Review article

# Systematic review and bibliometric analysis of the metabolome found in human breast milk from healthy and gestational diabetes mellitus mothers

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## *Abstract*

**Introduction.** Human breast milk is considered the gold standard of nutrition, given that thanks to the diversity in the metabolome it manages to meet the individual needs of each infant by providing essential metabolites that contribute to and intervene in optimal growth and development. Few factors can modify the composition of breast milk and, simultaneously, its benefits. However, the increase in maternal metabolic diseases such as gestational diabetes mellitus raises the question of whether it can be one of the factors that condition the quality and quantity of metabolites contained in breast milk. **Objective.** To identify the metabolome of breast milk from healthy mothers, its influence on the growth and development of the infant, and to recognize those that are altered because of gestational diabetes mellitus. **Methodology.** A systematic review was carried out using multiple databases. For the bibliometric analysis, we used the results of Web of Science and Scopus and the Tree of Science and Bibliometrix software.

**Keywords:** metabolome, breast milk, diabetes mellitus.

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https://doi.org/10.22490/24629448.7545

## Introduction

Metabolomics is a branch of the omics sciences responsible for studying the set of metabolites present in biological systems known as the metabolome (1,2). In it, low molecular weight molecules can be identified in each matrix at a given moment in time, generating an overview of the sample that determines the physiological or pathological state of the sample, which can contribute to the detection of risk factors in populations (3).

From the metabolic and nutritional point of view, one of the populations of special care are infants between 0-6 months; during this stage of life, it is proposed that feeding should be exclusively through breastfeeding. This is defined as a physiological act that provides the baby with specialized and individualized nutrition, by containing the optimal distribution and quantity of nutrients that are adapted to the nutritional and energetic needs of the infant, It is considered the gold standard of nutrition in this first stage of life and this is how metabolomics in neonatology offers an important approach to investigate the link between nutrition and infant health. (4) The first study to analyze the metabolome of breast milk, according to researchers, was conducted by Cesare Marincola et al. in 2012 (5)(6).

Breastfeeding is the main protective factor against infant health; therefore, its promotion, protection, and support have been a priority for all countries. In Colombia, breastfeeding is initiated from the very instant of birth and currently, 43% of mothers use it as exclusive infant feeding according to a report published in 2017 by Unicef, the World Breastfeeding Collective, and the World Health Organization.

The world is currently undergoing a public health problem generated by epigenetic conditions in people. This problem encompasses metabolic diseases, which include diseases such as obesity, type 2 diabetes, heart and/or metabolic disease, and non-alcoholic fatty liver disease (7,8).

The metabolic diseases with which the mothers arrive at pregnancy or develop during pregnancy can influence the quality of the nutrients in breast milk and in the same way have repercussions on the health of the infant, mainly leading to metabolic disorders in the short and medium term (9). Recently, the variation in the metabolome of breast milk in diabetic mothers in relation to healthy mothers was published, finding a high variation in amino acids and lipids that are fundamental in the development of the child (10).

Gestational diabetes mellitus (GDM) is understood as "an intolerance to carbohydrates that is discovered or manifests itself for the first-time during gestation" (11). In Colombia, the prevalence is currently estimated to be between 10.3% and 15% of which 5% are type II diabetics, 7.5% are type I diabetics who become pregnant, and the remaining 87.5% are true diabetes of pregnancy (12). All these figures are on the rise due to poor eating habits, the predominant sedentary lifestyle and environmental conditions that generate even more metabolic diseases such as gestational diabetes mellitus (GDM), so it is vital to know the impact of this disease on the first protective factor of childhood, which is breast milk.

The objective of this review is to identify the different metabolites in breast milk from healthy mothers, their influence on the growth and development of the infant and to recognize those that are altered because of GDM.

## Methodology

Systematic research was carried out by means of a literature search in databases such as Web of Science, Scopus, Scielo, PubMed, and Google Scholar using the keywords “Metabolome”, “Gestational diabetes mellitus”, “Human Breast milk metabolome”, “metabolomics profile” “growth and development” “neonatal health”. Using the connectors “AND” and “IN”.

Articles whose content dealt with the keywords were chosen, as well as epidemiological studies on prevalence rates in this geographical area and associated factors.

The reviewed publications were selected with a maximum age of 6 years, except for root articles.

For a complete understanding of the studies, the criteria considered for inclusion were: (i) published primary research (original), (ii) systematic reviews, (iii) reviews, and (iv) research or clinical trials. The literature search was conducted in a period between January 2020 and July 2021.

### Search equation

A bibliometric analysis was performed using the packages “tosr” and “bibliometrix” (13,14) for this, the search was performed in Web of Science (WoS) using the following equation: Topic: (metabolomic* OR metabonomic*) and Topic: (“breast milk” OR “human breast milk”) in a period from 2001 to May 15, 2023, in the indexes SCI-EXPANDED, SSCI, A&HCI, ESCI. Likewise, a search was performed in Scopus using the same equation in the fields Title, Abstract, and Keywords. From these searches, 165 results were obtained in WoS and 272 in Scopus. All the results were downloaded with the references cited in each of the articles, to construct a citation network for the prioritization of the documents. The WoS and Scopus results were merged into a single data file using the R package “tosr”.

### Citation analysis

Each of the references and citations of each document were analyzed by means of a citation network using the SAP algorithm of Tree of Science, which is an open code for prioritizing publications (15). This systematic search methodology is based on the prioritization of documents using indicators of the topology of the citation network explained in detail by Zuluaga et al. (16) and making an analogy with the tree of knowledge. Briefly, the methodology consists of identifying the classic articles (roots), the articles that gave growth to this area of knowledge (trunk), and the recent articles with the greatest impact (leaves). The citation network is used to extract the documents with a high degree of entry, the documents with high intermediation, and the most recent ones that are linked to the root and the trunk. Likewise, the modularity algorithm (17) is applied to select different clusters or perspectives in the knowledge area. The number of clusters is selected using the tipping point algorithm; (13,18). All the code for this citation analysis is available through the following link: https://github.com/coreofscience/tosr. Additionally, 104 articles found in the PubMed database that were not prioritized in the methodology described above were manually added (Figure 1).

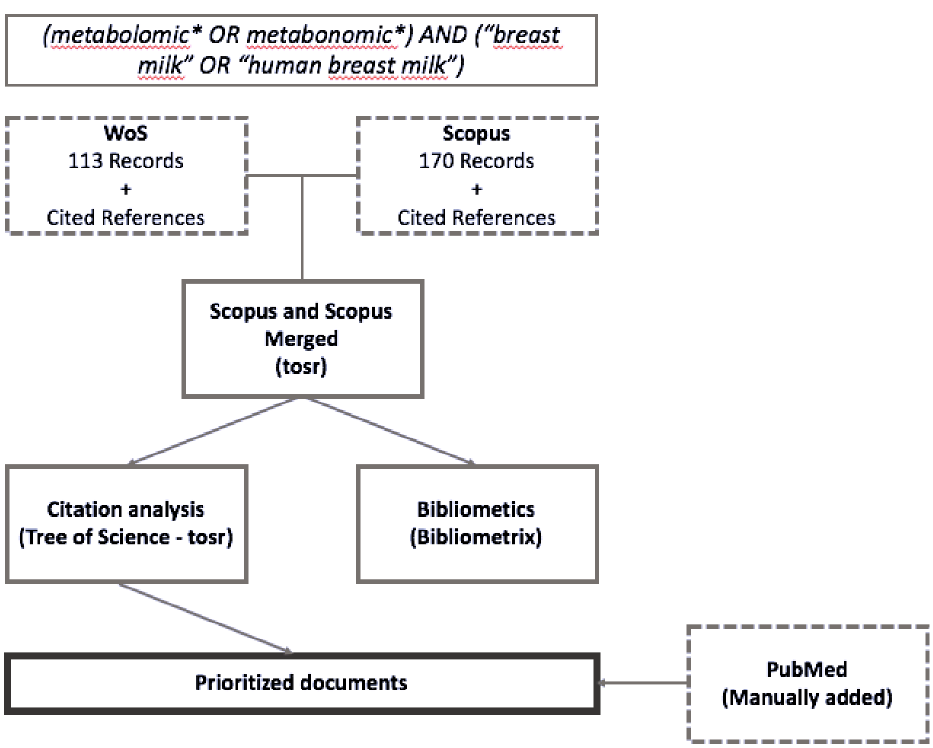


**Figure 1.** Methodology for the search and selection of articles. Own source.

### *Bibliometric Analysis*

Bibliometric analysis was performed using the Biblioshiny tool, an application of the R package Bibliometrix (14), from which indicators such as annual scientific production, number of citations per journal and per author, publications per author per year, trend of keywords per year and the collaboration network were extracted.

## *Results*

### *Search equation and citation analysis*

The literature review was carried out in WoS and Scopus since between these two databases they integrate more than fifty thousand scientific journals (19) and additionally allow the user to download the metadata and references cited within each article. The search equation resulted in 272 references in WoS and 165 in Scopus, which were downloaded in plain text with all the metadata and citations reported in each of the articles resulting from the search. These results generated an initial network of 805 nodes (articles) and 1878 links (citations); after using the network indicators and filters to extract the groups or perspectives, a final network of 347 nodes and 582 links was obtained (Figure 2).

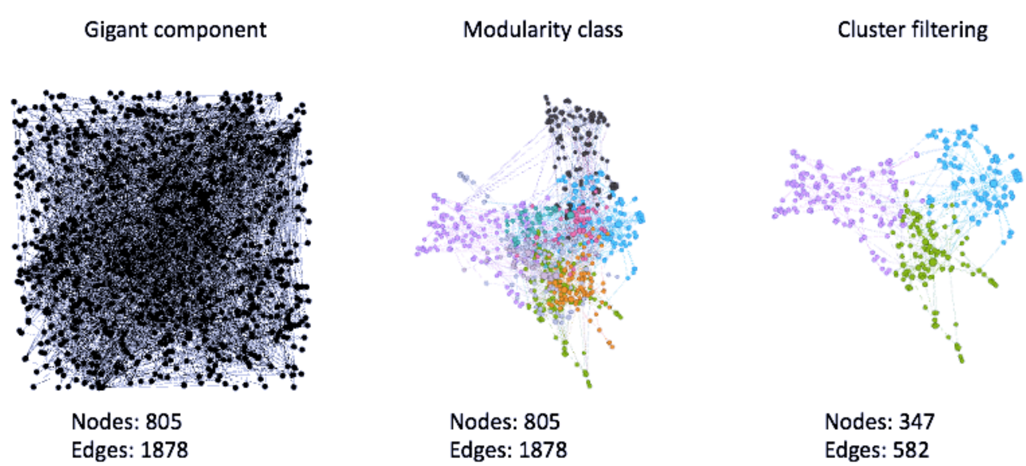


**Figure 2.** Prioritization of documents by citation analysis. Own source.

The title of each group was extracted to generate the word cloud for each perspective (Figure 3). Likewise, the articles were organized according to their importance by means of the root, trunk and leaf indicators as explained in the methodology section:

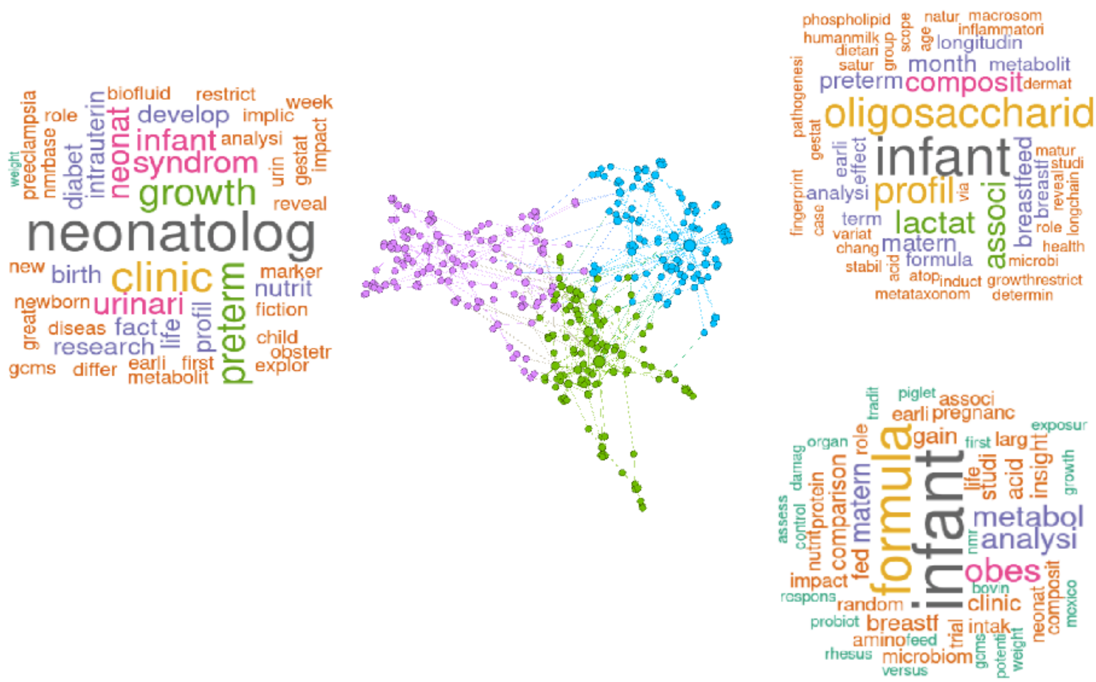


**Figure 3.** Citation network and word cloud. Own source.

The first perspective represented in Figure 3 by the purple cluster represents 16.89% of all the articles in knowledge. According to the word cloud, it can be inferred that the articles are related to the influence of breast milk on growth, and the clinical behavior of neonates in situations such as preeclampsia, diabetes, and preterm birth. The second perspective determined by the green cluster represents 13.54% of the total number of articles and refers to the study of formulas in different animal models and metabolomic analysis by NMR. The third cluster represents 12.67% and is represented by the blue color, and is related to the oligosaccharide profile, lactation studies, and longitudinal studies of newborn feeding.

### *Bibliometric analysis*

The bibliometric analysis was divided into four different aspects, the first related to publications and journals, the second related to authors, the third to the documents with the highest impact and the fourth to the social structure. The metabolomic studies of breast milk show according to the number of publications per year an increasing trend, which during the year 2022 presented the highest number of publications (Figure 4a). Figure 4b. The five articles with the highest number of citations are shown. Marincola et al., (2012) and Marincola (2015). Spevacek (2015). Sundekilde (2016).

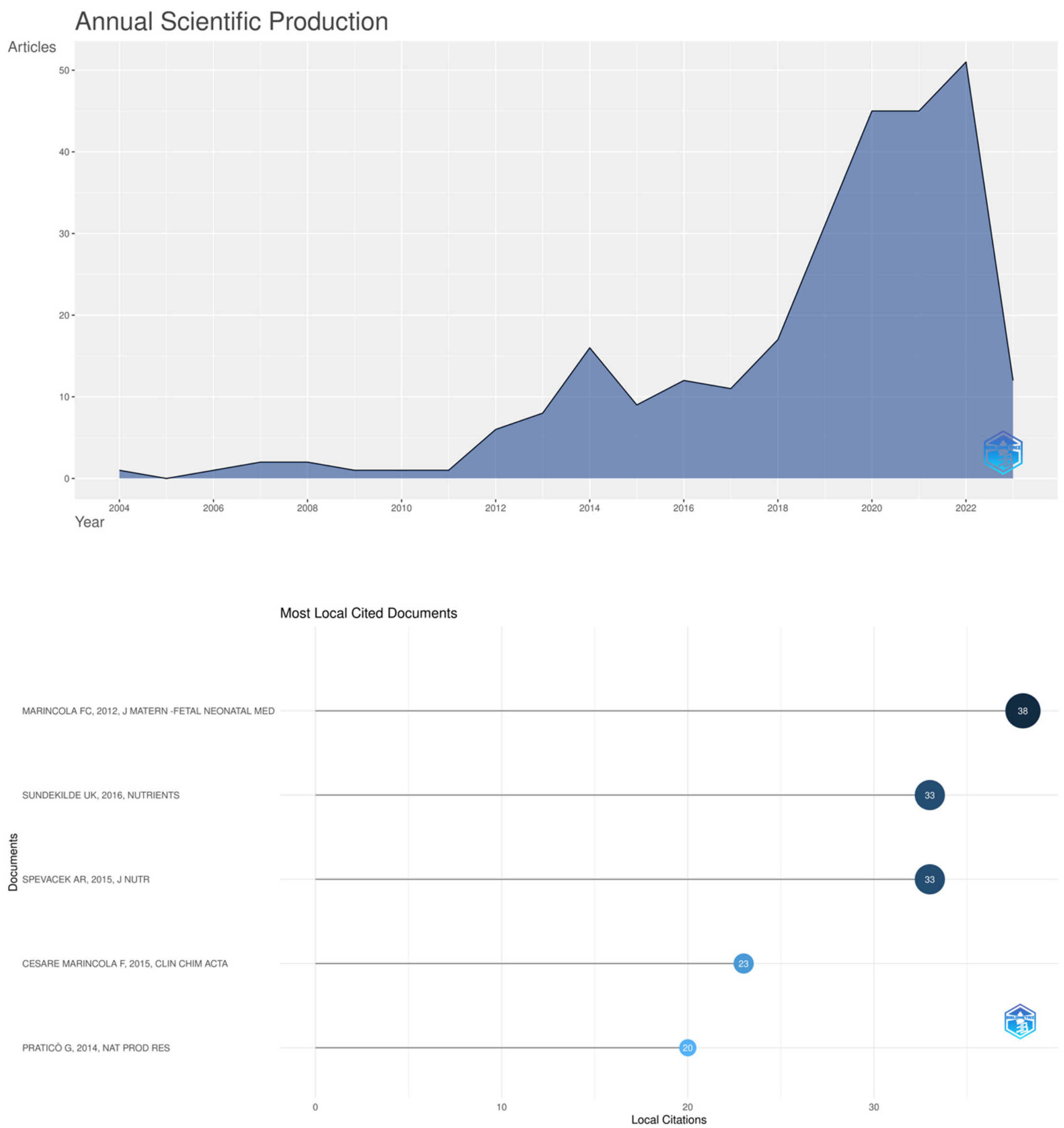


**Figure 4.** a. Annual scientific production. b. Papers with highest citations. Own source using bibliometrix software.

On the other hand, the five authors who have published the most in metabolomics and breast milk are shown in Figure 5a, where the total number of published documents related to the area of knowledge is indicated. Figure 5b shows the frequency of publication of each of the five authors, with the number of documents published per year.


**Figure 5.** a. Most relevant authors. b. Authors' production over time. Own source using bibliometrix software.

The research of Fanos and collaborators has been based on the study of the components of breast milk, their bioactives and their interaction with the development of the infant. Atzori and coworkers delved into the application of metabolomics in maternal-fetal medicine and the recognition of metabolome changes in mothers with gestational diabetes mellitus. The focus of Bardanzellu et al. is on the characterization of the metabolome and microbiome of breast milk and its comparison with formula milk, where the influence of each nutrient on neonatal metabolism is evaluated. Dessí et al. focused on the clinical impact of metabolites contained in breast milk, their intra- and intervariability; and Reali et al. investigated the metabolomic differences between colostrum and mature human milk and their functions at each stage of development.

Collaborative networks are of co-authorship analysis where clear evidence and results are shared that allow a greater association and deepening of the present subject studied.

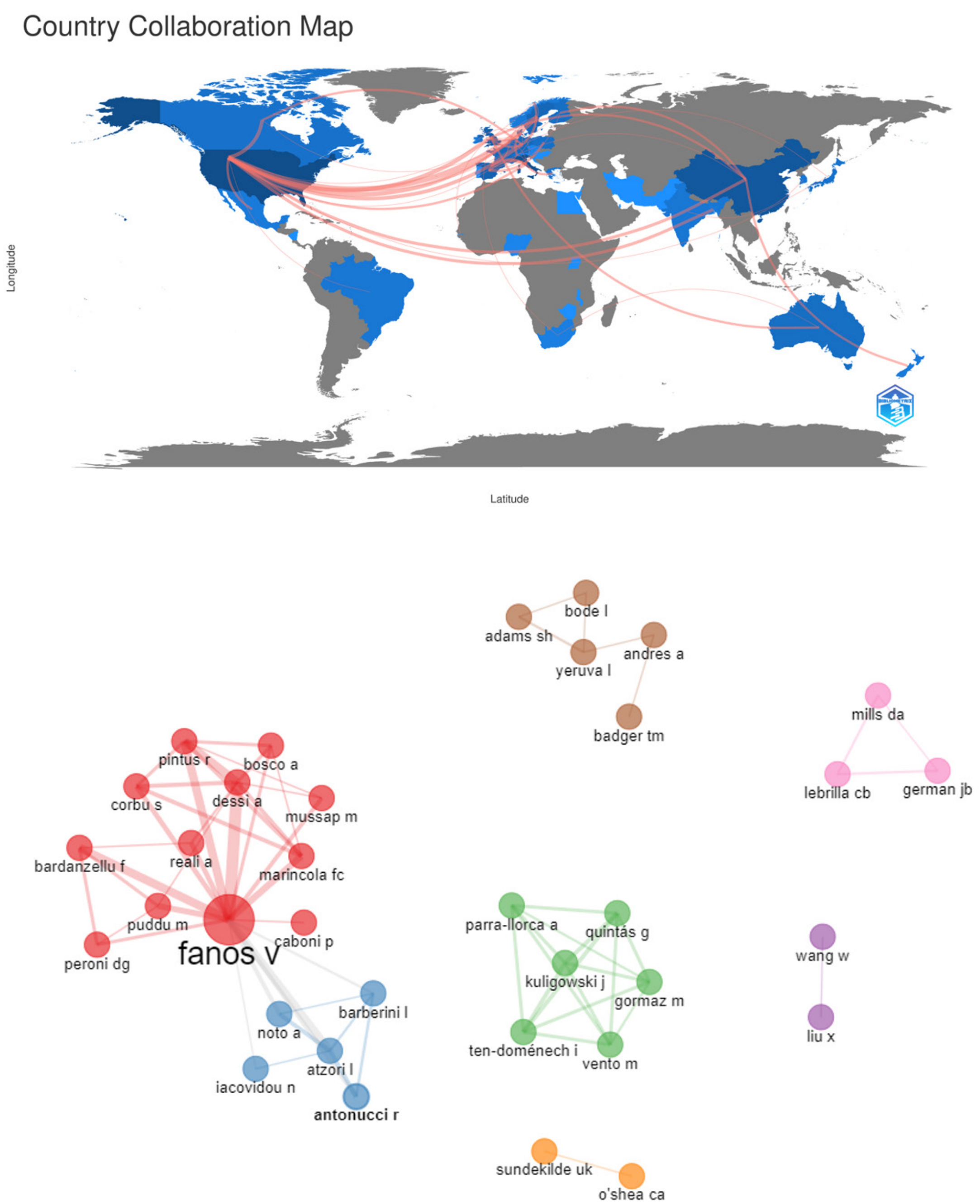


**Figure 6.** a. Collaboration networks. b. Contribution between countries. Own source using bibliometrix software.

### *Analysis of the documents*

The multiple properties and components of breast milk in the neonatal stage are crucial for optimal growth and development since this is one of the most critical periods of life (20).

However, breast milk contains about 600 species and it is stipulated that a breastfed infant ingests up to 10 million live bacteria per day (6), in addition, this liquid has special characteristics that allow the neonate to adapt to extrauterine life and influence the rest of it, thanks to its capacity in the activation of metabolic processes such as growth, development of the immune, nervous and cognitive systems, colonization, intestinal microbiological maturation, among many others in which the bioactive compounds of breast milk intervene (21,22).

Although the industry has made efforts to create formula milk similar to breast milk, it has been identified that this objective has not been achieved so far, in view of the fact that there are marked differences both qualitatively and quantitatively in relation to the metabolites that compose it, being amino acids and fatty acids the ones with the greatest variability (23-26).

In a comparative study of the year 2021 between the metabolites of human milk, goat's milk, equine milk and cow's milk, 37 metabolites were found to be significantly different among the four samples, and complementarily, seven main metabolic pathways were identified in the milk of these animals that allow a global analysis of their metabolome (27). However, in the study conducted by PHAN M and collaborators, it is evident that after an analysis of 261 metabolites, 151 of these, present in formula milk, are closely related to human breast milk (28). This is evidence that the efforts made by the industry to achieve formula milk similar to breast milk are progressing more and more, but, even so, an exact similarity has not been achieved, so it cannot replace it.

The composition of breast milk is not stable; on the contrary, it undergoes a series of transformations according to the biological age and physiological needs of the infant (5,29,30). It has even been shown that the external environment (geographical location), the internal environment (mode of delivery), the state of mind, and the dietary intake of the mothers can alter the composition of the milk (31)(32)(33).

When the lactation process begins, the liquid coming from the mother between the first and fifth or sixth day is known as colostrum, characterized by its thick and yellow appearance, contains a large number of proteins and minerals, as well as many immune active substances, such as macrophages and lymphocytes, complement systems, lysozyme, oligosaccharides and antimicrobials such as lactoferrin, which give an immunoprotective action to the newborn and decrease the production of inflammatory cytokines (34,35).

After the first six days of lactation, some authors describe the existence of transition milk, in which nutrients and caloric content stabilize to give way to mature milk with a completely different metabolomic composition to meet the requirements of this stage of the infant (36). After the first month of lactation, breast milk reaches its maximum point of maturity in metabolites and energy which tends to optimal development and growth in this first stage of life (35,37).

It should be clarified that human milk metabolites are derived from three sources: (i) nutrients from synthesis in the lactocyte, (ii) those of dietary origin where the concentrations in the milk from the mammary gland are strongly related to the mother's intake (38) and (iii) those from maternal reserves regardless of the state of the milk (39). The mother's health and nutritional status, all this to guarantee one hundred percent of the caloric and nutritional intake demanded by the infant (29,30,39).

Mature milk is the food that is provided for a longer period in infant lactation compared to the other phases of milk, which are transitional and of short duration. In fact, it has been suggested that the metabolites and microbiome of mature milk remain stable for up to 24 months (40). Therefore, it is of vital importance to characterize this biofluid and the relationship it has with the growth, and development of the infant and its physiological functions (41).

For an adequate metabolomic analysis of breast milk, several aspects must be considered, from its extraction, preparation, storage, and study. The most critical parts of the process are storage since this step must be carried out quickly and at appropriate temperatures, and study since it identifies polar and non-polar elements (42).

One of the techniques for the analysis of these compounds that provides a complete view of the dynamics of breast milk is liquid chromatography adapted to mass spectrometry, since it guarantees a wide coverage of the metabolome of breast milk, especially for its fatty part (glycerophospholipids and sphingolipids of low abundance), in addition, it allows monitoring short-term changes and the composition of variable metabolites. However, it should be clarified that the analysis to quantify HMO can only be performed in those that have a reference standard (26,43,44).

Currently, there are new study techniques such as mass spectrometry with capillary electrophoresis that allow an evolution in the study of the metabolome of diverse fluids, given that it identifies highly ionic and polar elements such as organic acids, sugars, and amino acids that cannot be appreciated through liquid chromatography, however, being a recent technique, it is difficult to access and presents a higher cost (45).

Considering that this liquid is stable throughout the months, its classic distribution of metabolites is highlighted. The main component and the one which is present in greater quantity is water with 88% - 90%, as quality it presents an osmolarity like that of the blood plasma which allows the infant an optimal hydroelectrolytic balance without having additional water requirements (41).

Lipids are the most abundant solid fraction during this stage of breast milk, contributing 40% to 55% of the total energy of the milk, followed by carbohydrates, considering that the most abundant in mature milk is lactose (6.7 g/100 ml). This disaccharide provides the infant with a high energy demand for brain function and when metabolized, it is the most important source of galactose for the maintenance and development of the central nervous system (30,41).

The protein contained in mature milk is produced in 80% to 90% by the lactocytes of the mammary gland, making it the third most abundant solid in breast milk (41,46). In addition, breast milk is characterized by the presence of different compounds, not nutrients, which together with the macronutrients will be essential in the infant's nutrition, fulfilling physiologically specific functions.

Although the systems of the term neonate are complete, many of them are immature. There is a variety of evidence correlating the metabolites in breast milk and the influence it has on maturation.

Growth and development is an event that occurs during the stage of conception and ends with adolescence, having one of its highest peaks during the first year of life, since it influences the exponential maturation of all organs and tissues to ensure proper functioning in the rest of the stages of life (47).

There are specific metabolites for the different adaptations of the infant's systems; however, the growth factors in breast milk play a transversal role in all the developmental processes of the child, since among their fascinating characteristics is their ability to reach the target organs intact, such as the nervous, gastrointestinal, respiratory, epithelial, and circulatory systems (48). Metabolites in the gastrointestinal tract

The infant's stomach has a reduced gastric capacity, and the intestine has not yet reached its potential in size. This is where the importance of feeding with breast milk lies since its composition contains growth factors such as vascular endothelial growth factor (VGEF), which interacts with specific receptors of the intestinal mucosa, particularly with those of the epithelial lineage. This generates hyperplasia and hypertrophy favoring the absorptive capacity of nutrients (22,49) It should be noted that the newborn has been exposed to microbial flora since the uterus, however, there is no established symbiosis (50).

Breast milk from the early stages of lactation provides the amount of enterobacteria necessary to enhance the immune response and metabolic processes. For these bacteria to survive, the metabolites that function as prebiotics are mainly HMOs, which are the third most abundant solid unit in breast milk, presenting up to 200 different HMOs, which makes it the mammalian milk with more of these compounds in a concentration of 16 g/L in milk (51,52).

On the contrary, they are used by non-pathogenic bacteria (53), especially by those belonging to the bifidobacteria genus, providing substrates which, when metabolized, confer beneficial effects (52), such as the generation of short-chain fatty acids (SCFA), which create a stable intestinal ecosystem (30,54).

The growth and maturation of the neonatal intestinal mucosa play a primordial role in the capacity of nutrient absorption and confer health and immunity to the infant. Certain growth factors and cytokines present in breast milk by mammary secretions such as TGF- β 2, EGF or FGF21, TGF β 2, and IL-10 promote intestinal maturation, configuring a uniformity in the microbiome by means of particular interactions between probiotic bacterial strains and these elements, thus guaranteeing diversity in the microbiota (55,56).

The same happens in pre-term neonates, who need to increase the rate of early growth and brain development due to their physiological conditions, so the milk of pre-term mothers is adapted to these rapid growth needs (57) thanks to its content of insulin-trophic and branched-chain amino acids, lacto-N-fucopentaose, choline and hydroxybutyrate, which are fundamental metabolites in energy utilization, protein synthesis, oxidative status and maturity of intestinal epithelial cells so important in prematurity (58).

Breast milk is the main exogenous source in the infant of compounds called polyamines. Among them, spermine and spermidine stand out in this biofluid, which generates growth, mucosal maturation, and cellular proliferation in the neonate (59). The tissues lining the baby's intestine are labile grow at high rates and have a very high turnover activity, which is why breast milk offers high concentrations of these molecules (30).

The enterocyte is the cell that makes up the intestinal parenchyma and its optimal function, maturation, and activity are directly related to the substrates that reach it (60). The free amino acids that reach the intestine through the ingestion of breast milk are, with the greatest abundance, glutamine, and glutamate, comprising 70% (61). Glutamine is a precursor of intestinal health, so its absence as an exogenous supply will limit the functioning of enterocytes, due to the fact that its endogenous synthesis is limited (62). It has the capacity to be oxidized by intestinal cells and immune tissue present in the intestine, which makes it an energetic substrate for the periods of rapid and exponential growth that this organ undergoes in the first months of the life of the infant (61,62). Additionally, glutamine in the company of microbiota supports the intestinal barrier function by modulating certain specific intracellular pathways and decreasing the permeability of the wall (62).

Glutamate, an amino acid that increases throughout lactation, contributes to the necessary production of energy to support intestinal functions, and neurodevelopment, in addition to promoting the microbiota in the infant's intestine and regulating appetite (30,63,64).

For an adequate absorption and facilitation of the metabolism of nutrients, there are substrates that intervene in this task, such as lactoferrin, osteopontin, milk fat globule membrane (MGFM), and palmitic acid, this helps the absorption of calcium and magnesium, and jointly decreases constipation in the newborn (30,65-67).

The maturation and integrity of the gastrointestinal tract are of vital importance since this system performs the uptake and absorption of nutrients, directing them into the bloodstream to reach the other sites in the body, in seeking to satisfy the needs of the organs for optimal development (68).

### *Metabolites in the immune system*

At birth, the infant is directly exposed to all the pathological factors that previously protected it in the amniotic sac, placenta, and intrauterine environment (69). The newborn presents a complete immune system, however, portrays some specific deficient functions such as "physical and chemical barriers, deficient function of innate effector cells, limited and delayed production of secretory immunoglobulin A (IgA), complement cascade function, and insufficient anti-inflammatory mechanisms of the respiratory and gastrointestinal tracts" (66).

The main immunological protectors of breast milk are immunoglobulins, including IgG, IgM, and IgA. The latter is the most prevalent and inhibits the binding of pathogens to the intestinal epithelium by trapping them in layers of mucus. IgM is responsible for binding identified pathogens and thus activating the complement factor. IgG transports, by phagocytosis, the antigens to the lamina propria, and has the capacity to activate the complement factor (66).

One of the fundamental components in this stage for the strengthening of the immune system is the growth factors since they have an immunoregulatory function due to the action they exert on the cells of the mesenteric lymph node, thus allowing a notable increase of the natural killer (NK) cells, maturation of lymphocytes and reduction of cytokines which are directly related to allergic reactions (56). The function of both epidermal growth factor (EGF) and transforming growth factor (TGF) are involved in modulating the inflammatory response and improving the response to pathogens thanks to the production of B lymphocytes, immune cells of the thymus and lymphoid tissues (12,34,70). Macrophage colony-stimulating factor (M-CSF) and granulocyte colony-stimulating factor (G-CSF) contribute to the survival of macrophages in breast milk (69).

The latter together with neutrophils, T cells, stem cells, lymphocytes, antimicrobial factors, immunoglobulins, and cytokines operate in networks and orchestrate the functions of the infant's immune system (71). Speaking especially of stem cells, they trigger the beginning of the immune system response in the newborn, since they have the capacity to migrate to different organs and differentiate, among which is the thymus which, being a lymphoid and specialized organ of the immune system, its development will be directly related to the cellular immune response of the neonate's organism (72)(73).

Breast milk contains cytokines which are pluripotent peptides that have an immunostimulatory and immunomodulatory effect, inducing phagocytosis and antigen presentation, growth, and differentiation of immunoglobulin, and suppressing the production of immunoglobulin E (IgE). In summary, they are not only responsible for passive protection but also intervene in the immunological development of the recipient infant (52,69). Within the mammary gland, IL-6 participates especially, generating, in an increased manner, IgM and IgA immunoglobulins and decreases IL-1 and TNF α (74).

The metabolite responsible for iron deprivation, alteration of membranes of pathogenic microorganisms, microbial receptor analogs, and favoring the growth of epithelial cells is lactoferrin and its derivatives which have antimicrobial and fungicidal functions (65,75).

Lipids also modulate the immune system. For example, phospholipids, although they are in smaller quantities compared to colostrum and transitional milk, play an indispensable role in immune and anti-inflammatory responses of the infant together with omega 3 and omega 6 fatty acids since they are fundamental in anti-inflammatory and inflammatory processes respectively (76-78) Other lipids involved in the immunity of the infant are short chain fatty acids from the metabolism in the microbiota of HMO. Their mechanism is to acidify the intestinal lumen and thus prevent bacterial growth (52).

Other influential metabolites are osteopontin which performs immune regulation by interacting with cell surface integrins and CD44 receptors (65,79) and simultaneously, glutamine and glutamate are immunomodulators preventing neonatal allergies and infections (61).

Many pathogens, to infect, adhere to the glycocalyx, which is a layer that covers epithelial cells and is formed by glucans, proteins, and lipids. HMOs are structurally like glucans, which is how pathogens and toxins identify them as sites of adherence and thus transit the gastrointestinal tract without causing disease (29,52,80). Metabolites in neonatal metabolism.

At this stage of life, the newborn requires precise amounts of energy from breast milk nutrients for proper organ function and thus promotes constant growth and development. The lipids in breast milk are the main metabolites in the production of energy that the newborn needs to fulfill and maintain the body's functions. About 98 to 99% of these are composed of triacylglycerols, whose properties are derived from the fatty acids of which they are composed (67,81).

The lipid fractions in breast milk are distributed as follows: "34% -47% Saturated fatty acids being mainly palmitic acid with 17% -25%; 31% to 43% are monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) with 12% -26% omega 6 (n-6) and 0.8% -3.6% omega 3 (n - 3)" (30). Concluding that the ratio of unsaturated versus saturated fats is higher than in other mammals (67).

Mature human milk stands out for containing a higher concentration of lipids, which form the structure of the cell membrane, are responsible for signal transmission and cell recognition in signaling pathways, as well as influencing lipoprotein metabolism, being transporters and sources of fat-soluble vitamins (81).

Some of the fatty acids that make up breast milk are palmitic acid, which, in addition to providing energy, has a surfactant function within the respiratory system, preventing the collapse of the alveolus and, at the same time, contributes to the absorption of magnesium and calcium (30,67). The long-chain polyunsaturated fatty acids involved in the growth and maturation of various organs in the infant are n-3 (α-linolenic acid) and n-6 (linoleic acid). However, not all lipids have an impact on adiposity gain. On the contrary, many of them will fulfill other tasks within the newborn's organism. The only fatty acid that has been directly related to adipose gain is linoleic acid (82).

There are endogenous lipid mediators derived from long-chain polyunsaturated fatty acids called endocannabinoids. Although their function has not been extensively studied or understood, Gaitan et al. established that mature milk contains lower levels of arachidonoylglycerol, which plays an important role in the sucking pattern of the infant (83). An important metabolite in energy generation is carnitine, since it promotes lipid utilization by controlling the entry of fatty acids into the mitochondria, facilitating the oxidation of pyruvate and large amounts of branched-chain amino acids (67).

Another large group of metabolites favoring energy production in the neonate are carbohydrates. Their total amount is related to the infant's length, weight gain, and percentage of fat-free mass (84). Lactose is positively associated with weight gain and adipose reserve in the period from 3 to 12 months of the infant (82), this is closely related to the increase of this disaccharide in human milk as the lactation time passes; and to maintain the osmotic state in balance, citrate, which is part of the intermediates in the energetic metabolism of the cells and in tricarboxylic acid, in this phase its quantity decreases (23). Its influence on infant growth is fundamental since it allows the absorption of nutrients that are directly related to bone health and growth such as calcium and others such as copper and magnesium involved in the generation of energy through phosphorylation (85).

Amino acids that promote energy generation by providing nitrogen necessary for growth in the newborn are alanine, glutamate, glutamine, isoleucine, threonine, valine, methionine, and the organic compound creatinine, the latter of which transports energy to sites of ATP synthesis (36). Glutamine participates in growth by enhancing the effects of growth factors including insulin-like growth factor and epidermal growth factor, while glutamate acts on weight gain (22,30,36,61).

Metabolically active proteins that are synthesized in the adipocyte have important metabolic functions, these are called adipokines formed by leptin that produces satiety, leads to high energy consumption, and increases serotonin availability; adiponectin increases insulin sensitivity; ghrelin favors regulation of energy metabolism and stimulates the secretion of growth hormone (GH) and obestatin modulates cell proliferation (86,87).

The growth factors play a fundamental role in the formation and maturation of various organs that consequently avoid any alteration in the baby's organism and promote adequate growth, the main ones are: vascular endothelial growth factor (VEFG), hepatocyte growth factor (HGF), epidermal growth factor (EGF) and insulin growth factors (IGF) (22) At the same time, polyamines that regulate development and stress response are necessary for cell growth and proliferation (59,77).

Each organ or system within the newborn's body uses specific metabolites for healthy development and within normal parameters. Thus, micronutrients such as zinc, iron, and copper are used for the functions of most of them because they are part of enzymes, biocatalysts, and cofactors of different reactions (88). On the other hand, vitamins B5, B6, B9 and B12 perform biological activities in nucleic acid production and influence metabolic pathways such as glycolysis, gluconeogenesis, and amino acid metabolism. Together with other cofactors, they can influence DNA methylation by carbon metabolism (89). Organs such as the brain, pancreas, thymus, spleen, kidney, and liver mature mostly due to stem cells contained in breast milk (22,73).

Visual development is strongly influenced by the contribution of DHA and taurine (90) and the circulatory system benefits from choline and betaine for the prevention of cardiovascular diseases that may affect the health of the newborn (91). Hence lies the importance of the contribution of breast milk, since it has been shown that infants fed with this biofluid have a higher excretion of choline than those fed with formula milk, which implies better coverage (36,92).

### *Metabolites in bone development*

Ossification in infancy is dependent on calcium and phosphorus (in a ratio of 1.2 to 2 respectively) and on vitamin D which is found in its original form in very low levels in breast milk but is sufficient to perform the functions of calcium homeostasis and bone metabolism accompanied by high concentrations of parathormone (PTHrP) which reduces the risk of hypocalcemia due to rapid accumulation in the bone (93,94).

### *Metabolites in the nervous and cognitive system*

The brain is one of the organs that requires specific nutrients and metabolites for its development. The neonate needs high demands to fulfill the 4 stages of maturation that may not be consecutive, but simultaneous, they are cell proliferation; migration; organization and lamination of the brain; and finally, myelination. These are affected by internal or external factors so that an inadequate supply of nutrients would delay optimal maturation (95).

Neural myelination allows an adequate transmission of nerve impulses. During the lactation period. The baby receives sphingomyelin through the mother's milk which promotes the coating of neurons and improves neurobehavioral development in infancy (96). With the help of B vitamins, brain plasticity, and neuronal differentiation are regulated, promoting other brain functions (71). Particularly vitamins B9 and B12 are involved in homocysteine metabolism and myelin preservation. Their deficiency may lead to neurodevelopmental disorders (89).

Lipids and HMO are the metabolites with the greatest incidence in brain health, both in its development and in good neuronal functioning, because the brain is the organ that houses a large amount of lipids after adipose tissue, the difference being that brain lipids are mainly polyunsaturated fatty acids and structural phospholipids of the membranes (97).

This is demonstrated by linolenic acid by the contribution of DHA which particularly targets the gray matter areas involved in motor control, sensory integration, and tension. In turn, arachidonic acid (ARA) derived from omega 6 is responsible for the plasticity of the hippocampus (89) and together with linoleic acid promotes brain development, neurotransmission, and normal functions of the cell membranes of the central nervous system (CNS); as well as HMO, in view of the fact that when fermented in the intestine they induce a regulation in brain signaling and inflammation that can generate some irreparable lesion (48,53,71).

Palmitic acid, although it is not a polyunsaturated fatty acid, is part of the lipids that play an essential role in the transport of proteins throughout the nervous system by a process called palmitoylation (98).

The main source of energy in the brain is attributed to glucose, which is obtained from various carbohydrates, and the normal processes of synapses and nerve impulses depend on its constant supply (89).

One of the oligosaccharides in human milk is 2'-fucosillactose (2'FL) and together with a metabolic resultant of them, sialic acid, which participates in the synthesis of gangliosides and glycoproteins to which important roles in neural growth are attributed, have evidenced notable improvements in later ages in memory, attention, and cognitive results (52,53,97). However, for these processes to occur, brain maturation must be properly established (53).

A large group of diverse metabolites stimulates the above-described, such as creatine, betaine, choline, lactoferrin, osteopontin, fat globules, leucine, isoleucine, and valine. Any error in the metabolism of these triggers direct neurological disorders. It should be noted that the amino acids, together with others such as methionine, participate in the regulation of the sleep-wake cycle in infants (22,36,67,99). While taurine allows a balance in terms of neural cell volume (41,100). The organization and distribution of the nervous system (NS) are modulated by the brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), S100B, and neurotropin (NT-3) which are part of the growth factors and are highlighted as they help vascularization, maintenance and development (101-104). Vitamin D, although not part of the growth factors, its deficiency may cause behavioral and learning disorders later, because it directly induces the activation of nerve growth factors and, additionally, promotes neurite outgrowth and protects hippocampal neurons from undergoing apoptosis (89).

There is much evidence correlating breast milk metabolites and their influence on the growth, development, and health of the infant, but it is even more important to evaluate whether there are maternal factors or conditions that may alter this composition and at the same time its benefits.BF in gestational diabetes mellitus (GDM)

GDM is a pathology defined as "carbohydrate intolerance that is discovered or manifests itself for the first-time during gestation" (12). Metabolic diseases condition maternal metabolism, which could affect the quality of breast milk during the lactation period since it is made from maternal substrates (9,39,105). GDM brings sequelae to both the mother and the newborn, which consequently can alter the composition of human milk and the effects of lactation such as delayed lactogenesis (103).

Some of the metabolites that are affected in mature milk will be described below, considering that 17 metabolites are altered at this stage of the 28 in total that are altered throughout lactation because of GDM (10).

Although energy does not correspond to a single metabolite, but is the contribution of macronutrients comprised in breast milk, according to Shapira and collaborators, the energy content in maternal mature milk was shown to be higher in healthy mothers when compared to mothers with GDM. While another study conducted in 2017 mentioned that in women with high pregestational BMI and GDM presented increased concentrations of fat and energy levels (27,106,107). It is speculated that the hypoglycemic diets that mothers with GDM undergo may be related to the decrease in energy content in this group, however, the mechanism has not been defined and the correlation is not established (106).

Regarding protein, it should be clarified that the literature is not yet so specific. However, Dritsakou and collaborators made significant findings regarding reduced levels of protein in mature milk in mothers with a controlled diet in GDM (107). When evaluating the quantity and concentration of lactoferrin and IgA in the milk of these mothers, it was demonstrated that GDM did not affect these metabolites, therefore, there were no changes, even if they were insulin dependent. It should be clarified that when a survey of the studies was made, no significant changes were found in terms of protein in mature breast milk, although it is important to report that GDM generates modifications in the proteins of human colostrum (103).

Six of the lipids suspended in the breast milk of mothers with GDM showed alterations. Five of them decreased in quantity, being 1 a saturated fatty acid (pimelic acid) and 4 unsaturated fatty acids (9-heptadecanoic acid, 10-pentadecanoic acid, 2-hydroxyglutaramic acid, and nervonic acid), while only one of saturated character increases its concentration (lignoseric acid)

(10). Lipid changes are considered significant in the study of Dritsakou et al. and Shapira et al. in terms of increased concentrations (106,107).

It has been shown that infants breastfed with milk from a mother with GDM lead to excessive weight gain at one year of life, directly related to the high lipid content. It is for this reason that the importance of an adequate intake of breast milk from mothers with GDM together with specialized and modified formula milk especially for these infants has been described to optimize results in body composition in later stages of lactation (27). The carbohydrate content is modified since 3 metabolites corresponding to this group are increased, they are iditol, galactitol, and sorbitol (27). In contrast, the study "The effect of gestational diabetes mellitus on human milk macronutrient content" found a lower concentration of all saccharides in the mature milk of GDM.In parallel, the study "The impact of maternal- and neonatal-associated factors on human milk's macronutrients and energy" reports no evidence of significant changes in these (106,107). In general, studies found no modifications in total carbohydrate levels between milk from healthy mothers and mothers with GDM, including insulin-dependent mothers (103). As for the hormones in the mature milk of mothers with this pathology, the variability found in insulin was related to an increase, especially in those who received exogenous insulin doses, while adiponectin was decreased, but in a specific period of time (day 90 of mature milk supply) (108). The hormone responsible for heat production in the neonate and for the conversion of white adipose tissue to brown adipose tissue is irisin, which according to Fatima and coworkers, is reduced in the BF of mothers with this disease (109).

Metabolites related to anti-infectious activity such as quemerin and dermicin, which stimulate the action of macrophages in inflammation, show higher amounts in the milk of mothers with GDM than in healthy mothers. Their importance in the prevention of diabetes in the offspring is highlighted (110).

The metabolomic profile of breast milk in GDM is varied and very broad; however, there are no recent studies that confirm the changes and associations of this metabolic alteration of the mother with the metabolites in the milk produced postpartum, especially in the mature milk stage. Although there is evidence of an incidence of alteration of the microbiota that can modify this profile (111,112).

## Conclusion

As a result of an exhaustive and detailed search of recent studies on breast milk and the effects on the metabolome that it can suffer due to a metabolic alteration such as

GDM, it is concluded that breast milk continues to be the gold standard of nutrition in the first months of life, which is constantly ratified in research on the benefits in growth, development, and health of the neonate and in later stages due to its specialized metabolite content.

Perinatal physiology prepares the mother to respond to nutritional needs after birth, which will be satisfied through breastfeeding regardless of a pathological condition such as GDM. In short, the mammary gland and maternal metabolism generate adaptations to provide the infant with all the necessary metabolites without significant changes that could demonstrate alterations in the growth and development of the offspring of mothers with GDM. Despite this, there is no clear evidence to certify that GDM breast milk induces the development of metabolic diseases in infants at future ages.

In view of the above, more clinical trials are needed to corroborate in a broad and deep way the variations in the metabolome of breast milk in this pathological condition, its relationship with being a conditioning factor for the development of future metabolic diseases in the newborn and whether they are influenced by external factors, mainly the mother's diet, which has heterogeneous characteristics such as culture, geographic region, socioeconomic situation and other social determinants; In addition, to inquire about the metabolism and adaptations of the mammary gland that responds to the needs of the infant even in this pathological condition.

## *Acknowledgments*

The authors would like to thank the Universidad Libre for funding the project, the research group ADN (Food, Diet and Nutrition) for their academic contributions, and the Universidad Nacional Abierta y a Distancia for their collaboration in co-authorship.

## *Funding*

This work was funded by the Universidad Libre through project Code 00522, entitled "Analysis of metabolomic data in breast milk from healthy mothers and mothers with diabetes".

## *Conflict of interest*

The authors declare that they have no conflict of interest.

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