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# Differences in Current Procedures for Handling of Expressed Mother's Milk in Danish Neonatal Care Units

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**ABSTRACT**

**Background:** Mother's own milk (MOM) is preferred when feeding preterm infants. When expressed mother's milk is stored and handled, there is a risk of bacterial contamination, decreased immunological activity, and less nutritional potential.

**Purpose:** The aim of this study was to investigate current routines when handling MOM in Danish neonatal intensive care units (NICUs).

**Methods:** A survey was sent to all 17 NICUs in Denmark in which current practices regarding human milk handling, storage, and preparation were evaluated. Furthermore, one question sought to establish when mother's milk was believed to be colostrum. Respondents of the survey were neonatal nurses.

**Results:** All 17 units responded to the survey. Only 5 of 17 units answered that human colostrum was defined as milk from the first week after birth. Refrigerator storage time varied between 24 and 72 hours. In 6 of 17 units, parents were in charge of mixing milk and fortifier. Heating of human milk was done by using microwave ovens in 4 of 17 of the units.

**Implications for Practice:** This national survey established that there is significant variability in the way mother's milk is handled. Some of the procedures performed may affect the quality of the milk. It is important to implement evidence-based practice regarding storage and handling of expressed mother's milk to ensure that the quality of the milk is the best possible alternative for all preterm infants.

**Implications for Research:** Prospective studies are needed to examine the association between handling of human milk and changes in composition and nutritional potential of the milk.

**Key Words:** feeding, guidelines, human milk, mother's own milk handling and storage, very preterm infants

## BACKGROUND AND SIGNIFICANCE

In Denmark, 1.5% of all births occur before the gestational age (GA) of 32 weeks. Necrotizing enterocolitis (NEC) is present in 5% to 6% of all very or extremely preterm infants born in Denmark,[1] but the incidence varies between countries. In an attempt to minimize complications such as NEC and feeding intolerance and improve growth in preterm infants, all aspects of their treatment should be evaluated continuously in order to optimize procedures. Type of enteral nutrition and feeding routines (heating and adding fortifier) have been suspected to have an impact on the risk of NEC among very preterm infants.[2]

Human colostrum, the milk produced until secretory activation (lactogenesis stage II), is particularly high in immunological components including leucocytes, macrophages, IgG and IgA, antioxidants, and proteins that initiate growth, protect the infant against infections, and provide maturation of the intestinal mucosa.[3,4] Women who deliver preterm often have a compromised initiation of lactogenesis stage II,[5] which makes it difficult to determine when their milk transitions from colostrum to more mature milk. Lactation stage influences how milk should be stored.

When expressing, storing, and handling mother's own milk (MOM) in neonatal intensive care units (NICUs), studies have shown that there is a risk of bacterial contamination, lowering of the nutritional potential, and even destruction of some of the most important immune components.[6-9] In addition, storage time, temperatures, heating method, and fortification can influence the quality of the milk.[6,10,11] This is particularly important to consider when feeding preterm infants, who generally have a high risk

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of infection due to their immaturity. Other Nordic countries (eg, Sweden) have detailed national guidelines regarding management of expressed MOM.[12] In Denmark, there are no national guidelines regarding handling and storage of expressed MOM for preterm infants, which leaves it to the individual unit to make its own guidelines, potentially resulting in 17 different ways to handle, store, and prepare mother's milk.

However, the Danish Health Authority has developed recommendations on breastfeeding, along with human milk handling, but they are primarily aimed at mothers of term born infants.[13] Before developing national guidelines, we planned to investigate how current procedures on human milk handling and storage are performed in Danish NICUs.

In Denmark, 17 NICUs attend to the medical care of preterm infants. The GA of the infants treated in these units depends on the level of intensive care. Four of the NICUs are levels 3 and 4 and are thereby allowed to treat extremely premature infants (GA $\leq$ $27^{6}/_{7}$ weeks) or infants with severe complications: Rigshospitalet; Odense University Hospital; Aarhus University Hospital Skejby; and Aalborg University Hospital. The remaining 13 are level 2 NICUs.

The aim of this study was to describe MOM handling, storage, preparation, and fortification routines in the NICUs in Denmark and to compare these findings with existing knowledge on how to maintain components and composition of human milk. No current data are available about the handling procedures of mother's milk in Denmark. Findings of significant variability between units could be due to the lack of a national guideline and thus indicate the need for such guidelines.

## METHODS

This descriptive study comprised a survey to determine handling, storage, and feeding practices of MOM. In April 2017, we (the first and last authors) developed a questionnaire consisting of 31 questions to collect information from the neonatal nurses about their current practices of handling, storage, and preparation of MOM. Furthermore, one question sought to establish how long the nurses considered the milk to be human colostrum since there is no agreement on this in Denmark and could affect the way the milk is stored.

The target population was neonatal nurses working in the 17 NICUs of Denmark.

Validation of the survey was done by 5 neonatal nurses in the region of Southern Denmark in May 2017 to ensure questions were relevant and that all main themes were covered and written in adequate and comprehensible terms. They contributed new perspectives and questions, which were incorporated in the final version of the survey. The final survey was sent by e-mail to the neonatal nurses in charge of the unit in all 17 NICUs in August 2017. Two neonatal nurses were asked to answer the survey individually. In October 2017, the leading nurse of the units who had not responded was reminded either by telephone or by mail. Data from the surveys were collected and entered into an electronic database (www.epidata.dk). The answers were entered twice to avoid errors.

### Statistical Analysis

This was a descriptive analysis. Categorical variables were summarized as numbers and percentages. Statistics and graphic illustrations were performed in STATA 15.0. (StataCorp, College Station, Texas).

## RESULTS

Neonatal nurses answered the questionnaire in all 17 units. We found that units had different procedures for human milk handling. Twelve percent (2/17) of the units had their own human milk bank, and 88% (15/17) used donor milk (DM) obtained from 1 of 2 existing Danish DM banks. Six percent (1/17) of the units did not use DM because the age of the treated infants was more than 32 weeks' GA, and 1 unit did not answer the question regarding the use of DM. One of these human milk banks (at one of the neonatal units) handled and stored MOM and mixed fortifier to milk in the milk bank kitchen, whereas the other units (including the other unit with a milk bank) handled and stored MOM and mixed fortifiers to milk in the units (in a separate milk kitchen or at bedside).

One hundred percent (17/17) of the units collected and stored MOM in the unit or the milk bank. In all units, freshly expressed milk was used immediately if possible and, if not used immediately, it was stored for later use. Freshly expressed MOM was stored under different temperatures and for different time intervals among the participating units. Table 1 shows information about the units, storage times, cooling, and freezing temperatures of MOM.

In 53% (9/17) of the units, the milk had to be frozen within 24 hours from expression. In the remaining 47% (8/17) of the units, the freezing procedures varied between freezing directly after expression (1/17), 30 minutes after expression (1/17), 48 hours after expression (2/17), and 72 hours after expression (2/17). In 12% (2/17) of the units, they did not reply to this question.

In 76% (13/17) of the units, parents had access to the refrigerator in which MOM was stored. In 59% (10/17) of the units, parents also had access to the freezer in which MOM was stored.

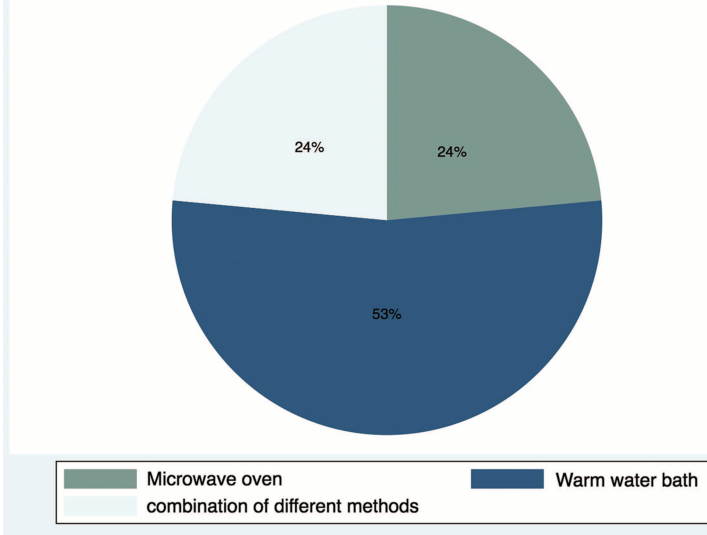
In 35% (6/17) of the units, they thawed frozen milk in refrigerators. In 29% (5/17) of the units, they thawed in cold water baths. In 12% (2/17) of the

TABLE 1. Descriptive Information About the Participating Units and Storage Conditions for Mother's Own Milk

| Region/hospital (N = 17) | Gestational Age of Infants | Profession of Person Who Answered the Survey | MOMCooling Temperature | MOM Cooling Time in Refrigerator | MOM Freezing Temperature | MOM Freezing Time (Durability) |
|---|---|---|---|---|---|---|
| Capital region | | | | | | |
| Rigshospitalet | ≤ $27^{6}/_{7}$ wk | Anesthesia nurse | 4°C-5°C | 72 h | −20°C | 6 mo |
| Hvidovre hospital | ≥28 wk | Nurse, health coordinator | 5°C | 72 h | −20°C | 6 mo |
| Herlev hospital | ≥28 wk | Nurse | 5°C | 72 h | No reply | No reply |
| Hilleroed hospital | ≥28 wk | Nurse | 4°C-5°C | 72 h | −18°C | 6 mo |
| Region Zealand | | | | | | |
| Roskilde University Hospital | ≥28 wk | Nurse | 5°C | 72 h | No reply | No reply |
| Holbaek Hospital | ≥28 wk | Nurse | 5°C | 72 h | −18°C | 6 mo |
| Naestved Hospital | ≥28 wk | Nurse | 5°C | 72 h | −20°C | 6 mo |
| Region of Southern Denmark | | | | | | |
| Odense University Hospital | ≤ $27^{6}/_{7}$ wk | Nurse | 5°C | 72 h | −18°C | 6 mo |
| Kolding Hospital | ≥28 wk | Nurse | 4°C | 72 h | −21°C | 6 mo |
| Esbjerg Hospital | ≥28 wk | Neonatal nurse incharge | 5°C | 72 h | −18°C | 6 mo |
| Aabenraa Hospital | ≥28 wk | Nurse | 4°C | 24 h | −18°C | 6 mo |
| Region of Central Jutland | | | | | | |
| Skejby University Hospital | ≤ $27^{6}/_{7}$ wk | Nurse | 5°C | 72 h | −18°C | 6 mo |
| Herning Hospital | ≥28 wk | Nurse, IBCLC educated | 4°C | No reply | No reply | No reply |
| Randers Hospital | ≥28 wk | Nurse, IBCLC educated | 5°C | 72 h | −18°C | 6 mo |
| Viborg Hospital | ≥28 wk | Nurse | 5°C | 24 h | −18°C | 6 mo |
| Region of Northern Jutland | | | | | | |
| Aalborg University Hospital | ≤ $27^{6}/_{7}$ wk | Nurse | 5°C | 72 h | −18°C | 6 mo |
| Hjoerring Hospital | ≥32 wk | Nurse | 5°C | No reply | −18°C | 6 mo |

*Abbreviations: IBCLC, International Board Certified Lactation Consultant; MOM, mother's own milk.*

FIGURE 1




Mothers milk heating procedures (n = 17).

units, they used warm water baths, and in the remaining 24% (4/17) of the units, they used a combination of the 3 methods.

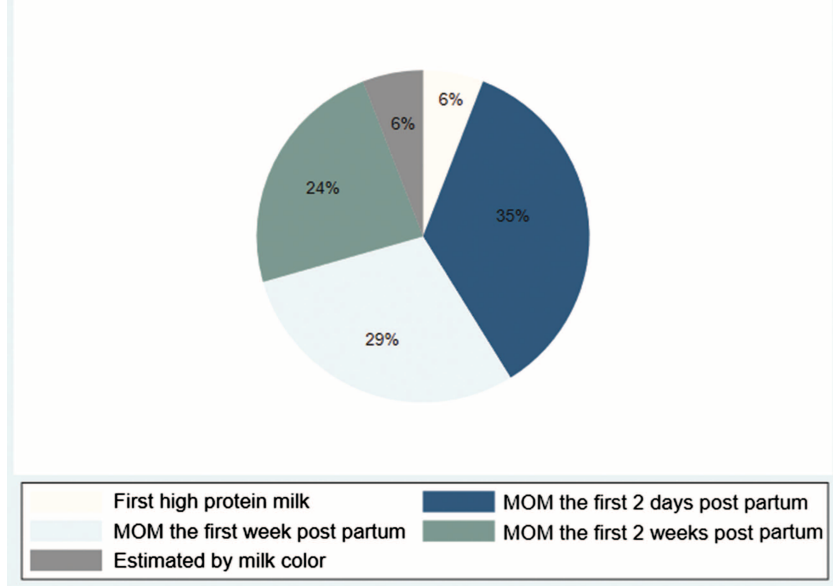
Different methods were used to heat the MOM (see Figure 1). In 12% (2/17) of the units, they answered that they used a combination of the methods (microwave and water bath) or bottle warmers (Medela, Baar, Switzerland) to heat the milk before feeding the infant.

The definition of human colostrum was not consistent among the units. How the units defined colostrum is shown in Figure 2.

## Feeding Practice and Fortification

All units answered that they intend to use all human colostrum, fresh as well as stored (refrigerated or frozen), before using transitional or mature fresh milk for all meals. Furthermore, they answered that

FIGURE 2




Definition of colostrum among Danish neonatal intensive care units. MOM indicates mother's own milk.

TABLE 2. Fortification Routines Among the Participating Units

| Hospital | Fortification Method | Fortification Mixed for | Fortification Preparation | Probiotics/Type |
|---|---|---|---|---|
| Rigshospitalet | BUN, blindly analysis of MOM, growth | 24 h | Milk kitchen staff | Yes, Bifiform |
| Hvidovre Hospital | Analysis of MOM, growth | 24 h | Milk kitchen staff | Yes, Bifiform |
| Herlev Hospital | BUN, growth | For several meals | Staff only | Yes, Bifiform |
| Hilleroed Hospital | Growth | For several meals | Parents + staff | Only if started at Rigshospitalet |
| Roskilde Hospital | Analysis of MOM, growth | For several meals | Staff only | Yes, Bifiform |
| Holbaek Hospital | BUN, growth | For several meals | Parents + staff | Yes, Bifiform |
| Naestved Hospital | Growth | missing | Staff only | Yes, Bifiform |
| Odense University Hospital | BUN, analysis of MOM, growth | For several meals | Staff only | No |
| Kolding Hospital | Blinded, growth | For one meal at a time | Parents + staff | No |
| Aabnenraa Hospital | BUN, blinded growth | Pr. meal | Staff only | No |
| Esbjerg Hospital | BUN, blinded growth | For several meals | Staff only | No |
| Herning Hospital | Blinded | Pr. meal | Parents + staff | No |
| Skejby University Hospital | Growth | | Parents + staff | No |
| Viborg Hospital | BUN, blinded growth | Pr. meal | Parents + staff | Yes, Dicoflor |
| Randers Hospital | Starts blindly, adjust after analysis of MOM | Pr. meal | Staff only | Yes, Ido Form |
| Aalborg University Hospital | Analysis of MOM | 24 h | Milk kitchen staff | No |
| Hjørring Hospital | Blinded, growth | For several meals | Staff only | No |

*Abbreviations: BUN, blood urea nitrogen; MOM, mother's own milk; Pr. meal, ....*

MOM was heated to body temperature (37°C) when fed to the infant. In 71% (12/17) of the units, they had procedures regarding use of human colostrum and fresh expressed MOM in the first 3 weeks of infants' lives. In 23% (4/17) of the units, they had no procedures, and in 6% (1/17) of the units, they answered that there was disagreement between the nurses concerning whether to use or not to use all frozen human colostrum after the first and/or second weeks.

All units fortified human milk (MOM and DM) with the powder, bovine milk–based fortifier Pre-NAN FM85 (Nestlé, Vevey, Switzerland). Fortification routines varied among the units. Table 2 shows fortification methods, including the amount of milk mixed and milk preparation and whether probiotics were used. In some units, it was standard procedure to individualize fortification according to the amount of protein in mother's milk (targeted mother's milk analysis), whereas other units fortified blindly (fixed amount of protein) or by evaluation of growth or according to blood urea nitrogen level.

## DISCUSSION

Results of this study indicate significant variability between the 17 NICUs in Denmark regarding human milk handling, storage, and feeding procedures and also in the definition of human colostrum. In the following discussion, current literature is presented in relation to the focus points regarding handling of MOM in the NICU with the potential risks of bacterial contamination, decreasing immunological components, and lowering the nutritional potential.

### Bacterial Contamination

Human colostrum contains a higher concentration of bactericidal components than transitional and mature milk. Human colostrum has also been shown to be more stable during storage with regard to bactericidal effects.[14] The perception of how long mother's milk is considered to be colostrum could influence the way in which milk is stored and thereby could lead to an increased risk of bacterial contamination. We found that NICUs had 5 different

definitions of colostrum including time ranging from 2 days postpartum to the first 2 weeks postpartum, color of the milk, or the first high protein milk. A study from 2013 analyzed preterm human milk samples and divided the milk samples into 3 groups. The study defined the lactation stages as early milk (0-2 weeks), transitional milk (2-4 weeks), and mature milk (>4 weeks).[15] A meta-analysis from 2014 defines colostrum as milk from day 0 to 3 postpartum but concludes that both colostrum and transitional milk from mothers who delivered preterm overall contain higher amounts of protein than milk from mothers who delivered at term.[16] This reflects the lack of international agreement on how long preterm human milk is defined as colostrum.

Five units (29%) allowed human colostrum to be stored at room temperature for 4 to 6 hours after expression. Four of these 5 units restored the human colostrum in a refrigerator in case it was not used, and 1 unit disposed of it if not used within 4 hours. It is important that the milk stored at room temperature (if any) is human colostrum because bacterial proliferation is more likely to occur in transitional and mature milk than in colostrum. Both American and English guidelines recommend MOM to be refrigerated immediately if not used directly after expression.[17,18]

## Immunological Components

Preservation of immunological components and risk of bacterial contamination seem to be correlated. Both human colostrum and to a greater extend transitional and mature milk show decreased activity in antioxidant and immunological components after storage.[8,19-22] Several studies have examined the influence of storage temperatures on the immunological components of milk. Three studies do not recommend freezing as a method for preserving antioxidant levels.[8,21,22] One of these studies showed that storage at −20°C decreased activity of peroxidase, immunoglobulin A, lactoferrin, lactoperoxidase, and lysozymes.[21] The authors conclude that this decrease appears to be connected to increasing bacerial activity. In contrast, 2 studies found that freezing protects the antioxidant capacity of the expressed MOM.[23,24] Furthermore, studies have investigated the influence of refrigerator storage duration on antioxidants and immunological components. Three studies showed a decreased activity after only 48 hours at 4°C,[6,14,24] 1 study showed a reduction in antioxidant capacity in both human colostrum and mature milk after 72 hours of refrigerator storage,[20] and 2 studies demonstrated only minimal changes in MOM composition after 5 days of storage at 4°C.[11,25] However, these studies are difficult to compare because the milk analyzed was from different stages of lactation and due to differences in the definition of when the milk is considered to be colostrum, transitional milk, and mature milk. Furthermore, heating methods can affect components in MOM. Microwave radiation has been shown to reduce lysozyme activity and the amount of specific IgA in human milk.[7,10] Heating in microwaves can also result in uneven heating of the milk and lead to hot spots, which can harm the infant.[17] Another study investigating the processing in human milk banking recommends not exceeding 62.5°C because the concentrations of IgA, lysozyme, and lactoferrin drop significantly.[12,19] Therefore, it is important that temperatures be carefully considered and that microwave radiation is kept to a minimum in an attempt to minimize loss of immunological components. The most recent recommendation from 2018 on human milk handling by the Danish Health Authority[13] does not directly advise against heating expressed MOM in microwave ovens. However, it states that heating could destroy some of the immunological components and that temperature is very hard to control. The guidelines from the United Kingdom advise against using microwave ovens for heating and recommend only to use water-free methods for thawing and warming MOM to prevent contamination with waterborne organisms.[18]

## Nutritional Quality of the Expressed Milk and Feeding Practices

With regard to the nutritional quality of the milk, it is important to provide the preterm infants with the best possible energy and protein supply under circumstances where expression and storage of MOM are needed. Freezing at −20°C for 3 months causes a significant loss in fat and caloric content.[26] However, freezing at −20°C appears to be better than −80°C concerning the preservation of macronutrients.[27] We did not analyze any human milk samples (fresh or frozen) for this study.

## Fortification

Fortification of mother's milk was done in different ways in the Danish NICUs. Some units mixed fortifier at each meal, and some units mixed amounts for 24 hours. One study investigated the association between osmolarity of fortified human milk and storage time and found a significant rise in osmolarity in the milk after 24 hours of refrigeration compared with immediately after mixing the milk.[28] Knowledge regarding fortification of MOM and/or DM and whether fortification should be mixed per meal or per day might have an impact both on osmolarity and the risk of bacterial contamination, but data are very limited.

## Strengths and Limitations of Our Study

We found inconsistent management of expressed MOM in Danish NICUs, which strongly supports the hypothesis that there is a need for national guidelines on handling and storage of expressed MOM.

| Recommendations for Clinical Practice and Research | |
|---|---|
| **What we know:** | • MOM is the preferred type of nutrition for all preterm infants.<br>• Fortifiers are often added to MOM to improve intake of both micro- and macronutrients for preterm infants.<br>• During storage of MOM, there is a risk of bacterial contamination and a risk of lowering both immunological activity and the nutritional potential. |
| **What needs to be studied:** | • Fortification products and routines might have an impact on the osmolarity of MOM when feeding preterm infants.<br>• Is there any association between fortification routines and NEC?<br>• Appropriate storage recommendations for milk from different stages of lactation (colostrum, transitional milk, and mature milk). |
| **What can we do today:** | • Make sure to have national guidelines regarding handling and storage of MOM; eg, correct temperatures of freezers and refrigerators.<br>• Ensure the healthcare team is educated in handling and storage of MOM. |

A limitation is that no validated survey existed on handling and storage of expressed MOM and therefore a new survey was developed. This could introduce informational bias due to potentially missing answer categories and wrong classification of answers. Two random neonatal nurses from each unit were asked to answer the survey. In some units, the responding nurses were lactation specialists (International Board of Lactation Consultant Examiners education) and in other units they were nonspecialized neonatal nurses, which could lead to selection bias.

The survey did not include questions regarding testing MOM for cytomegalovirus (CMV). In Denmark, it is not a standard procedure to screen MOM in the NICU for CMV. CMV can cause serious and severe symptoms in premature infants including sepsis-like symptoms, although this is rare.[29] Studies have shown that it is possible to inactivate the virus in MOM by performing short-time heat treatment of the milk, which may maintain the immunological and nutritional quality of the milk. Whether this should be routinely performed in NICUs in Denmark requires further investigation.

### Future Perspectives

In general, a high standard is required when handling food products in the food industry. Some of the principles used could be applied to the handling and storage of expressed MOM. Hazard analysis and "Critical Control Points principles" comprise a systematic approach designed to identify, evaluate, and control possible risk factors in food processing and storage. Such an analysis has been conducted in Belgium to standardize handling of expressed human milk at every stage and to ensure the safety and quality of milk and same strategy was used prior to the writing of the UK guidelines for handling of MOM.[18,30]

## CONCLUSION

There are many important aspects to consider when handling and storing MOM for preterm infants. In Denmark, there is a lack of evidence-based practice on which procedures are the best for preserving the components of MOM. We found differences in handling, storage, and preparation of MOM. Developing Danish national guidelines could lead to safer handling of expressed MOM.

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