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The breastmilk as guardian of an immune treasure

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ABSTRACT

Breast milk is the most complete food for feeding neonates. In addition to its nutritional richness, it has multiple immune components that will participate both in the passive and active responses of the newborn and in the proper development and maturation of the immune system. Milk banks are institutions for collection, processing, and distribution of breast milk voluntarily donated to be used in cases where newborns cannot be fed by their own mother, at least during hospitalization period. This review aims to highlight the benefits of breast milk on the nutrition and maturation and functioning of the infant's immune system and therefore highlight the role of milk banks. The pasteurization process produces a decrease in the milk immune components, which is why these are kept in lower concentrations and still fulfill their functions in the newborn's immune system. The effect of this process on the cells of breast milk in newborns are not yet known.

Keywords: Breast milk, pasteurized human milk, human milk composition.

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INTRODUCTION

Breastfeeding is the process of feeding the newborn through the maternal breast, which strengthens the mother-child bond (1–4). It offers benefits for both. For the mother, puerperal bleeding, the risk of developing breast cancer, the incidence of postpartum depression, obesity and metabolic syndrome are decreased. For the child, it is a source of nutrients and reduces the risk of digestive and respiratory infections, reduces the risk of developing obesity, asthma and other allergies (5). Breast milk is the most complete and ideal food for the newborn during the first 6 months of life (6,7). Therefore, exclusive breastfeeding at libitum it is recommended for the newborn in the world (5) and in Cuba (7). Afterward, it is supplemented with other foods, but it continues to be an important source of nutrients and immune components during the first two years of life. Breast milk, in addition to nutrients and immune components, contains hormones such as melatonin, which regulates the circadian rhythm and is an antioxidant substance (8).

Breastfeeding is a complex process that involves the psyche and the nervous, endocrine, and immune systems, referred to as psiconeuroendocrineimmunology suprasystem (9). To produce milk, it is essential that the mother wishes to breastfeed her child and is psychologically prepared for it. Aspect where the mother's age, school level and commitment to the newborn, among others, influence the milk production (10). Prolactin (PRL), secreted by the anterior pituitary gland, is the main hormone that stimulates milk production. Its secretion is stimulated from the nerve impulses generated in the breast by the suckling of the baby that stimulate the hypothalamus, which, in turn, induces an increase in PRL synthesis (9). The lactic characteristics change according to the development of the baby and three types of milk are described: colostrum; transitional, and mature one (11). Colostrum contains more lymphocyte, proteins, macrophages, neutrophils, oligosaccharides, fat soluble vitamins, carotenes, minerals (such as sodium and zinc), maternal commensals (stimulate the mucous immune system), immunomodulators (glutathione peroxidase, cytokines such as TGF-β and IL-10). However, it contains less lactose, fat, and water-soluble vitamins than mature milk. This allows the innate and acquired protection of the child (12,13).

Transitional milk varies its composition until it reaches the characteristics of mature milk (11). Mature milk has a variable composition. Variations occur between individuals, between the breasts of the same mother, during the day, during the same feeding, and at different stages of lactation. These variations are not random, but functional and it is increasingly clear that they are directly related to the child's needs (11). However, despite the benefit of breastfeeding and the many programs in this regard, the number of women who breastfeed has not been increasing as expected. This is a fact both worldwide and in Cuba. Fact that hinders the proper maturation of the immune system and, therefore, favors the appearance of respiratory, digestive, and allergic diseases, among others. Breast milk banks are institutions that store and process breast milk from healthy donor mothers to meet the needs of newborns who for different reasons cannot be fed by their mothers. During the pasteurization process, the milk decreases the enzyme and antibody concentrations. This decrease can be significant (14).

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This review aims to highlight the benefits of breast milk on nutrition and maturation and functioning of the infant's immune system and therefore, highlight the role of milk banks and prevention of multiples diseases and alterations of immune's system.

METHODS

A search of the literature was performed in the following databases: Pubmed, SciencDirect, and LILACS. The search strategy was according by date posted without limit. The languages were Spanish, English, or Portuguese. Phrases such as "breast milk", "breast milk composition", "human milk banks" and their counterparts in Spanish and Portuguese were sought. Publications from the last 10 years and others that contain important content for this review were selected.

Historical background on breastfeeding and human milk banks

Breast milk results from an evolutionary process estimated to take more than 200 million years. It has been shown that the primitive mammary gland arose in terrestrial ancestors of mammals, and it has been speculated that the secretion of these primitive glands was gaining in complexity (15). The mammary glands of mammals derive from the ectoderm and are in the so-called "milk line", on the anterior aspect of the thorax or abdomen. Human milk is the food offered by nature for humans and other mammalians, also called white blood (16). In the world, from the 18th century, events occurred that were both for and against breastfeeding (4,6,15–17) (Tab. 1).

Since ancient Egypt, wet nurses were hired to feed infants whose mothers did not breastfeed due to illness, abandonment, death or because of their social status, so since that time the donation of milk was a common practice (17,18). For this reason, banks of milk from the 20th century. At present, Brazil has the largest network of milk banks (19) (Tab. 1)

Due to human immunodeficient virus (HIV) many of the milk banks were closed. There are several viruses that, like HIV, are transmitted through breast milk (herpes simplex, cytomegalovirus, rubella, hepatitis B, etc.) for which alternative techniques analogous to those used in blood banks have been sought to eliminate pathogens such as ultraviolet filters (17,18,20).

Breastfeeding

Breastfeeding term is used to denote the feeding to the neonate/infant through the mother's breast (2,16). According to the administration of other foods together, it can be classified in exclusive, almost exclusive or partial/mixt (Fig. 1) (6,16).

The milk production process mediated by the action of oxytocin and prolactin is called galactopoiesis. The suction of the child generates nerve impulses to the hypothalamus where the production of oxytocin and prolactin releasing factor are stimulated.

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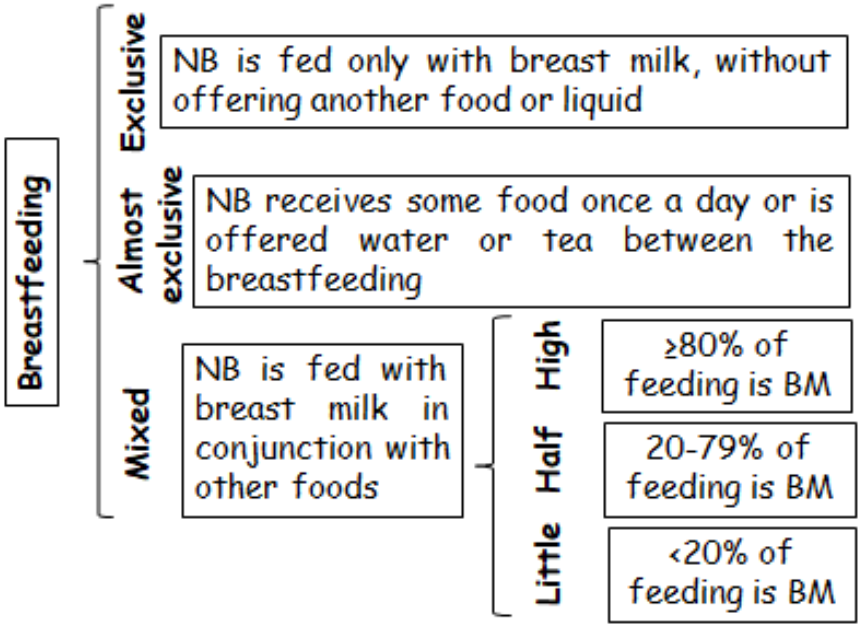
Tab.1. Historical breastfeeding and milk bank worldwide. Ref, reference; BC, before Chris; GNBHM, Global network of human milk banks

Date	Breastfeeding	Ref	Date	Milk bank	Ref
1794	First evidences of the benefits of milk	16	**2250 BC**	Hammurabi code shows characteristics of a good nurse	19
1rd half 20th century	Technological development. Emergence of milk substitutes. Decrease breastfeeding	16	**1909**	Vienna	18
			1910	Boston	18
			1912	Germany	18
			1943	Brazil	19
From the 2nd half of the 20th century	Beginning of actions in favor of breastfeeding Mother-child friendly hospital initiative	4,16	**2000`s**	Cuba (2 milk banc)	42
			At present	GNBHM, 23 countries Cuba have more than 10 milk banks	19,42

Fig.1. Classification of Breastfeeding. NB, newborn; BM, breast milk



Prolactin is released in the anterior pituitary and acts on the milk-producing cells, while oxytocin acts by causing the contraction of myoepithelial cells and the ejection of milk through the alveoli, ducts and milk sinuses towards the nipple (oxytocin reflex). On the other hand, prolactin is inhibited by dopamine. Its secretion is a consequence of the balance between prolactin and releasing factor. (Fig. 3) (9,16).

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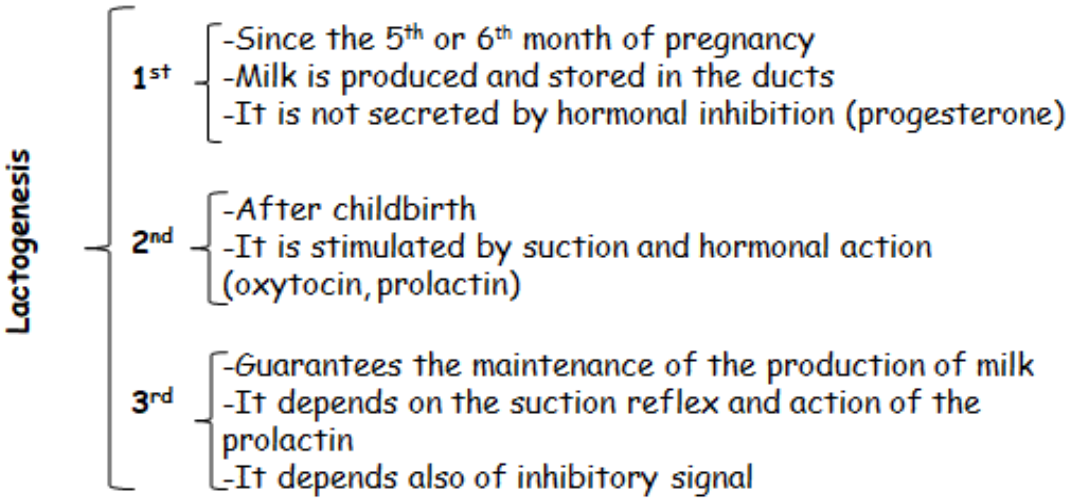
Milk production or lactogenesis consists of three stages (Fig. 2) (4,5).

Fig. 2. Stages of Lactogenesis

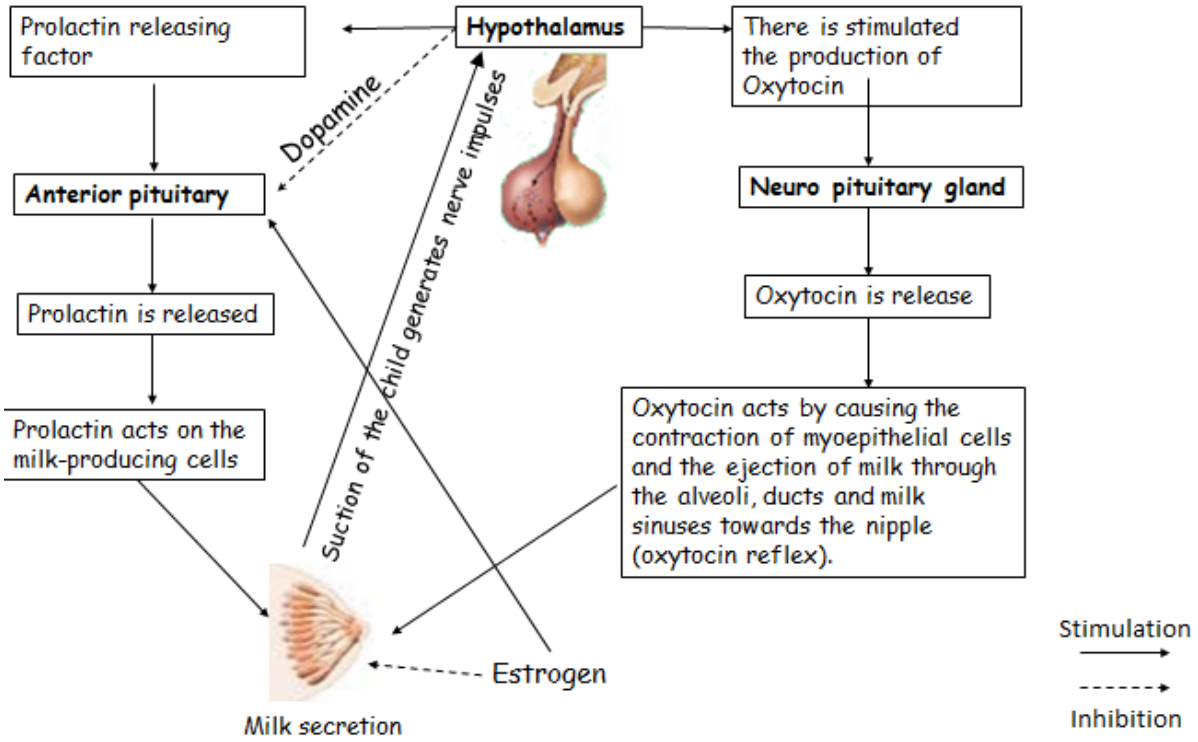


Breastmilk

Breast milk is adapted to the needs of the newborn so its composition varies according to the age or special needs of the infant and the time of day that it is offered (11,14,21).

Breast milk is easily digested and assimilated by the newborn. It consists of nutritional and immunological elements that contribute to its growth and development (17). The immunological components of milk replace the neonate's own components in functions until it can produce them by itself (22).

Fig. 3. Maternal milk production



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Breast milk has multiple antioxidant properties that reduce the oxidative stress generated in neonates at birth. Antioxidants may act synergistically. These include vitamins (ascorbic acid, retinol, etc.), minerals (copper, selenium, zinc, etc.), antioxidant enzymes (glutathione peroxidase, superoxydodismutase), and non-enzymatic antioxidants (glutathione) (12).

According to the nutritional characteristics, 4 types of breast milk are defined (Fig. 4) (5,11,13,14,17,23–25)

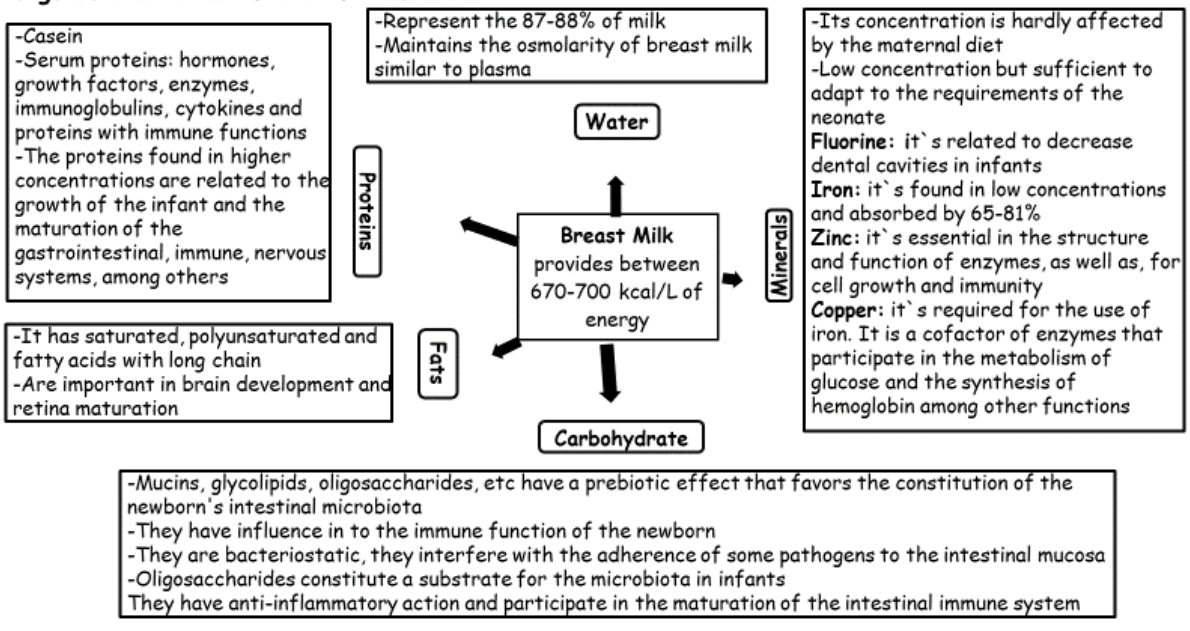
Fig. 4. Classification of breast milk

Colostrum	Transition Milk	Mature Milk	Preterm Milk
• 3-7 days postpartum • High density, thick, yellowish and salty • Volume: 2-20 mL/day reaches 580 mL/day on the 6th day • High content of proteins, fat-soluble vitamins, carotenes and minerals • High concentration of lactoferrin, IgA and oligosaccharides • Approximately 100,000 cells /mL	• After colostrum, 5-10 days • Volume: 600 to 700 mL/day • Daily variations of the components until reaching the characteristics of mature milk • Fat, lactose and water-soluble vitamins increase their concentration • Proteins decrease their concentration progressively	• It is produced from 15 days postpartum • Volume: from 750 to 1200 mL/day and neutral pH • Variable quantity of elements • Functional variations related to the needs of the newborn	• It occurs approximately during the first month postpartum • Produced by women with delivery before 37 weeks of gestation • High concentration of proteins and immune components • Variations in the components of preterm milk depend on the degree of prematurity of the newborn and are adapted to the needs of the newborn

Composition of breast milk

The composition of breast milk undergoes variations both within lactation, during the day and even presents differences in the composition between both breasts, between different women and within a population (26,27). It has multiple components: proteins, carbohydrates, fats, cells, water, vitamins, among others (Fig. 5) (5,11–13,15,21,23,25,27–30).

Fig. 5. Nutritional facts of Human Milk



Immunoglobulins

- IgM: it is found in breast milk in concentrations between 0.3 and 0.9 mg/mL with higher concentrations in colostrum (2.5-3.2 mg/mL). It has a lower affinity for antigen than IgG. It is important in the humoral response and for its shape it is capable of activating complement and phagocytosis. Neutralizes large amounts of viruses and is effective against Gram-negative bacteria (16).
- IgG: its concentration in colostrum is 0.43 mg/mL and in mature milk 0.04 mg/mL[12]. It is vital in the humoral immune response. It is capable of activating complement, participates in antibody-dependent cellular cytotoxicity events, and acts as an opsonin to facilitate phagocytosis (16).
- IgA: colostrum has an approximate concentration of 17.4 mg/mL and in mature milk the concentration is 1 mg/mL (11). It constitutes 90% of the immunoglobulins, being the most abundant one in breast milk. It is the most important in mucosal immunity. It has two subclasses IgA_1 and IgA_2 and the latter has a greater resistance to enzymatic degradation (14,15). It does not activate classical complement system, at least as monomers. At mucosa it has at least three levels of action: it prevents the penetration of antigens into the wall of the intestine, contributes to the elimination of immune complexes and prevents the adherence to the mucosa of some microorganisms (31,32). In breast milk it predominates as secretory IgA (IgAS). The secretory component (SC) plays an important role in the elimination of different microorganisms in the mucosa (16,33). IgA in breast milk is produced by the broncho-entero-mammary axis. This immunoglobulin is produced in response to the antigens with which the mother has come into contact (34). It is also resistant to acid pH and proteolysis (11,17). IgAS occurs as a polymer made up of two or three IgA monomers. Each IgA molecule is made up of two light chains and two heavy chains, a connecting segment (Chain J), and a SC. This SC is a highly glycosylated protein that stabilizes the polymeric structure of IgAS and it also has direct antibacterial action (14,32,35). *In vitro* IgA has antibacterial action (*Escherichia coli, Salmonella, Clostridium tetani, Shigella, Streptococcus, Haemophilus influenzae,* among others), antiviral action (*Coxsackie, Herpes simplex, Poliovirus, Cytomegalovirus, mumps, Rubella, Rotavirus, influenza,* among others), and antiparasitic action (*Entamoeba histolytica, Giardia lamblia,* among others) (11). IgA can modify the composition, gene expression and metabolic activity of the microbiota through binding to bacteria in the intestinal lumen (29).

Currently, in the context of Covid-19 pandemic, IgM, IgG and IgA antibodies with cross-reactivity against SARS-Cov-2 have been found in breast milk of women who have not had the disease. This reactivity is higher in IgA molecules than in the rest (36). In women who have suffered from Covid-19, IgA has been detected against different antigens of the virus, especially against the receptor binding domain (RBD) (36,37). The IgA against SARS-Cov-2 present in the human milk have a great capacity of neutralization o the virus (36)

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Proteins with innate functions

1. Lactoferrin. It is an iron-binding glycoprotein (22) with bacteriostatic action on some ferrodependent germs (eg. *E. coli)* and help with the absorption of iron in the intestine of the newborn (11). It has been associated with decreased free radical production (12).
2. Lysozyme. It has bacteriolytic action against *Enterobacteriaceae* and Gram-positive bacteria. Supports the maintenance of the infant's intestinal flora and has anti-inflammatory properties (11). It is a heat stable protein and is resistant to stomach acid (14,23).
3. Enzymes. Milk contains many enzymes such as trypsin, chymotrypsin, plasmin, elastase, cathepsin D, pepsin, melatonin, among others. These enzymes participate in the digestion of the milk's own proteins (15).
4. Antimicrobial peptides. Many of them are encrypted in the structure of milk proteins and are released through enzymatic hydrolysis. Peptides derived from the β-Casein`s hydrolysis have high antimicrobial activity. Val-Pro-Pro and Ile-Pro-Pro are inhibitors of the angiotensin-converting enzyme and therefore regulate blood pressure. κ-Casein-derived peptides have an antithrombotic activity by inhibiting platelet aggregation. Some of them stimulate phagocytosis and proliferation of T lymphocytes and natural killer cells. In addition, they regulate gastric emptying and modulate intestinal peristalsis (15). Some peptides can act by activating the genes of the antioxidant system in the cells of the intestinal epithelium that protect the intestine from the action of free radicals (12).
5. Cytokines, chemokines, and receptors. Newborns have a limited ability to synthesize some cytokines, the main source of which is breast milk (38). Cytokines present in breast milk could stimulate the newborn's immune system (39). These cytokines have immunostimulatory and immunoregulatory functions (40). Breast milk is an important source of anti-inflammatory cytokines (TGF-β and IL-10), which are important in the prevention of inflammatory bowel disease (39). In addition, there are low concentrations of pro-inflammatory cytokines, which, together with TGF-β, can prevent allergic diseases in neonates (40). Breast milk also contains chemokines that attract immune cells to the site of inflammation (16) and colony stimulating factors that stimulate the growth and differentiation of immune cells(38).

Other components

- Hormones. Many hormones are found in milk in higher concentrations than in maternal plasma (gonadotropin-releasing hormone, insulin, somatostatin, neurotensin, calcitonin, and relaxin) and others in lower concentrations (stimulating thyroid hormone, thyroxine, triiodothyronine, erythropoietin, etc.) (11,13).
- Cells of human milk. A heterogeneous cell population has been described in human milk that includes leukocytes, epithelial cells, hematopoietic progenitor cells, and lactocytes. Little is known about the origin and function of each of these cells (24,41). Recent studies have shown that around 6% of the cells in breast milk are progenitor cells and mesenchymal stem cells with high differentiation potential have been isolated to different

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tissues. These cells are suspected of being of crucial importance in the growth of immune cells in the neonate, ensuring adequate maturation of the immune system and the protection of the infant (40).

Studies in animals have shown that maternal immune cells contained in breast milk are resistant to passage through the baby's gastrointestinal tract and from the intestine pass into the blood and secondary immune tissues of the infant (41). Maternal cells have the ability to participate in the processes of active immunity of the infant, although it is unknown how exactly the maturation of the immune system and the gastrointestinal tract of the neonate (41). Maternal major histocompatibility complex (MHC) molecules are also presented to the neonate as cells pass into the neonate's body. It is still unknown if this generates a defensive immune response in the newborn or if it generates tolerance.

- Microbiota. Milk also contains probiotic microorganisms among as *Lactobacillus, Lactococcus, Enterococcus,* among other species. It is suggested that the most common route of arrival of these bacteria to milk is the entero-mammary route, through a process that involves dendritic cells and $CD18^{+}$ cells that capture non-pathogenic bacteria from the intestine and transfer them to the mammary gland (29,41). Lastly the transfer to fetus of microbiota since the second trimester have been shown (42).

Milk banks: they are specialized institutions where milk donors are selected, milk processing is carried out following quality and safety standards. Milk is also stored and dispensed. In addition, these institutions promote breastfeeding and educate women regarding the feeding of newborns (14,17,18,20,43).

The workflow in banks can be summarized as (44):

- Donor Selection: to choose donors it is necessary to know the obstetric history of the possible donor. As well as your personal history and the results of the serological tests for HIV, hepatitis B, among others (14).
- Milk extraction: at the time of extraction, all hygienic measures must be followed, both for the donor and for the collection utensils. Milk can be expressed manually or mechanically (14,17). Milk can be donated in milk banks, in collection areas of the health area or at home (44).
- Milk classification: after collection, the milk is classified as preterm or term milk, depending on whether the donor's delivery was at term or preterm. It is also classified as colostrum, transitional or mature according to the time elapsed since delivery (14).
- Storage: after collection, the milk should be stored in refrigeration at 0° if it is collected at home or at a temperature lower than -18° in the samples stored in the milk banks. It should be stored for a period of less than 13 days before pasteurization (44).
- Milk selection: it is carried out following three aspects:

  1-Physical characteristics: the color of the milk, the smell, taste, and the hygiene of the container are evaluated. Assessment of the presence of physical contamination (14,44);

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2-Acidity by the Dornic method: it is used as a quality control. It is a measure of contamination by microorganisms (14). It is the main control used by the GNBHM for milk selection. This network is a world reference for the operation of milk banks (17). Normal acidity should be approximately 4.79 ± 1.75(44); and

3-Crematocrit: it is the proportion of cream and is used to specify the calories of milk in 100 mL (14,17,44).

- Pasteurization: it is carried out by different methods (Pretoria Pasteurization, Flash-heat, High pasteurization (HTLT), Holder pasteurization) for the elimination of microorganisms. Pasteurization produces a decrease in different components of milk (14,17,34). Among the elements that can be affected are the proteins of the immune system (lysozyme, IgAS, cytokines, etc.). It is presumed that the bactericidal capacity of milk after pasteurization is diminished, although there are not many studies to verify this. The antioxidant capacity is also diminished after pasteurization (12).

It has been shown that in some cases oral tolerance has been induced in patients with organ transplants (45). That's why, as has been expressed, the pasteurization process reduces the concentration of different milk components, especially proteins. Until now, the effect of pasteurization on human milk cells and whether MHC molecules are presented in the context of the cell or as an independent protein to neonates have not been addressed. It is also unknown whether the response it generates against them is defensive or tolerogenic.

Lastly, it is important to follow the kinetic of arrival of human bank milk to the host newborn. It is preferable to donate to newborn from only one breastmilk or several ones. This aspect is no been addressed. So, if we administered several halogenic milks to the same newborn probably is more dangerous than only from only one mother.

Conclusions

Breast milk has many nutritional components, microbiota, and both innate and adaptive immune system that also participate in its maturation and function. Despite the decrease in several components due to pasteurization methods, the milk donated to milk banks preserves many of their components, even in a low concentration. For this reason, it continues to be the best option, at least during hospitalization, compared to artificial breastfeeding in those cases where newborns cannot be fed by their own mothers.

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