medela

Research review
Human milk and breastfeeding logistics

The logistics of providing human milk in the NICU can be complex. This review describes the current evidence for optimising the logistical pathway in the NICU, aiming to maximise quantity and quality of human milk available to the preterm infant.

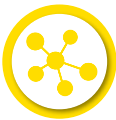
Medela: Comprehensive solutions for human milk and breastfeeding

For more than 50 years Medela has strived to enhance mother and baby health through the life-giving benefits of breastmilk. During this time, the company has focussed on understanding mothers' needs and infants' behaviour. The health of both mothers and their infants during the precious breastfeeding period is at the centre of all activities. Medela continues to support exploratory research into human milk and breastfeeding, and incorporates the outcomes into innovative breastfeeding solutions.

Through new discoveries surrounding the components of human milk, the anatomy of the lactating breast and how the infant removes milk from the breast, Medela has developed a set of solutions to support Neonatal Intensive Care Units (NICUs) in providing human milk and improving breastfeeding.

Medela understands the challenges of providing human milk in the NICU. There are challenges from the mother's side to reach an adequate milk supply and from the infant's side to ingest the milk; plus there are issues of hygiene and logistics when meeting these challenges. The portfolio Medela offers is directed towards obtaining human milk, promoting human milk feeding, and supporting all infants in achieving breastfeeding as early as possible.

Medela aims to provide the most recent, evidence-based knowledge to support breastfeeding and human milk use in the NICU. The goal of the innovative, research-based products, together with the educational materials, is to overcome the difficulties associated with human milk provision in the NICU.



Scientific research
Medela strives for excellence in scientific research – an attitude that has enabled the company to develop advanced breastpump and breastmilk feeding technologies. Medela works with experienced medical professionals and seeks collaboration with universities, hospitals and research institutions worldwide.

Products
Helping mothers to express milk is Medela's core competency. This includes careful and hygienic collecting of breastmilk in BPA-free containers. Easy solutions for labelling, storing, transporting, warming and thawing – all help to safely manage precious human milk. And for human milk to reach the infant, Medela has developed a range of innovative products for different feeding situations.

Education
Within Medela, research and education are closely linked. Medela connects clinicians and educators in ways that lead to professional growth, exchange of knowledge and interaction with the broader scientific community.

To put available solutions, their functionality and their interaction into the context of the overall hospital processes and evidence-based decision making, Medela has developed a series of research reviews. The reviews are available for NICU processes in which human milk and breastfeeding play a significant role. These include feeding development of the preterm infant, human milk logistics and infection control of human milk.

Human milk and breastfeeding logistics

Abstract

Human milk is critical to the developmental and health outcomes of the preterm infant. When fed directly from the breast, human milk is in its safest and optimal format. However, for many preterm infants breastfeeding is delayed, making expressed human milk feeding a priority in the NICU. In order to provide milk in a form that is closest to fresh milk at the breast, evidence-based practices must be implemented. These include expression protocols that maximise breast drainage; storage and handling practices that minimise the loss of milk components; and fortification procedures that enhance infant nutrition. These practices aim to optimise the entire human milk pathway by maximising both the quality and quantity of human milk in the NICU.

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Introduction

Worldwide, the benefits of breastfeeding are unanimously agreed upon [1–5]. Breastfeeding provides optimal nutrition, immunological protection [6] and enhancement of the mother-infant pair immediately after term birth and therefore is recommended as the sole source of nutrition for the first six months of life [1–4]. However, after preterm birth, breastfeeding is often challenging in the beginning [7]. Imperative development that normally occurs late in gestation is interrupted and, instead, must be accelerated in the post-natal environment. Since the provision of human milk for preterm infants is especially important in the first months of life [1], it is critical that NICU practices optimise the use of human milk.

The NICU plays an important role supporting mothers and infants in the provision of human milk. As a result, the NICU must rely on the most up-to-date, evidence-based practices that ensure that human milk is of sufficient quality, volume and integrity. This research review aims to provide the NICU professional with an in-depth understanding of the current research encompassing the benefits of human milk for preterm infants; interventions that support mothers to initiate, build and maintain their milk supply and the logistical issues that the NICU faces when it comes to the safe collection, handling and feeding of human milk.

The value of breastfeeding and human milk

Breastfeeding not only provides human milk with all the necessary components for optimal growth and development of the infant. It also provides immunological protection [6], and bonding of the mother-infant pair immediately after birth. Due to the profound benefits, human milk is recommended for all term and preterm infants.

Health outcomes of breastfeeeding

Close body contact between the mother and infant during the early post-partum period enhances and regulates the newborn's temperature, respiration, acid-base balance [8], and soothes the infant [9, 10]. During sucking, the close body contact also helps prolong the lactation period, and helps adapt the mother's gastrointestinal tract to meet increased energy demands during lactation [7]. In particular, breastfeeding facilitates a bond between the mother and infant [11]. Oxytocin, released during the milk ejection reflex as a result of infant sucking (Figure 1), increases blood flow to the chest and nipple area, increasing the temperature of the skin and creating a warm and nurturing environment for the infant [11]. Mothers having newborns skin-to-skin immediately after birth spend more time with their babies, interact more with their infants during breastfeeding [12] and breastfeed for longer [13]. Although this scenario is different for mothers of preterm infants, due to physical separation from their infant and other medical issues, skin-to skin contact is still associated with increased milk production, earlier onset of lactation, and improved physiological stability in preterm infants [14–16].

The benefits of breastfeeding extend to the long-term health of both the mother and infant. For the mother, breastfeeding accelerates uterine involution after birth, reduces the risk of haemorrhage, and helps with re-gaining pre-pregnancy weight [17]. In addition, lactation reduces the mothers' risk of ovarian and breast cancer, osteoporosis, type II diabetes, cardiovascular disease and rheumatoid arthritis [1, 18, 19]. For infants, breastfeeding reduces the risk of acute otitis media [19] and promotes normal oral facial growth [20], including improved dentition, perioral and masseter muscle activity and palatal growth [21, 22]. Human milk feeding is further associated with a reduction in the risk of gastrointestinal tract infections, respiratory tract infections, atopic dermatitis, childhood asthma, childhood leukaemia, type I diabetes, obesity, necrotising enterocolitis (NEC) and sudden infant death syndrome (SIDS) [1, 19, 23].

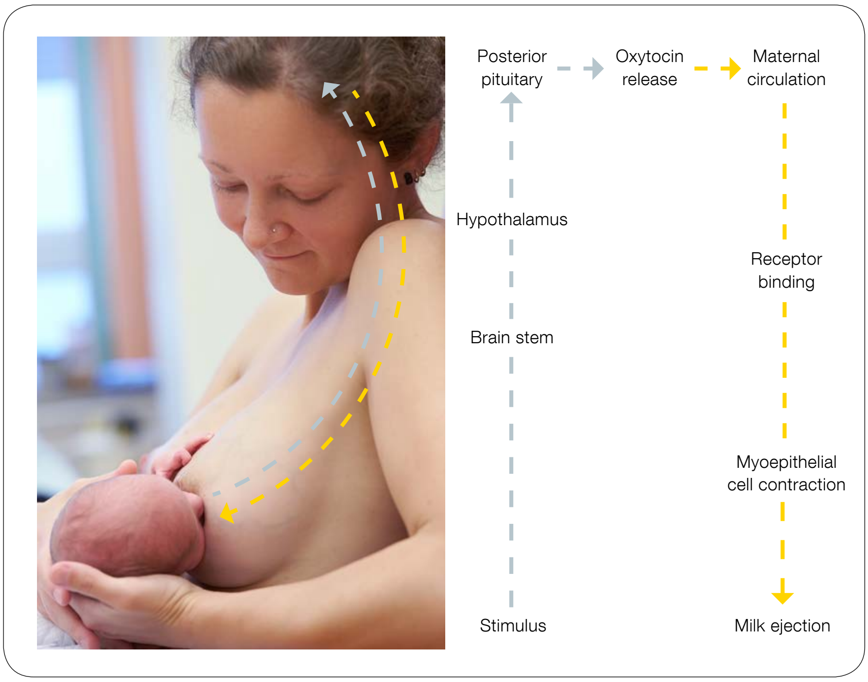

Figure 1 – Milk ejection reflex
In response to a stimulus, oxytocin is released from the posterior pituitary into the maternal circulation. Oxytocin binds to the receptors on the myoepithelial cells surrounding the alveoli. These cells contract and force milk out of the alveoli into the ducts towards the nipple.

Bioactive components of human milk

Human milk provides all the necessary components for optimal growth and development of the infant. This includes the essential macronutrients (fats, carbohydrates and proteins), micronutrients (vitamins and minerals), and developmental factors (long chain polyunsaturated fatty acids (LCPUFA), growth factors and cytokines). Human milk also contains bioactive components that protect the infant against infection and promote gut maturation.

Multifunctional proteins including sIgA, lactoferrin and lysozyme, as well as free fatty acids in human milk, act as anti-infective agents that are essential to the preterm infant [24]. These agents work together to inactivate, destroy or bind to specific microbes, preventing their attachment to mucosal surfaces [25]. Living maternal cells (Figure 2) are transferred through the milk to the infant. These include blood-derived leukocytes, cells of the mammary epithelium, stem cells and cell fragments, which provide immune-protection to the infant [26–28]. A large number of human milk oligossacharides are also transferred to the infant and have been reported to have an important immunological function acting as probiotics that promote the intestinal growth of commensal bacteria [29] (Table 1). They also act as decoys or receptor analogues to inhibit binding of pathogens – including rotaviruses – to intestinal surfaces [30–32]. Human milk also contains commensal bacteria that become part of the gut microflora and influence inflammatory and immunomodulary processes. Not only do commensal bacteria prevent overgrowth of pathogenic bacteria, they also acidify the gut, ferment lactose, breakdown lipids and proteins, and produce vitamins K and biotin [33–35].

In light of the diverse and bioactive nature of human milk, it is important that any processing of human milk should aim to maintain the activity and integrity of these components.

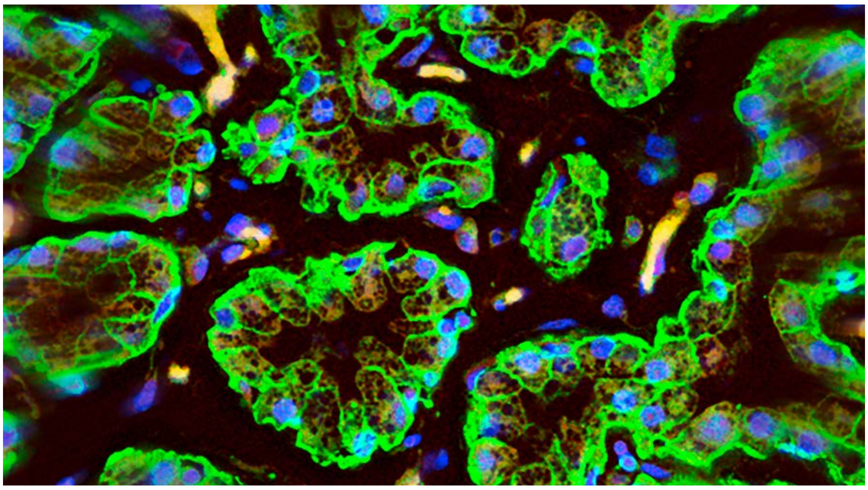

Figure 2 – Example of lactating mammary tissue – A source of stem cells found in human milk.

The milk of a mother who delivers a preterm infant is different from that of a mother who delivers at term. Compared with term milk, preterm milk has higher levels of energy, lipids, protein, nitrogen, some vitamins, and minerals. In addition, preterm milk has higher levels of immune factors, including cells, immunoglobulins, and anti-inflammatory elements [36, 37]. The composition of preterm milk is especially important for gastrointestinal and neurological development and for conferring immunological protection of preterm infants [6]. Although human milk is recommended for all preterm infants [5] the nutritional composition of human milk cannot completely meet the high nutrient demands for preterm infant growth, especially in infants born very low birth-weight (< 1500 g) [37, 38]. Fortification of human milk with protein, nutrients, vitamins and minerals is therefore recommended for all infants born < 1500 g to ensure the best possible growth and developmental outcomes [39].

Table 1 – Bioactive components of milk with overlapping effects on protection against infection and intestinal development of neonates [25]

Function	Component
Compensates for developmental immaturity from the intestine	sIgA, lactoferrin, lysozyme, platelet-activating factor acetylhydrolase, cytokines, enzymes
Assists with development of the immature intestine	nucleotides, oligosaccharides, growth factors
Prevents infection and inflammation	sIgA, lactoferrin, lysozyme, platelet-activating factor acetylhydrolase, cytokines, milk fat globule membrane, oligosaccharides
Promotes establishment of beneficial microbiota	sIgA, lactoferrin, lysozyme, oligosaccharides, α- linoleic acid

Health-care benefits of human milk

Human milk feeding has been shown to reduce the incidence, severity and/or risk of prematurity-related morbidities in a dose response manner, most notably during the first months of life. Research by Patel *et al.* [40] demonstrated that the dose-response relationship between morbidities and average daily dose of human milk (ADDHM) in the NICU is such that with every increase of human milk by 10 mL/kg/day, there was a 19 % decrease in the odds of sepsis. Infants who received the lowest daily dose of human milk (< 25 mL/kg/d ADDHM) not only had the highest risk of sepsis, but also the highest NICU costs (Figure 3). The authors demonstrated that the hospital could save 20,384 USD per infant or a total of 1.2 million USD by increasing their ADDHM to 25–49 mL/kg/d in the first 28 days of life, and by increasing the ADDHM to ≥ 50 mL/kg/d, 31,514 USD per infant and 1.8 million USD in hospital savings could be made.

These cost savings have been replicated with other prematurity-related morbidities. Since human milk feeding significantly reduces the incidence and severity of late-onset sepsis, bronchopulmonary dysplasia, NEC, and retinopathy of prematurity, the incremental costs of these morbidities are also reduced. The incremental direct costs of these morbidities were shown to range from 10,055 USD for late-onset sepsis to 31,565 USD for bronchopulmonary dysplasia during the NICU stay. By reducing both the incidence and severity of these diseases, human milk feeding was shown to indirectly impact the cost of NICU hospitalisation, while also reducing other NICU hospitalisation costs independent of its impact on these diseases. Even though there are some costs to the NICU in providing human milk [41], in terms of logistics, the economic benefits of providing human milk significantly outweigh the relatively low costs to the mother and institution [41].

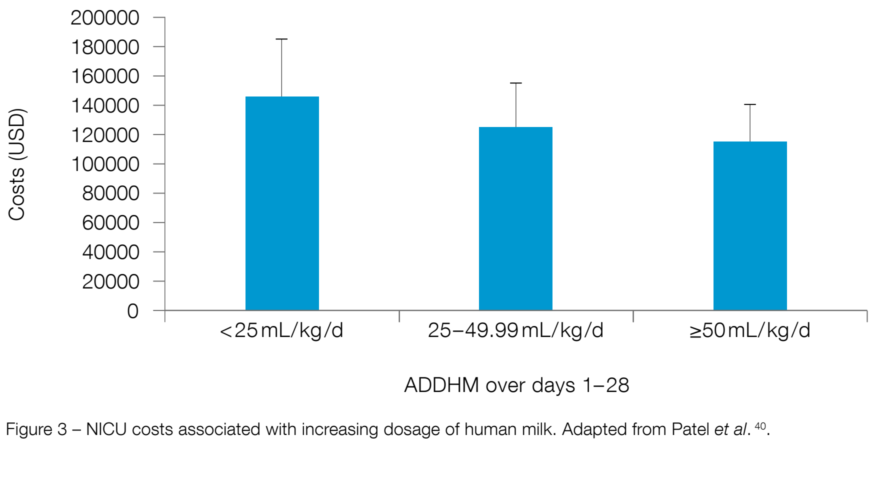

Figure 3 – NICU costs associated with increasing dosage of human milk. Adapted from Patel *et al.* [40].

Human milk pathway in the NICU

Although breastfeeding may be challenging for the preterm infant initially, there is a compelling body of evidence to support human milk feeding as the nutrition for all preterm and hospitalised infants while breastfeeding is being established. Unlike breastfeeding, human milk feeding in the NICU requires multiple levels of processing and preparation. As mothers are encouraged to pump, collect and store their milk for enteral or oral feeding, some of the essential components of milk may be jeopardised. Since the collection, storage and processing of human milk comes with risks of nutrient loss, volume loss and milk contamination [42], efforts must be made to minimise macro and micronutrient loss, while maximising the volume of human milk available to the preterm infant.

Establishing clear protocols for the entire milk pathway is therefore an essential process that begins with utilising evidence-based practices. Maximising the volume of own mother's milk for feeding includes up-to date interventions for initiating, building and maintaining an adequate milk supply. Enhancing NICU practices to maintain milk quality includes hygienic expression and cleaning. Similarly, it is essential to understand the literature behind best-practice guidelines for the safe storage and handling of milk; this may encompass thawing, warming and fortifying for appropriate feeding (Table 2).

Table 2 – Human milk pathway in the NICU and the logistical considerations

Human milk pathway in the NICU		**Logistical considerations**
Expression:	Express at home or in the NICU	I breastpump I breastshields I maximise milk removal I hygienic collection I storage containers
Transport:	Transport from home or storage in the hospital	I cooling I labelling I pooling
Storage:	Room temperature, refrigerated or frozen	I optimal storage times I fortification I pasteurisation
Prepare for feeding:	Thawing and warming	I optimal temperature I water vs non-water devices

Milk pumping

In many mothers of preterm infants, the milk pathway begins with expression to initiate and build lactation. Due to their neurological immaturity, respiratory illness, and other medical complications, preterm infants born less than 34 weeks may be unable to breastfeed initially [43], and must instead rely on expressed human milk. Mothers may experience challenges initiating, building and maintaining lactation due to an immature stage of mammary development, the lack of infant's ability to feed, emotional challenges resulting from preterm delivery, and lack of access to appropriate equipment and timely support [44].

Initiate, build and maintain lactation

Lactogenesis begins with secretory differentiation (formerly termed Lactogenesis I) during pregnancy, when the mammary gland develops the capacity to secrete milk. This includes significant growth of the glandular tissue of the breast and, in the second half of pregnancy, the differentiation of alveolar epithelial cells into milk secreting cells known as lactocytes [45] (Figure 4). The first two weeks post-partum are thought to be critical to the initiation and programming of lactation [46, 47]. In mothers of term infants, milk volume rises rapidly from around 36 hours post-partum. While volumes vary greatly between women, on average they begin at ~50–100 mL/day on day 1, ~500 mL/day by day 5, and ~750–800 mL/day by 1 month post-partum [48, 49]. Pump-dependent mothers, however, are at risk of delayed initiation and have been shown to be 2.81 times more at risk of not producing an adequate volume of milk (less than 500 mL/day) by one month post-partum, and show more varied milk productions compared to term mothers [50]. In addition to this, it has been suggested that milk production of pump-dependent mothers of preterm infants tends to level off between 340–640 mL/day rather than increase over time [50, 51].

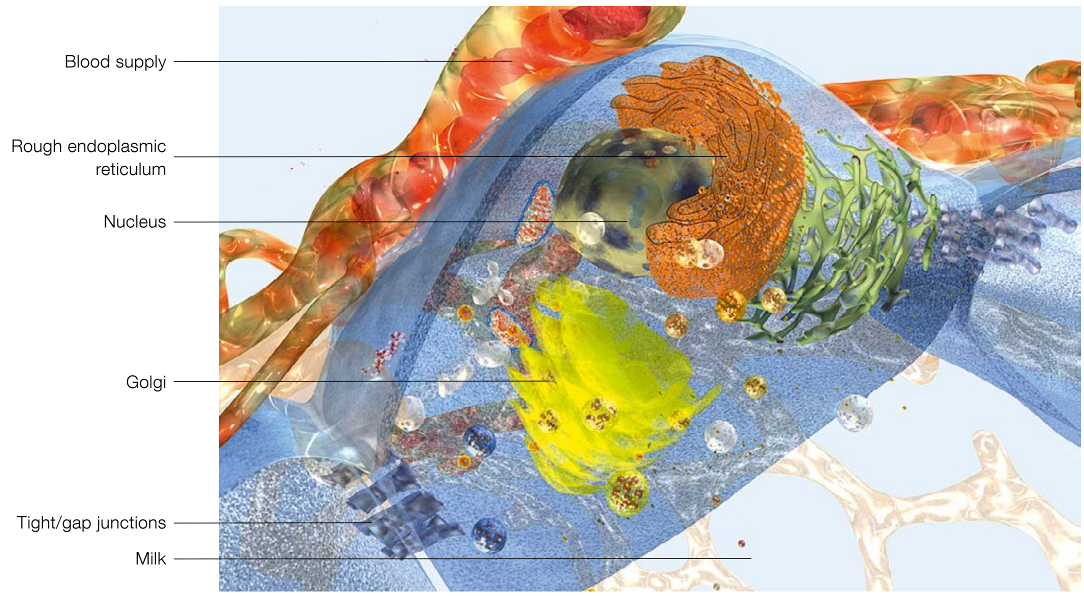

Figure 4 – Components of the milk-secreting lactoctye lining the alveoli

Regular and frequent milk removal *via* breastfeeding or by expression is critical to support a steady increase in milk volume over the first week post-partum. Mothers of term infants show increased milk production when they express after breastfeeding and further drain the breast [52]. Effective drainage of the breast is therefore thought to be essential for pump-dependent mothers to increase milk synthesis and production. Nevertheless, for many mothers of preterm infants, effective removal of milk during this period is particularly difficult and can result in inadequate milk production [38].

Maximising milk production

Assisting mothers *via* early, frequent and efficient pumping significantly improves the timing of secretory activation and milk output after preterm birth [53–55]. Previous data has consistently supported the notion that early pumping improves milk output, classifying early pumping as within the first six hours after preterm birth [53–55]. However, initiating pumping within the first hour after birth has shown even greater improvements in milk production of preterm mothers [56, 57]. The results of a pilot study have shown that mothers who initiated pumping within the first hour of birth (compared to 2–6 hours after birth) have greater total milk output over the first 7 days (1374 vs 608 mL/day), a greater daily production at 3 weeks post-partum (614 vs 267 mL/day), and an earlier time to secretory activation (80 vs 136 hours) [56]. Although these results need to be replicated in a larger study, they highlight the importance of early pumping in pump-dependent mothers.

Pump-dependent mothers who express their milk frequently (more than 6 times a day), have greater milk production at 5 and 6 weeks than mothers who pump less frequently [53, 58]. Increased frequency of daily pumping has also been correlated with an extended lactation of more than 40 weeks in mothers of preterm infants [55]. Although this benefit has been seen with at least 6 pumping sessions a day, general clinical recommendations suggest mothers should pump between 8–10 times per 24 hours [59] to prevent down regulation of milk synthesis [60].

Breastpumps are thought to be more effective when they utilise vacuum patterns that are similar to infant sucking during established breastfeeding. Prior to the first milk ejection, infants have been shown to suck rapidly during breastfeeding. After milk flow, the sucking frequency slows, and the infant applies a stronger level of vacuum to remove milk [61]. Hospital-grade electric pumps that utilise this 2-phase pattern to stimulate the flow and expression of milk, have been shown to be as effective as, and more comfortable than, single phase electric pumps. The 2-phase pattern that was used in this study began with a higher frequency stimulation phase of greater than 100 cycles per minute to elicit a milk ejection and milk flow. Mothers were then required to switch to the expression phase, which consisted of ~60 cycles per minute. Mothers who used this 2-phase pattern at a vacuum level that was considered their maximum comfortable vacuum, demonstrated more effective and efficienct milk removal from the breast compared to mothers using lower vacuum levels [62–64].

More recently, an expression pattern that mimics newborn sucking during the first days of lactation has been incorporated into an electric breastpump. This initiation pattern used until secretory activation, consisted of three phases, varying over fifteen minutes. This included two stimulation phases with frequencies of 120 and 90 cycles per minute, an expression phase with a frequency between 34–54 cycles per minute, and intermittent pauses. Mothers who used this initiation pattern prior to secretory activation, followed by the 2-phase pattern, exhibited a greater daily milk production between days 6–13 post-partum, and an increased milk output per minute spent pumping, compared to mothers using only the 2-phase expression pattern (Figure 5)[65]. In addition, pump-dependent mothers of term infants in the cardiac intensive care unit, have demonstrated adequate milk productions by day 7 post-partum, when using the same initiation pattern[66].

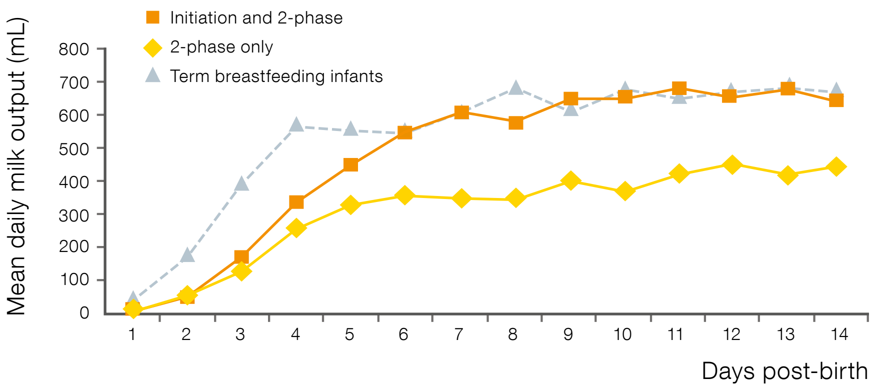

Figure 5 – Randomised controlled trial demonstrating mean daily milk output was significantly higher ($p<0.05$) from days 6–13 when using the initiation pattern followed by 2-phase pattern, compared to the 2-phase pattern alone[65]. This higher output is comparable to reference data of term breastfeeding infants[49].

Although electric breastpumps are recommended for pump-dependent mothers, it is essential that the breastshields used during pumping are the correct fit for each breast[67]. Poorly fitted breastshields may result in incomplete milk removal, nipple trauma and pain[68, 69]. While NICU mothers are often clinically evaluated for shield fit initially, the appropriate size can change more than once over the course of extended breast pumping and therefore different shields may become more appropriate over time[68]. Similarly, the degree of nipple expansion, the amount of breast tissue entering the tunnel, and the extent that the breastshields push into the breast tissue all may compromise milk flow due to compression of the superficial milk ducts[70], however no studies have provided an evidence-based guideline for the appropriate fitting of breastshields.

It is also important that the shield matches the anatomy of the breast and nipple to minimise friction and damage of the nipple and areola tissue against the sides of the tunnel [69, 71, 72]. Clinical indicators of a properly-fitted breastshield include the nipple moving easily in the tunnel, none of the areolar (or only a small amount) being pulled into the tunnel, no blanched, painful or cracked nipples, and the pumping mother feeling comfortable [68]. The use of warm breastshields (39 °C) during electric pumping may also be helpful since they show a faster time to 80 % milk yield than ambient temperature shields. However, no difference in milk output after 15 minutes has been found [73].

Double pumping with electric breastpumps has been consistently demonstrated to be more efficient at removing milk than sequential single pumping. Double pumping results in a greater milk output (Figure 6) in mothers of both preterm [69, 74] and term [75] infants. Mothers have also been shown to have an additional milk ejection during double pumping compared to single pumping, along with a higher caloric content of expressed milk [75]. Other factors that may assist milk production of pump-dependent mothers include pumping at the bedside, or in a more relaxed environment to reduce maternal stress [76]; skin-to-skin contact or kangaroo care, which is associated with increased production and prolonged lactation [14, 15, 77, 78]; non-nutritive sucking at the breast, which is thought to stimulate the release of oxytocin and prolactin and improve milk production; [76] and breast massage during pumping, which is associated with increases in milk volume removed [69, 79] and milk caloric content [80].

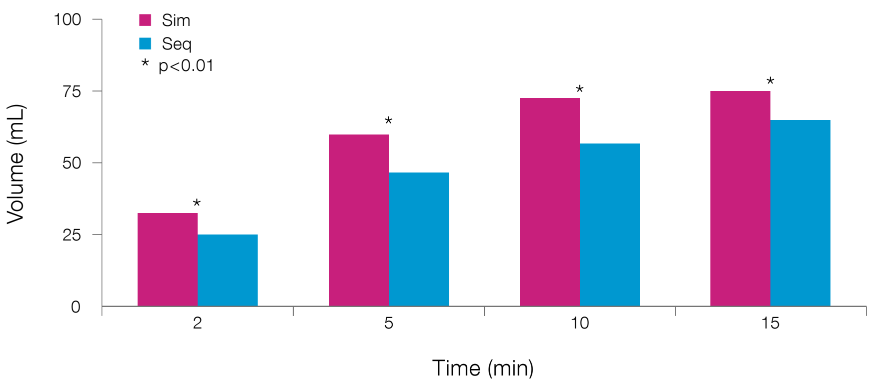

Figure 6 – Double pumping (Sim) results in significantly more milk output at 2, 5, 10 and 15 minutes than sequential single pumping (Seq). Adapted from Prime *et al*. [75].

It is recommended that mothers be taught the skill of hand expression in the early post-partum period [69, 79]. This early one-to-one support usually includes an explanation to mothers of how their breast functions and what to expect. Hand expression as a sole method of expression has shown mixed results in studies assessing mothers of preterm infants. While it has been associated with an increase in colostrum output in the first 2 days post-partum [81], it has also been associated with decreased milk volume output in the first 8 days post-partum compared to expressing with an electric breastpump [82]. Mothers should be informed of the different options available to them for expressing milk.

Hygienic collection practices

Hand washing is the first line of defence in reducing pathogens and bacteria [83]. Pumps, pumpsets and bottles are potential sources of contamination during pumping [84, 85]. Pumpsets usually consist of breastshields and tubing to be used with an electric pump. Tubings exposed to aerosol of milk or water are of concern if they become contaminated with bacteria or mould [42]. In terms of cleaning, mothers can either disinfect pumpsets between uses, or use disposable pumpsets that can be disinfected between uses and disposed of after one day. Discarding the pumpsets after a day's use may also be preferable over autoclaving, since autoclaving is generally expensive and may risk return of incomplete sets [86, 87].

Pooling and tracking expressed milk

Hospitals traditionally store mother's milk in individual containers after each pumping session [42]. However, whether mothers should store their milk individually after every pump session, or pool their milk over 24 hours has been questioned. In particular, pooling milk has been suggested as it has potential to ensure milk is more nutritionally consistent between feedings. Certainly one study has shown that pooling milk over 24 hours results in no differences in bacterial colonisation, as well as a reduced variability in the caloric, protein, fat and carbohydrate content of the milk, compared to individually stored milk, which varied in caloric content up to 29 %. As the nutrient content of individual pump sessions differed significantly from the 24-hour nutrient content, it was suggested that inaccurate nutrient and calorie supplementation may take place. Interestingly, pooling milk also resulted in greater maternal satisfaction than individual collection. Pooling milk may therefore provide the opportunity to tailor fortification and improve nutritional delivery to the infant [88].

Pooling milk also has advantages in terms of labelling only one bottle compared to labelling multiple bottles or containers after every expression. As the NICU must track and store human milk, the handling of human milk may be prone to errors if containers are not labelled appropriately [89]. Appropriate labelling with the patient's name, milk type, date of expression and volume expressed may assist in minimising milk mix-ups. Methods like individual storage boxes for each mother in a freezer or fridge, as well as bar codes (more commonly used with donor milk), may be additionally advantageous [42, 90, 91].

Storage of milk in the NICU

Safe storage of milk in the NICU is essential to ensure optimal nutrition for the infant. Fresh milk contains live maternal cells [28, 92] and the highest amounts of nutrients, growth factors, and many other protective components [25]. Over time and with exposure to varying temperatures, these components decrease in potency while the risk of bacterial contamination and growth of pathogens increases. Fresh human milk is not sterile but rather contains a wide variety of organisms including non-pathogenic bacteria, pathogenic bacteria, viruses, mycobacteria and fungi [93–97]. While quantities of bacteria in human milk vary widely, in general, the majority of identified organisms are non-pathogenic normal skin flora from the mother's nipple or breast, or are organisms that protect the newborn's gastrointestinal system after having migrated to the breast *via* the enteromammary pathway [98].

The effect of storage on the microbiological content, lipid composition, cellular components, anti-bacterial properties and antioxidant capacity has been investigated extensively, however, many factors still remain unknown. Along with changes occurring with time, different problems arise from storage at various temperatures, including room, refrigeration and freezing temperatures.

Room temperature

Milk degradation at room temperature, defined by various studies between 25–38 °C, has been studied over various time frames. One key study asessed milk degradation at 15, 25 and 38 °C, over 24 hours. The authors showed that although proteolysis and digestive enzyme changes were minimal at 15 and 25 °C after 24 hours, lipolysis occurred rapidly within a few hours of storage resulting in a 440–710 % increase in free fatty acid concentration. Similarly, bacterial growth, which was mainly restricted to non-pathogens, was minimal at 15 °C, and remained low at 25 °C for the first 4–8 hours, but increased rapidly after 4 hours at 38 °C. The authors concluded that milk at 15 °C was safe for 24 hours, and at 25 °C for 4 hours [99]. More rigorous methods used to target protein activity in milk have since shown further reductions in ß-casein at 25 °C over 24 hours [100, 101], and reductions in lipase within 2 hours of storage at 25 °C [100]. Optimal storage conditions for room temperature (25 °C) are therefore <4 hours, especially in the NICU [42]. However, for healthy term infants in extremely clean environments up to 6–8 hours is considered acceptable [42] (Table 3).

Refrigeration

Refrigeration temperature, usually defined as 0–4 °C, preserves the integrity of human milk longer than when it is left at room temperature [102]. The most comprehensive study assessing storage at 4 °C suggests the maximum time fresh milk should be stored under refrigeration conditions is 96 hours (4 days) [103]. At 96 hours and 4 °C, fresh refrigerated milk showed no significant changes in osmolality, total and gram-negative bacterial colony counts, macronutrients and immune factors, including fat, sIgA and lactoferrin. In addition, refrigeration has been shown to inhibit gram-positive bacterial growth [104], indicating that the live host defence system in milk prevents contamination [105]. Rises in free fatty acid concentrations and subsequent increases in acidity as a result of lipolysis have also been consistently observed in refrigeration studies [103, 106]. Products of lipolysis, however, are not considered to be of risk as they are associated with antimicrobial activity against bacteria, viruses and protozoa [103, 106–109]. Loss of white cell counts, including macrophages and lymphocytes, as well as total proteins, has been observed at 48 hours [103]. Based on these studies, optimal storage at 4 °C has been suggested at < 4 days, especially for NICU infants [42] with acceptable storage from 5–8 days under very clean conditions for term infants [110] (Table 3).

Freezing

Freezing at –20 °C for up to 3 months has been recommended as optimal in the NICU [42]. At 3 months, vitamins A, E, and B, total protein, fat, enzymes, lactose, zinc, immunoglobulins, lysozyme, and lactoferrin are maintained, although there may be loss of vitamin C after 1 month [111–114]. Bacterial growth is not a significant issue up to 6 weeks [115, 116]. The bactericidal capacity however is generally less than that of fresh milk [117, 118]. Up to 12 months at < –20 °C is considered acceptable in the NICU [42]. Deep freezing at –80 °C, may be more appropriate to maintain the bactericidal capacity of human milk, especially in NICU settings [116]. During freezing, live cell loss, for example the destruction of phagocytes, and changes in taste and smell may occur as lipase continues to break down fat into fatty acids [110]. Re-freezing milk after thawing in the fridge has been shown to maintain a safe bacterial load [119], however milk that has been completely thawed to room temperature has been suggested to be unsafe, and should not be re-frozen [42]. There is limited evidence for appropriate storage times after thawing to rooming temperatures, as well as for the effect on milk quality that various transfers between containers and temperatures have [42]. However, even milk that has been frozen for several months is more beneficial than formula. Refrigerated milk is considered fresh, so it should be used before milk that has been frozen [42].

Table 3 – Human milk storage guidelines for NICU infants. Adapted from HMBANA [42].

Human milk	Optimal storage time
Freshly expressed milk	
Room temperature:	≤ 4 hours [117, 120]
Refrigerator:	≤ 4 days [103]
Freezer:	≤ 3 months. Acceptable ≤ 12 months [111–114]
Previously frozen	
Room temperature:	Thaw to room temperature use within ≤ 4 hours [117, 121]
Refrigerator:	Thaw to fridge, use within ≤ 24 hours
Freezer:	Do not refreeze
Freshly expressed, fortified	
Room temperature:	Do not store at room temperature
Refrigerator:	≤ 24 hours [105, 122–125]
Freezer:	Do not freeze
Previously frozen, fortified or pasteurised	
Room temperature:	Do not store at room temperature
Refrigerator:	≤ 24 hours
Freezer:	Do not refreeze
Warmed towards body temperature	
Room temperature:	For completion of current feed
Refrigerator:	Discard
Freezer:	Discard

Handling

Preparing milk for feeding requires a series of processes including thawing, warming and fortifying. Each process may affect milk composition and increase the risk of contamination.

Thawing and warming milk

Thawing milk is necessary after freezing and is generally performed by leaving the milk in the refrigerator or by gently warming it. Although there are few studies investigating the optimal method to thaw milk, it is well known that pasteurisation (milk heated to 62 °C for 30 minutes) of donor milk results in significant losses to immunological and anti-inflammatory components of milk, including sIgA, lactoferrin and lysozyme, as well as probiotic bacteria and white blood cells. These losses are reduced when pasteurising at lower temperatures [126] (Figure 7).

Milk is often thawed in the NICU by leaving it in the refrigerator, at room temperature, or by being placed in warm water. Microwaving, hot or boiling water are not recommended since these methods destroy the anti-infective properties of milk [127, 128]. Water-based methods, commonly used for both thawing and warming, usually involve placing bottles or containers of milk in water baths, or water-filled containers [42]. However there is a contamination risk with water as it can potentially get under or inside the bottle lid and into the milk [42, 129]. Human milk banking guidelines [42] recommend thawing milk quickly in a water-filled container not exceeding 37 °C, taking care to prevent the water touching the bottle lid. Milk should be thawed until ice crystals remain and placed in the refrigerator. Thawed milk is not recommended to be left at room temperature for more than a few hours to prevent bacterial growth [118].

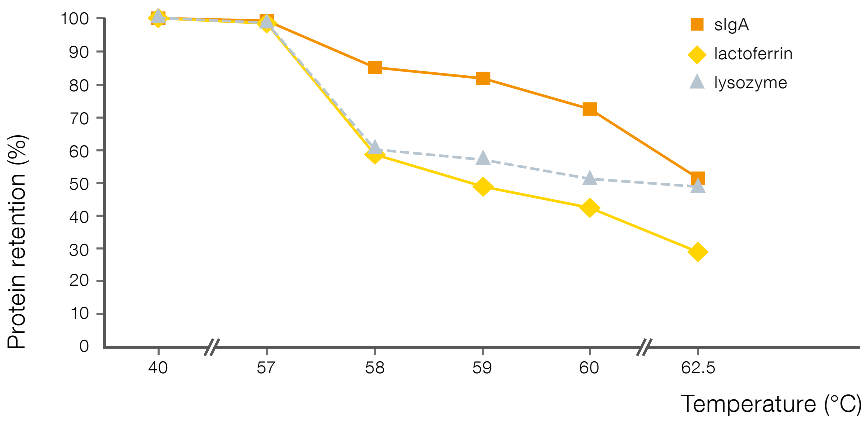

Figure 7 – Calculated retention of proteins: lactoferrin, sIgA and lysozyme after 30 minutes of pasteurising at various temperatures between 40 and 62.5 °C using an experimental pasteuriser. Adapted from Czank *et al.* [126].

Milk temperature may also play a role in assisting the infant's ability to tolerate gavage feeds. It has been hypothesised that milk temperature can influence infant body temperature. Since infant temperature has been shown to decrease when room temperature intravenous fluids are administered, it has therefore been recommended that intravenous fluids, such as blood and saline, are warmed towards body temperature prior to infusion [130, 131]. In many NICUs warming of feeds is considered an important step of the milk pathway. However, a series of studies assessing the effect of milk warming on preterm infant stability and gastric residuals has shown mixed results. Rectal and stomach temperatures have been shown to be lower after room temperature gavage feeds compared to body temperature feeds; however, no differences in metabolic rates have been observed [130–132]. While one study showed preterm infants' axillary temperature increased up to 0.44 °C during warmed feedings, the authors found no changes in heart rate, respiratory rate or oxygen saturation with the increased temperatures [133]. On the other hand, preterm infants who were gavage fed milk at cool temperatures, room temperature, and body temperature, had lower gastric residuals and improved feeding tolerances when receiving milk at body temperature (37 °C), compared to cool temperatures (10 °C); however, the type of feed was not controlled for [134]. Other studies assessing preterm infants have not shown any differences in body temperature, gastric emptying and heart rate between cold, room and body temperature during gavage feeds [135, 136]. While term infants are able to drink milk at cool, room or warmed temperatures [110], the evidence is less clear for preterm infants.

Current recommendations for warming milk highlight that milk should be warmed in a container of warm water or by being held under running warm water, while also keeping the bottle lid dry to avoid water contamination [42]. Regulating and achieving optimal temperature with water-based methods is challenging. Achieving optimal temperature requires consideration of several factors, including milk volume and milk temperature at the beginning of the warming process, the size of the milk container, and water temperature. Water bath temperatures from within one institution have been shown to range from 23.5 to 45.5 °C at the start of warming, and between 23.8 °C to 38.4 °C at the end of warming. Milk temperatures at the time of feeding subsequently showed large variations, ranging from 21.8 °C to 36.2 °C, therefore suggesting that determining when milk is at a desired feeding temperature is often not achieved [133]. In another study, similar variations in water bath temperatures over 419 milk feeds were shown to range from 22 °C to 46 °C, with an average of ~31 °C, highlighting the lack of standardisation in warming practices [137].

Fortifying milk

Human milk is strongly recommended for enteral feeding and all oral feeding in the NICU. However, whether fresh or frozen, it often requires fortification with protein, nutrients, vitamins and minerals in order to meet the high nutrient demands for preterm infant growth. Micro- and macronutrients, which are ordinarily deposited during the last trimester *in utero* [39], are substantially diminished at preterm birth, and must be replaced rapidly. Fortification is therefore recommended for all infants born <1500 g, but may also be recommended for other infants [138].

If own mother's milk is not available or is short in supply, donor milk is often used [37, 38]. Donor milk is generally lower in protein content compared to own mother's milk, and therefore requires a greater level of fortification [37, 38]. When preterm infants reach feeding volumes of approximately 100 mL/kg/day, many hospitals will fortify human milk to increase protein, calories, calcium, phosphorous and other nutrients; although, this is not a consistent practice universally [139]. In the US, a human-milk-based human milk fortifier is available to those hospitals wishing to avoid bovine-based fortifiers. Research thus far suggests that a 100 % human-milk-based diet reduces the risk of medical and surgical NEC [140, 141]. If human milk is unavailable, infants are given preterm formula, which has less nutrient bioavailability than human milk [142]. An exclusive human milk diet, including donor milk with human milk fortifier, has been shown to reduce the risk of NEC compared to preterm formula [140].

Despite its benefits, fortification is associated with changes in the functional value of human milk. Fortifying with bovine fortifiers has been shown to alter and interfere with the anti-bacterial actions of human milk [105, 125]. Since fortifiers can change the composition of milk, extra care must be taken considering contamination and storage risks. As contamination and osmolality increase faster in fortified milk [143, 144], guidelines and manufacturer's instructions must be observed. The addition of fortifiers using aseptic techniques [122, 123] at room temperature or cooler has been suggested to minimise the increase of osmolality levels [145]. Shortened storage durations have also been recommended with fortified milk. These change depending on whether milk is fresh or frozen, previously thawed, or the duration spent at room temperature [146] (Table 3).

Figure 8 – Example of early enteral feeding in the NICU

Feeding

The final step of the milk pathway is feeding the infant. As preterm infants face significant challenges to oral feed initially and often do not progress to breast-feeding until later in their NICU stay [43], they may need to rely on parenteral nutrition and enteral feeds at first (Figure 8). Preterm infants generally begin oral feeding at around 32 to 34 weeks gestational age or once their cardiopulmonary status is considered stable [43]. This varies significantly, depending on the infant's gestational age at birth [43, 147], birth weight, existing medical conditions, and health care institution. Since attainment of independent oral feeding is a key discharge criterion for preterm infants [148], developing oral feeding skills as early as possible is of vital importance. In addition to this, it is necessary to ensure that feeding methods are safe and at low risk to the infant. From a logistical standpoint this means ensuring the right milk is available for the right infant, and that milk is of optimal integrity, closest to that when fed directly from the breast.

Conclusion

In order to provide milk in a form that is closest to fresh milk at the breast, evidence-based practices are required to support the entire milk pathway. These maximise the use of human milk, while ensuring the quality and volume of human milk feeding in the NICU is maintained. Consideration of effective expression protocols that include frequent, double pumping to initiate, build and maintain the mother's milk supply is required. Ensuring that pump equipment is appropriately cleaned before and after expression is also necessary. Once milk is in the hospital, processes can be established for labelling, tracking and storing milk using the most up-to-date evidence. This includes refrigeration of fresh milk within 4 hours, and storing milk in the refrigerator or freezer for the shortest time possible to ensure maximal retention of nutrients, growth factors, and many other protective components in milk, whilst also minimising the risk of milk contamination.

Thawing and warming procedures should be standardised as they can negatively affect milk quality if the warming temperatures are too high; therefore, they are not recommended to exceed physiologic temperatures. Furthermore, often fortification is an extra step in milk preparation that is needed to meet the high nutrient demands for preterm infant growth. This should be carried out in a way that minimises the risk of contamination and mix-up whilst preserving the components of human milk. Despite the growing body of evidence documenting the importance of the human milk processing and feeding in the NICU, future research investigating methods to optimise human milk quality after expression is urgently required to assist the vulnerable NICU population in receiving the maximum benefits of human milk.

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