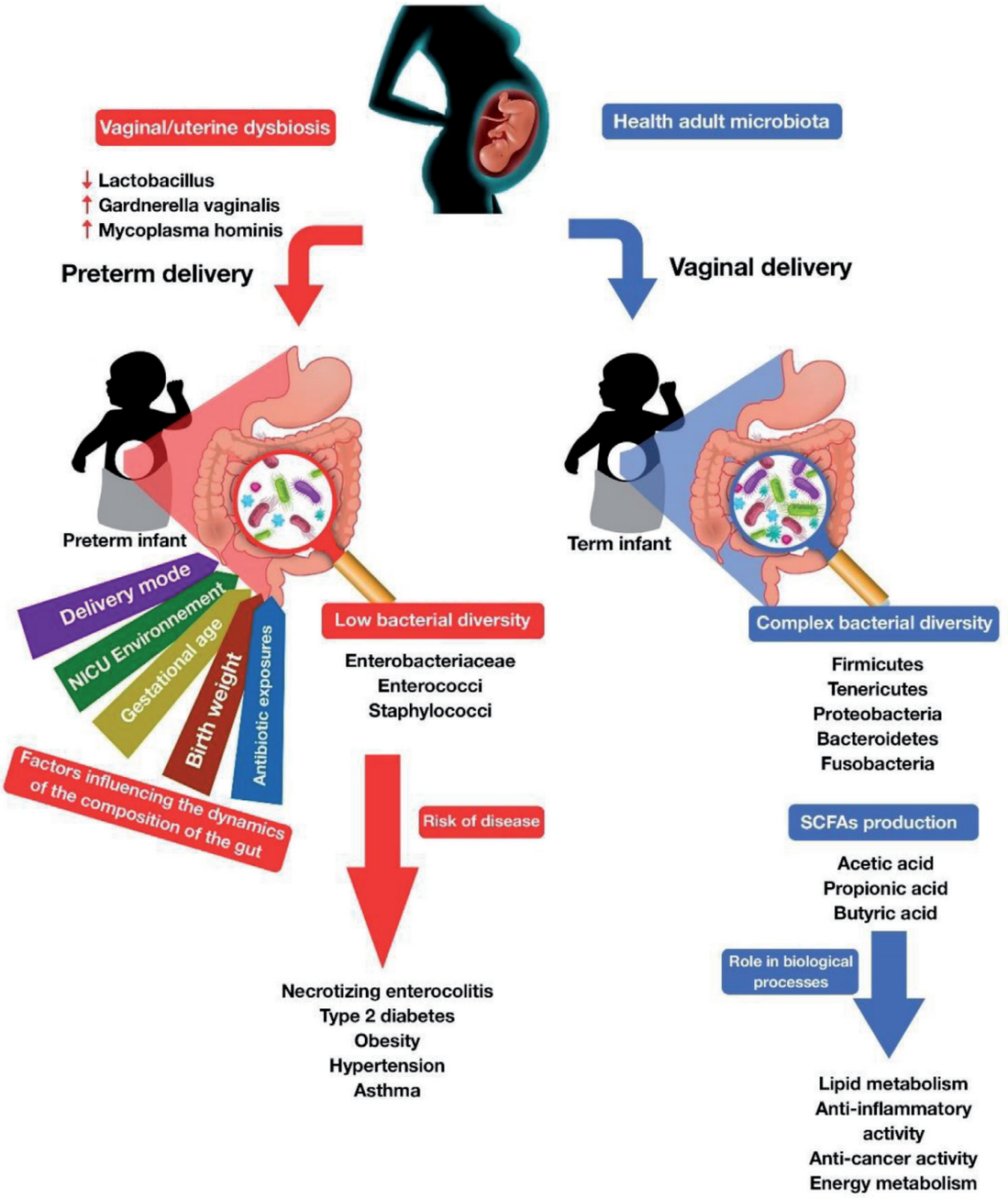

Figure 2.
Factors influencing microbial intestinal homeostasis of term and preterm infants.

4.2 Preterm intestinal microbiota

Premature infants may be admitted to a highly specialized area of the hospital, the Neonatal Intensive Care Unit (NICU). Prematurity and admission to the NICU are often unexpected and may occur before families have discussed the nature of feeding for their infants. Prenatal and postnatal exposure to antibiotics as well as other medical risks can affect the composition of the early gut microbiota in premature infants [89].

The immaturity of the intestine of preterm promotes pathogenic bacterial colonization due to the high permeability of the intestinal surface, which paves the way for a destructive dysbiosis. This dysbiosis, responsible for chronic inflammation and microbial translocation across the weakened intestinal barrier, is associated with life-threatening conditions of prematurity such as NEC and late-onset sepsis (LOS) [90].

The intestinal microbiota of children born prematurely has a low diversity and a reduced number of anaerobic bacteria. Two elements which would participate in the risk of developing pathologies of premature babies. In premature newborns, it was observed a significant delay in colonization compared to full-term infants as well as colonization by a smaller number of bacterial species [91].

The delay in colonization is especially marked for the anaerobic flora (*Bifidobacterium* and *Bacteroides*) while the aerobic flora (Enterobacteriaceae, Enterococci, Staphylococci) colonizes the premature baby fairly quickly [92–102]. This delay in implantation may be due to the fact that these children are more frequently born by cesarean section, are quickly separated from their mothers and placed in a highly aseptic intensive care environment and often subjected to broad-spectrum antibiotic therapy [89]. Several studies have compared the microbiota of NICU-cared preterm infants and healthy term infants after birth. A significant abundance of Staphylococcaceae has been observed in premature infants. In contrast, the abundance of Enterobacteriaceae, Streptococcaceae and Enterococcaceae is significantly lower in NICU preterm infants immediately after birth, and their level gradually increases from the 2nd week. The abundance of Bifidobacteriaceae in term newborns is greater than in preterms at birth, associated with a high concentration of acetate, a sign of good host health. However, higher levels of propionate and butyrate are noted in preterm infants, indicating less saccharolytic fermentation [103–105].

4.3 Breastfeeding in the intensive care unit: a way to support preterm infants

Breastfeeding in preterm infants is often initiated by administration through a nasogastric or orogastric probe. Infants switch to oral feeding when the coordination of sucking, swallowing and breathing is developed [106–108]. This oral feeding is a difficult stage for preterm infants [109], with almost 40% finding it difficult to transition to direct oral breastfeeding [110–113]. Olfactory stimulation [114–120], could remedy the difficulties of direct oral feeding and consequently reduce the risks of pathologies associated with prematurity [121, 122]. The results of several studies have shown that direct breastfeeding increases the likelihood of maintaining longer breastfeeding durations during NICU hospitalization and up to 4 months after discharge [93–95, 123–125].

In preterm infants, breast milk is associated with a significant reduction in NEC, and a reduction in other key morbidities, as well as improved neurodevelopmental outcomes [91]. These impacts have long-term benefits for the child (and mother) even after weaning. This advantage is probably due, in part, to the development of a healthy gut microbiota dominated by *Bifidobacterium* from breast milk and its bioactive components [126].

Breast milk could be a natural way to restore intestinal homeostasis newborn and meet the recommended health standards for newborn term and preterm [127]. Thus, awareness of the importance of breast milk for premature infants is essential for families whose premature birth is planned and for those whose child is in the NICU [128–132]. On the other hand, trained NICU personnel will help and encourage mothers to breastfeed more frequently during hospitalization. This breast milk and intestinal microbiota axis opens up promising research avenues for new therapies in premature babies and other high-risk infants.

5. Conclusion

The long-term health of the human being is programmed before birth where nutrition plays a crucial role. Indeed, the mother's lifestyle is a determining factor

in shaping her own microbiota, which will be transmitted in part to the fetus. From the moment of birth, breast milk is the optimal source of nutrition for the newborn, which will meet its nutritional and immune needs. It is recommended to be the only source of nutrition for the first six months of life and up to 2 years.

Preterm birth is a complex syndrome, the leading cause of morbidity and perinatal mortality. The immaturity of the preterm gastrointestinal tract and the initial dysbiosis are corrected by breastfeeding. Indeed, breast milk promotes an evolutionary dynamic of the intestinal microbiota and is truly a food for all newborns and particularly premature babies in intensive care. The cross-talk established between the bioactive components of milk, mainly the metabolites of the intestinal microbiota and the intestinal mucosa of the newborn, can compensate for the delay in colonization in preterm infants. This infant's breast milk-intestinal microbiota axis is a proven link between mother and baby. This symbiosis is essential for the maintenance of health and well-being.

Thus, promoting breastfeeding is the natural solution to preventing infant morbidity and mortality.

Conflict of interest

The authors declare no conflict of interest.

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