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# Fat and Caloric Content of Breast Milk of Mothers of Premature Infants: Comparison of High vs. Low Volume Producers and the Impact of Volume in Single Pumping Sessions and the Interval Between Pumping Sessions

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# FAT AND CALORIC CONTENT OF BREAST MILK OF MOTHERS OF PREMATURE INFANTS: COMPARISON OF HIGH VS. LOW VOLUME PRODUCERS AND THE IMPACT OF VOLUME IN SINGLE PUMPING SESSIONS AND THE INTERVAL BETWEEN PUMPING SESSIONS

by

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ABSTRACT

FAT AND CALORIC CONTENT OF BREAST MILK OF MOTHERS OF PREMATURE INFANTS: COMPARISON OF HIGH VS. LOW VOLUME PRODUCERS AND THE IMPACT OF VOLUME IN SINGLE PUMPING SESSIONS AND THE INTERVAL BETWEEN PUMPING SESSIONS

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Under the supervision of Professor Teresa Johnson.

Premature birth affects 8-18% of all infants worldwide and is the leading cause of death in children less than the age of 5 years. The premature birth rate has been on the rise between 2014-2016 in the United States and 1 in 10 infants are born prematurely. Infants who are born prematurely are at considerable risk of serious infections, visual impairments and neurodevelopmental problems. Human milk, specifically mother's own milk (MoM), is associated with decreased rates of necrotizing enterocolitis, pneumonia, chronic lung disease and late onset sepsis. MoM is also associated with improved neurodevelopmental outcomes in premature infants.

Although MoM is considered the gold standard of nutrition (with current fortification guidelines), it places the hospitalized premature infant at risk of insufficient postnatal growth due to maternal individual variability in the fat and caloric content of MoM. Premature hospitalized infants are already at risk of insufficient caloric intake due to iatrogenic factors in the storage, handling and delivery of human milk. Fat loss can also occur during the freezing, thawing and handling process and during gavage feedings.

Obstetrical, antepartum, postpartum and neonatal nurses play an integral role in supporting mothers to provide breast milk for their premature hospitalized infant. With a greater understanding of the numerous advantages infants have when provided with MoM and when evidence-based collection, handling and feeding methods are well understood, vulnerable premature infants are provided with practices that can optimize their health, growth and development.

Three key areas are presented in this dissertation. Chapter 1 summarizes the main study (*Fat and Caloric Content of Breast Milk of Mothers of Premature Hospitalized Infants: Comparison of High vs. Average to Low Volume Producers and the Impact of Volume in the Breast at Single Pumping Sessions and the Interval Between Pumping Sessions*). This chapter also introduces the second manuscript (Chapter 2: *Collection Techniques of Colostrum in the Postpartum Period from Mothers of Hospitalized Infants for Oropharyngeal Care and Trophic Feedings*) that discusses the composition of colostrum and techniques that nurses can employ to collect this highly viscous dense bioactive fluid from mothers in the postpartum period for early enteral feedings to improve neonatal gut maturation.

Chapter 3 (*Creamatocrit Measurement of the Fat and Caloric Content of Mother's Own Milk in the Neonatal Nursery: Clinical Pearls to Achieve Accurate and Reliable Results*) summarizes the research available on the accuracy, reliability and validity of the instrument used in this study. It also provides useful clinical pearls to achieve accurate and reliable results with the primary measurement instrument of this study (the Creamatocrit Plus, Separation Technology, EKF). This manuscript was written to describe best practices in the measurement and caloric content of MoM and in response to numerous questions I receive from NICU staff members throughout the world about how to use this instrument in the neonatal nursery setting.

Chapter 4 is the results manuscript of the main dissertation study. This study was developed based upon a review of the literature and our clinical experience that many mothers who produced high volumes of breast milk ($\geq$900 ml/day) had a premature infant with inadequate weight gain.

Separation or fractionation of breast milk (hind milk feeding) is a common practice in neonatal nurseries in the United States (US) that involves pumping the initial low fat breast milk, stopping the pumping session and then saving this low fat milk for later use. The mother then continues the pumping session to collect higher fat breast milk that is collected and fed to her infant. Although this may result in improved weight gain in the infant, it can involve wastage of MoM and is an arbitrary process if the MoM is not tested by a validated measurement instrument at several points in the day and after the separation of the higher fat portion of the maternal pumping session. Also, mothers who have a daily supply of breast milk that does not exceed the volume needs of their infant cannot participate in hind milk separation and feeding. This limits this feeding intervention to only infants of mothers with sufficient daily breast milk production. The main study of this dissertation (Chapter 4) attempted to answer key factors that may influence the fat and caloric content of MoM to provide baseline information to problem-solve inadequate or slow growth and inform best practices for this vulnerable population of infants.

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## LIST OF ABBREVIATIONS

APRN- advanced practice registered nurse

CRCT- creamatocrit

kcal- kilocalories

g/L- grams/liter

HM- human milk

HS- bedtime or time of onset of nighttime sleep stretch

MoM- mother's own milk

NEC- necrotizing enterocolitis

NICU- neonatal intensive care unit

oz- ounce

VLBW- very low birth weight

HMA- human milk analysis

US- United States

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This is a journey one would never know without embarking on it. What I have learned is that this work was not acquired nor achieved in isolation. I was introduced to the research process and this program from my first key mentor in graduate school, Karen Pridham, PhD, RN, Professor Emeritis at the University of Wisconsin-Madison. She continues to inspire me.

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the type of mentor I have been so gifted to receive from these intelligent, professional and gracious scientists. They have taught me that mentorship pays off in great dividends. The more we support and teach other, the greater the chance that quality knowledge development will occur that can ultimately and hopefully improve patient outcomes.

I am especially thankful to the wonderful mothers who agreed to participate in this study. Their dedication to the study and the time they carved out of their lives to record their pumping sessions and collect their samples of breast milk is so appreciated. I stand in admiration of their commitment to provided breast milk to their infant under challenging and difficult circumstances.

I also thank my colleagues, Jeanne Barreira, CNM,IBCLC, Martha Krauss, CNM,IBCLC, Ellen Linder, BSN,IBCLC and Molly Gros, BSN, IBCLC , for their ongoing support of my journey of learning. I am blessed to work with a fantastic group of women who care deeply about supporting our breastfeeding mothers, their infants and their families. They also know and understand how important it is to provide evidence-based and quality care for the people that we serve.

I also acknowledge and thank Medela, Inc. (McHenry, IL) for the in-kind loan of calibrated Creamatocrit Plus instruments (distributed by Separation Technologies, EKF, Inc), a Medela® Waterless Warmer (Medela, McHenry, IL) and creamatocrit supplies to measure the fat and caloric content of the breast milk samples.

CHAPTER 1

# INTRODUCTION

## Background

Greater than 450,000 infants are born prematurely in the United States (US) each year (CDC, 2017; March of Dimes, 2014) and more than 15 million are born per year worldwide (WHO, 2015). There has been an increase in the number of premature births in the US from 2014-2016 and currently one in ten infants are born at less than 37 weeks gestation (CDC, 2017). Across 184 countries, premature birth rates range from 8-18% of all infants born each year. Complications of premature birth is the leading cause of death in children less than the age of 5 years worldwide and is the leading cause of neurodevelopmental problems and chronic lung disease in children (CDC, 2017; WHO, 2015).

In addition to a significant risk of intracranial bleeding, visual impairments, and neurodevelopmental issues, premature infants are at risk of chronic lung disease and serious infections such as necrotizing enterocolitis (NEC), pneumonia, and sepsis (WHO, 2015). When they receive their mother's own milk (MoM), they are provided with numerous nutritional benefits as well as bioactive properties including stem cells, growth hormones, pre- and probiotics, and anti-infective and immune-building properties (Hassitou et al., 2013; Koletzo, Agostini, Bergmann, Ritzenhaler & Shamir, 2011). MoM for premature infant nutrition also promotes optimal brain growth, reduces the risk of retinopathy of prematurity and improves neurodevelopmental outcomes (AAP, 2012; Belfort et al., 2013; Belkind-Gerson, Carreon-Rodriguez, Contreras-Ochoa, Estrada-Mondaca & Parra-Cabrera, 2008; Delplanque, Gibson, Koletzo, Lapillonne & Strandvik, 2015; Hsiao, Tsai, Chen & Lin, 2014; Lucas, Morley & Cole, 1992; Patel, Meier & Engstrom, 2007).

Colostrum that is produced by mothers of premature infants in the early postpartum period is particularly rich in proteins, lipids, vitamins, minerals, growth factors, and immune cells (Hassiotou et al., 2013) and is recognized as early immune therapy that stimulates bioactivity to enhance the maturation of the neonatal intestines and decrease the gut permeability that can lower the risk of NEC and nosocomial infections (Lucas, Morley et al., 1992; Meier, Engstrom, Patel, Jegier & Bruns, 2010; Newburg & Walker, 2007; SIFT Investigators Group, 2013; Wagner, Forsythe & Pittard, 2007; Taylor, Basile, Ebeling & Wagner, 2009). In a retrospective chart review of 349 VLBW infants, Corpelijn et al. (2012) concluded that early enteral colostrum and mature breast milk feedings in the first 60 days of life was associated with a decreased risk of NEC, sepsis and death and is correlated with improved neurodevelopmental outcomes. The first manuscript (Chapter 2) of this dissertation summarizes the composition of colostrum with specific recommendations for collection of this rich and viscous fluid from hospitalized mothers of premature infants in the early postpartum period.

**Significance of the problem**

Although MoM is the first and the optimal choice for nutrition of the premature infant, it places the infant at risk of poor growth velocity due to the variability of fat content and caloric density in human breast milk due to maternal individual differences, pumping procedures (e.g. insufficient emptying of the breast or pumping with a substandard breast pump), diurnal differences in fat content throughout the day, storage and handling of breast milk and fat loss during gavage feedings (Atwood & Hartmann, 1992; Brenham-Behm, Carlson, Meier & Engstrom, 2013; Chang, Chen & Lin, 2012; Daley, Rosso, Owens & Hartmann, 1993; Harzer, Haug, Dietrich & Genter, 1993; Hassitou et al., 2013; Kociszewska-Najma, Borek-Dzieciol, Szpotanska-Sikorska, Wilkos, Pietzrak & Wielgos, 2012; Underwood, 2013). The caloric and

fat content of breast milk received by the premature infant can impact their growth and development. Kociszewski-Najman et al. (2012) reported that premature infants must receive 100-120 kilocalories per kilogram in 24 hours to achieve satisfactory growth with MoM.

**Diurnal variation of fat in MoM.** In a systematic review and meta-analysis of 41 studies of breast milk composition in mothers of preterm and full term infants, breast milk caloric and fat content varied from the beginning to the end of the feeding or expression session with the breast milk near the end having a higher caloric and fat content (Gidrewicz & Fenton, 2014). Diurnal differences were found in lactating women as the breast milk expressed in the morning was lower in caloric and fat content than breast milk expressed in the evening hours (Manley et al., 2011; Moran-Lev, Mimouni, Ovental, Mangel, Mandel & Lubetsky, 2015; Kocizewski-Najman et al., 2012; Weber, Jochum, Buhrer & Obladen, 2001; Lubetsky, Mimouni, Dollberg & Solomon, 2007).

**Influence of maternal diet to fat content of MoM**. A small prospective study conducted by Harzer, Haug, Dietrich and Genter (1983) found diurnal differences between women of German and English descent. All breastfeeding women in the Harzer et al. study had the lowest caloric and fat content in the morning hours, but German women had the highest caloric and fat content in the midday and English women in the evening hours. Investigators for this study postulated that caloric and fat content may vary based on maternal dietary intake throughout the day, but maternal dietary intake has not been demonstrated to be correlated with fat content in breast milk in the systematic review and meta-analyses conducted by Gidrewicz and Fenton (2014).

**Maternal age and fat content of MoM**. The age of women who breastfeed could also impact the caloric and fat content of breast milk. Hausman-Kedem et al. (2013) compared the

caloric and fat content of the breast milk of women who were less than or older than 35 years of age at the time of their infant's birth. They found that the breast milk of the older women had a higher fat content in colostrum yet no statistically significant differences in caloric and fat content were found between the two age groups of women in relation to mature breast milk.

**Iatrogenic (non-maternal) factors associated with loss of fat and caloric content**. A concern related to the caloric and fat content of breast milk is whether non-production factors can impact the amount of calories and fat gavage-fed infants receive. Lipid loss during gavage infusions presents an additional risk factor for poor growth velocity in tube-fed premature infants. Brennan-Behm et al., 1994 measured the caloric and fat content of single samples of maternal breast milk before and after a gavage infusion and compared the differences between standard and microbore gavage feeding tubes. Brennan-Behm et al. (1994) found a 1.59% loss of lipid concentration with standard gavage tubing and 5.24% decrease in lipids with microbore gavage tubing. It was postulated that lipid loss occurred during enteral infusions due to adherence of fat in the lumen of the tubing. Lipid loss during gavage infusions of premature infants presents an additional risk factor of impaired growth.

Breast milk that is given to hospitalized premature infants is frequently frozen in plastic containers to prolong its shelf life, and then thawed and rewarmed before it is given to infants (Koletzo, Agostini, Bergmann, Ritzenhaler & Shamir, 2011). Lucas et al. (1978, 1983) reported that the temperature to which milk samples are subjected to exert a significant effect on the fat values of breast milk and fresh breast milk has a fat content higher than breast milk that has been subjected to changes in temperature. Lucas et al. (1978) stated that the effect of heat disrupts the fat globule membrane. Chang et al. (2012) demonstrated that the freezing and thawing process of human milk results in a reduction of fat in human milk up to 9%. Breast milk samples of

(N=18) healthy lactating women were collected and baseline macronutrients were measured using mid-infrared spectroscopy prior to freezing in a variety of nine different containers (e.g. glass, polyurethane breast milk bags, and plastic breast milk containers). Human milk samples were then measured after frozen at -20 degrees Celsius for a period of two days. Chang et al. concluded that there was a statistically significant decrease in fat content in all storage containers ranging from 0.27 to 0.30 grams/dl ($p=0.02$) but no significant decrease in total energy content. The authors concluded that fat loss during the freezing process and thawing process may be related to adherence of human milk fat to the breast milk containers and lipolysis and lipid perioxidation.

**What is not known and in need of further study**. The physiologic reasons for the variability in the fat content and caloric density in maternal breast milk are unknown, but one theory suggests that milk fat globules adhere to alveolar membranes and are displaced when the mammary gland is near empty (Atwood & Hartmann, 1992; Neville, Allen & Waters, 1983). Atwood and Hartmann (1992) hypothesized that greater milk volumes in the breast have a high lactose and water content thus creating a dilution effect and lowering the caloric and fat content of the breast milk sample. To date and to our knowledge, there are no studies that have examined the fat and caloric content of the breast milk of breast pump-dependent mothers of premature infants who produce high volumes of breast milk each day and compared this to women who produce average to lower volumes each day. More than six decades ago, Gunther, Camb and Stanler (1949) demonstrated that a long night interval between breast feeding or pumping sessions lead to variation in fat content between morning and evening breast milk samples in mothers of full term infants. They demonstrated that a consistent and regular breast emptying schedule with similar time intervals between expression sessions lead to a more

constant fat content in breast milk samples throughout the day and night. In a more recent study of the components of the breast milk of mothers of infants 12 months or older, Perron, Fogelman, Newburg and Allen (2017) found that as the number of hours between pumping sessions increased the fat and caloric content decreased. To date, the impact of volume in the breast at single pumping sessions and the impact of the time interval between pumping sessions on the fat and caloric content of the breast milk of breast pump-dependent mothers of hospitalized premature infants has not been studied.

**Purpose of the study**

Hospitalized premature infants are at risk of poor growth velocity when fed their mother's breast milk due to the variability in the fat and caloric content of breast milk throughout the pumping or expression session, and possibly due to the interval or spacing between pumping sessions. Furthermore, premature hospitalized infants are at further risk of insufficient caloric intake as human milk fat content may decrease with iatrogenic factors that may occur during storage and handling and loss during gavage feedings.

The primary purpose of this study was to examine the possible relationship of the fat and caloric content of breast milk in breast pump-dependent mothers of premature infants with high daily breast milk volumes versus mothers with average to lower daily volumes.

Specifically, this study examined between–subject differences of those mothers who produce $\geq$ 900 milliliters (ml) per day with those who produce $\leq$500 milliliters. In addition, it examined the caloric and fat content of the breast milk of mothers of premature hospitalized infants and whether or not breast milk volume in the breast at single pumping sessions, and the time interval between pumping sessions was related to the fat and caloric content of their breast milk. In this study, the time interval between pumping sessions was defined as the period of

time lapsed from the last pumping session of the day until awakening to pump in the morning. Identification of mothers at risk for production of breast milk low in caloric and fat content can inform lactation-based nursing and dietary interventions to prevent and improve inadequate growth in premature infants. Results of this study may inform individualized lactation interventions specific to the needs of the infants to optimize their growth and development.

**Research questions and hypotheses**:

**Descriptive research questions**

1. Is there a difference in the fat content and caloric density of MoM of pumping mothers of premature hospitalized infants who produce $\geq$900 ml/day compared to those who produce $\leq$500 ml/day?

2a. Does the volume of breast milk in the breast at a single pumping session relate to the fat and caloric content of MoM?

2b. Does the interval (or time elapsed) between pumping sessions relate to the fat and caloric content of MoM? The time lapsed or interval is defined as the period of time from the last pumping session of the day (HS) until the first pumping session of the morning after the nighttime sleep stretch.

**Hypotheses**

**Study aim 1**: Primary null hypothesis: There is no difference in the fat content and caloric content of breast milk of pumping mothers of premature infants who produce $\geq$900 ml/day than those who produce $\leq$500 ml per day.

**Study aim 2a:** Secondary null hypothesis: There is no relationship of the volume of breast milk produced in the breast at a single pumping session to the fat content and caloric content of breast milk.

**Study aim 2b**: Secondary null hypothesis: There is no relationship between the interval or time lapsed (from the HS pumping time to the time of the first pumping session of the AM) and the fat content and caloric content of breast milk (defined as the first sample pumped in the AM after the nighttime sleep stretch).

**Definition of variables**

The independent variable for the first descriptive research question and the primary null hypothesis is mothers with breast milk volumes of $\geq$ 900 ml/day compared to those who produce $\leq$ 500 ml/day (Hassitou et al., 2013). Composite breast milk is the unit of analysis. This is the breast milk that is obtained from the entire breast pumping session and is the breast milk that is most routinely fed to premature infants who receive MoM (Daley et al., 1993; Hassitou et al., 2013). The dependent variable is the fat (grams/liter) and caloric content (kcal/ounce) of the breast milk that can range from 16 kilocalories per ounce to 32 kilocalories per ounce or more (Hassitou et al., 2013; Lin et al., 2011).

The independent variable for the second descriptive research question is the maternal volume of breast milk at single pumping sessions, and the dependent variable is the fat content and caloric content of breast milk. The interval of time between the pumping sessions in this study was defined as the time lapsed from the last pumping session of the evening (HS) to the period of time of the first pumping session of the morning after a nighttime sleep stretch. Systematic pumping support is critical to achieve a minimum supply of MoM with a goal of 500 ml/day or greater (Lucas, Paquette, Briere & McGrath, 2014). To achieve this, it is recommended that mothers pump 7-8 times per day with a hospital grade breast pump with a maximum sleep stretch of 4-5 hours per night (Doughtery & Luther, 2008; Jackson, 2010; Underwood, 2013).

## Methods

### Study design and sampling

A descriptive cross sectional design was used with the intention to observe, describe and document aspects of the fat and caloric content of breast milk of pump-dependent mothers of premature infants to provide data and information to generate future hypotheses and research (Hully, Cummings, Browner, Grady & Newman, 2013; Myers, Gamst & Guarino, 2013; Trochim, 2006). Descriptive cross sectional research designs describe relationships among variables and do not support inferences of causation (Hully, Stevens, Browner, Grady & Newman, 2013; Polit & Beck, 2012). Convenience purposive sampling was used in this study due to feasibility. Due to minimal funding, convenience purposive sampling was inexpensive and accessible and allowed for the study of a previously unexplored topic (Burns, Grove & Gray, 2013).

### Subjects

Sample size was determined by a power analysis. In a previous study that measured the fat and caloric content of mothers of breast milk expressed for a premature infant (Veira, Moreira, Rocha, Pimenta & Lucena, 2004), the fat and caloric content of MoM was normally distributed with a standard deviation of 8 fat grams/liter. Based on this study, we powered our study to see a difference of 6 grams/liter between groups. Therefore, we would have needed to study 29 experimental participants and 29 control participants to be able to reject the null hypothesis that the population means of the experimental and control groups are equal with a probability or power of 0.8. The type 1 error probability associated with the null hypothesis is 0.05. Data collection was stopped from the single recruitment site after one full year with the

agreement of the primary investigator's doctoral committee with 41% of the sample size achieved.

This study was approved by the MUSC Institutional Review Board (IRB PRO00056491). Mothers who were lactating in the neonatal nurseries at MUSC were known to the PI of this study in her work as a lactation consultant and nurse practitioner. Pumping logs were reviewed to determine their average daily milk supply prior to determination for inclusion into the study.

**Eligibility criteria**

- Any mother who was pumping and providing breast milk for of a hospitalized premature infant who was 25-36 weeks gestation at the time of sample collection that was producing a minimum of 100 ml more than the daily volume needs of her infant (Patel et al., 2007) and had a current daily breast milk supply of $\leq$ 500 ml/day or $\geq$ 900 ml/day. Mothers of the hospitalized premature infants in this study were not able to feed their infants at the breast due to developmental immaturity and the mothers were dependent on breast pumping to sustain lactation and provide their breast milk.
- All ethnicities were included, but mothers needed to be able to speak, read and write in English.
- The mother must have been at least 14 days postpartum as breast milk supply is usually established by 14 days postpartum (Bishara, Dunn, Merko & Darling, 2009; Meier, Engstrom, Patel et al., 2010).
- Mother must have been producing greater than 150 ml/day (at least 100 ml greater than the current daily volume needs of her infant) and not currently taking a medicine (galactogogue) to increase milk supply. Mothers with inadequate milk supply are in need of intervention and treatment and did not qualify for this study and were

therefore excluded from the study. Treatment with a galactogogue, a medication to increase breast milk supply, may present a confounding variable that affects fat and calories in breast milk (Jackson, 2010). Mothers must have been able to provide enough breast milk to meet the needs of her infant in addition to the breast milk samples required for the study at the time of breast milk sampling. There was to be no risk to the nutritional needs of the infant to provide additional breast milk samples for this study.

- Mothers must have been able to document their pumping times and volumes produced in milliliters over a three to four day period and label breast milk containers according to study protocol. The three to four day study period was chosen as studies of expressed human milk have shown low levels of bacterial contamination even after four to eight days of refrigeration (Eglash, Simon & ABM, 2017). To maintain the integrity of the samples, all MoM was tested within 24-48 hours of receipt of samples by the PI and to prevent a decline in fat content (Bertino et al., 2013). Fresh MoM samples were tested as previously frozen samples have demonstrated a reduction in fat content due to the freezing process. Chang et al. (2012) demonstrated that the freezing and thawing process of human milk results in a reduction of fat in human milk up to 9%.

**Procedures**

- Each mother was fully informed of the study and signed an informed consent per MUSC IRB procedures prior to participating in the study. Consent was written according to IRB regulations at the research site (MUSC) (IRB PRO00056491). The consent and procedures were also submitted and accepted for IRB approval at the University of Wisconsin-Milwaukee (where the PI is a doctoral student).
- Additional variables that were retrieved from the electronic medical record (EHR) included maternal age, BMI documented in EHR at time of data collection, marital/single/cohabitation status, and insurance status. Infant variables to be retrieved from the EHR include gestational age at birth, gestational age at point of breast milk sample collection, and birth weight.
- All research participants documented the time of each pumping session and volumes of breast milk produced at each pumping session for three to four consecutive days on a pumping log. Mothers were instructed to empty both breasts into a breast milk container sufficiently large enough to combine the breast milk of both breasts (composite breast milk sample) and then collect 10 ml just after their longest sleep stretch in the AM, and again between 9 AM -12 PM, and between 9 PM – 12 AM (a total of nine samples of 10 ml each). The collection of breast milk within the specified timeframes at three points in the day captured representative samples from each mother with respect to diurnal variations in fat and caloric content of MoM (Manley et al., 2011; Moran-Lev et al., 2015; Kocizewski-Najman et al., 2012; Weber et al., 2001; Lubetsky et al., 2007) and to provide two MoM samples per participant to assess the time interval (night time sleep stretch) hypothesis.

- Each mother was provided with an insulated carrying bag, gel freezer packs and hospital-approved breast milk sample containers to collect study samples. Instructions were provided to refrigerate the breast milk and not to freeze it. Mothers were instructed to refrigerate their breast milk samples at home and bring them to the primary investigator (PI) in an insulated cooler within the day of or day after of the three to four day sample collection period. The mothers transported their study samples to the research site within 24 hours of collection. If the mothers did not have sufficient transportation to bring the samples to the site within the specified timeframe, the PI met the mother at an agreed upon location to collect them.
- All samples were measured fresh, refrigerated and never frozen. The rationale for testing milk in the fresh state was based upon the work of Chang et al. (2012) and Veira, Moreira, Rocha, Pimenta and Lucena (2004) who demonstrated a reduction in fat after freezing and rewarming breast milk samples. Lucas et al. (1978, 1983) reported that the temperature to which milk samples are subjected to exert a significant effect on the fat values of breast milk and fresh breast milk has a fat content higher than breast milk that has been subjected to changes in temperature. Chang et al. (2012) and Veira et al. (2004) demonstrated a reduction in fat after freezing and rewarming breast milk samples.
- All samples were delivered to, handled and tested by the PI within 24 to 48 hours of receipt of the samples. The PI provided contact by cell phone and each mother was encouraged to call with any questions or review of instructions throughout data collection.

## Measurement

**Pumping log**: Maternal breast milk volume and timing of each pumping session was documented by the mother using a pumping log which is a standard log and educational tool provided to all mothers at the designated research site. The log is simple and easy to read and has been approved at the research site as a standard documentation tool that all participants are familiar with. Mothers were instructed to document the date, the time of each pumping session and the amount of breast milk produced at that session in milliliters over a three to four day period.

**Creamatocrit Plus instrument:** The Creamatocrit Plus (Separation Technology, EKF, Inc.) was the primary instrument of the study**.** The creamatocrit method for measurement of fat and calories in human milk was first introduced by Lucas, Gibbs, and Lyster in 1978 and further described by Lemons, Schreiner, and Gresham in 1980. It is similar to the hematocrit method and calculates the percentage of cream to total volume that is then substituted into an equation to yield energy or calories (fat grams/liter and kilocalories/ounce).

In Chapter 3 of this dissertation, a summary of the accuracy, reliability and validity studies of the creamatocrit method is discussed. A comparison and synopsis of the current manufacturer's recommendations for use of the Creamatocrit Plus (Separation Technology, EKF, Inc.) is discussed. In addition, clinical pearls in the handling and testing of MoM to achieve accurate and reliable readings of the fat and caloric content of breast milk with this instrument is also presented. In summary, four studies demonstrated the accuracy and validity of the Creamatocrit Plus instrument for the measurement of caloric and fat content in maternal breast milk compared to gold standard measurement techniques for human milk fat and caloric density

in the industry such as bomb calorimetry and laboratory assay analysis (Du et al., 2017; Griffin, Meier, Bradford, Bigger & Engstrom, 2000; Meier, Engstrom, Zuleger, et al., 2006; Lin, Hsieh, Chen, Chiu & Su, 2011). One study demonstrated mothers' abilities to perform the creamatocrit procedure in a hospital setting compared to advanced practice nurses' experienced in this measurement instrument (Griffin et al., 2000).

We have also found that using fresh MoM that may have been refrigerated but never frozen leads to more reliable readings with the Creamatocrit Plus. Each breast milk sample was warmed to body temperature (37$^{o}$C) using the Medela® Waterless Warmer (Medela, Inc., McHenry, IL) to homogenize the fat in the sample just prior to testing and each sample was swirled 5-8 times prior to drawing the breast milk into the creamatocrit tube. Two samples of the same MoM sample were crossed checked for accuracy due to the potential of spurious results between two measurements that may occur due to blow-out of the putty or leakage of MoM in the creamatocrit tubing during the centrifuge process. Therefore, creamatocrit readings that did not fall within the range of one kilocalorie of each other of the same MoM sample were retested.

**Data management plan**

- The PI (who is a doctoral student and experienced nurse practitioner and lactation consultant in high risk nurseries) worked directly with an experienced biostatistician.
- All data was stored in a RedCap database with storage and each participant had a unique study identifier that had no meaning to the study database. This enabled de-linking of the study data from personal identifiers for the purpose of maintaining confidentiality (Hully et al., 2013). Additional data gathered from the infant and maternal record was associated with their unique subject ID.

- The PI conducted all of the research procedures, including consent of participants, maternal and infant record reviews, and creamatocrit testing of the milk samples and entered all data into the RedCap data file. All data entries were cross-checked at least twice.

**Data analysis plan**

- Data analysis was performed using the latest version of SAS® by an experienced biostatistician (Kristen Morella, MPH).
- Descriptive statistics were used to describe the characteristics of the study sample.
- For categorical variables, a Fisher's exact test was used to test the probability of two groups having different proportions. For continuous variables, simple t-tests were performed (Kovach, 2015; Myers et al., 2013).
- A Student's T-test was used for the primary null hypothesis for the comparison of Groups 1 and 2 (high versus average to low producers of breast milk) to compare the mean score on an interval or continuous level variable (fat content and caloric content of MoM).
- Linear regression analysis was used for the remaining hypotheses (two to nine measures per hypothesis) as it holds that the assumption that the relationship between the covariate and the dependent variable is linear (Myers et al., 2013; Polit & Beck, 2012). It was performed to determine the relative contribution of the independent variable and its linear relationship with the outcome variable of fat content and caloric density of MoM in mothers who are pumping for a hospitalized premature infant. There were nine measures per participant (n=198 MoM samples) that were included in the analysis of the volume in the breast at single pumping sessions for the secondary null hypothesis (2a) and two measures per participant (n=44 MoM samples) included in the interval between pumping sessions for the secondary null hypothesis (2b).

Chapter 4 of this dissertation contains the final results manuscript that summarizes the background, methods, results and conclusions of this study. Chapter 5 covers recommendations for future research and policy and implications for clinical practice.

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CHAPTER 2

# COLLECTION TECHNIQUES OF COLOSTRUM FROM HOSPITALIZED MOTHERS OF VLBW INFANTS FOR OROPHARYNGEAL CARE AND EARLY TROPHIC FEEDINGS

Abstract

Administration of colostrum for early trophic feedings and (colostrum) oral immune therapy (C-OIT) for NICU infants enhances gut maturation and lowers the risk of infections.  All health care providers who care for mothers after birth face challenges to collect early colostrum due to the thick viscosity and low volume.  Mothers may be unable to sit during pumping sessions from post-surgical pain, acute or chronic illness, or birth complications.  Colostrum is essential for early trophic feedings and C-OIT, and specific collection techniques from hospitalized mothers are discussed.  Additionally, early collection and administration of colostrum to preterm infants has been demonstrated as a factor to engage and motivate mothers to continue pumping and providing breast milk through discharge.

Keywords: colostrum; trophic feedings; oral care; breast milk; VLBW infants; NICU infant; preterm infant

Precis: Nurses must assist hospitalized mothers of VLBW infants in the NICU as needed to collect colostrum for tropic feedings and C-OIT

Callout 1: The many important characteristics of colostrum for early trophic feedings and C-OIT and for VLBW, preterm, and sick infants are summarized.  21 words

Callout 2: Initial colostrum is viscous and may be challenging for women to collect who are unable to sit up immediately after the birth of their infant.

Callout 3: Methods to capture every drop of colostrum are described and shared with photographs.

Nurses have an important role to care for mothers of preterm infants in the early post birth period specific to colostrum expression. Nurses must educate and assist mothers with techniques to express and collect colostrum to provide immune therapy for their preterm infants Early trophic feedings and colostrum for oral immune therapy (C-OIT) for very low birth weight (VLBW) preterm infants are essential to enhance gut maturation and immune development (American Academy of Pediatrics (AAP), 2012; American College of Obstetricians & Gynecologists (ACOG), 2016; Association of Women's Health, Obstetric, and Neonatal Nurses (AWHONN), 2015; Gephart & Weller, 2014; Lucas & Cole, 1990; Meier, Engstrom, Patel, Jegier, & Bruns, 2010; Newburg & Walker, 2007; Sift Investigators Group, 2013; Wagner, Forsythe & Pittard, 1995; World Health Organization, 2009). Trophic breast milk feedings are not intended to be nutritionally meaningful; rather, they are intended to stimulate bioactivity in the neonatal gut and bridge the gap between the in-utero to external world. Premature infants have immature intestinal development and are at high risk for prolonged gut maturation that increases their risk of infections that include necrotizing enterocolitis (NEC) and nosocomial infections (NI) and increase the risk of intestinal malabsorption (Taylor, Basile, Ebeling, & Wagner, 2009). Thus, macrophages, present in colostrum, travel to the gut, survive up to one week, and secrete intestinal growth factors and anti-inflammatory cytokines as a way to prevent infections (Kobata et al., 2008; Wagner, Taylor, & Johnson, 2008). The result is that human milk provides growth factors and gut peptides that facilitate the maturation of the neonatal gastrointestinal tract (Wagner, 2002, personal communication, Wagner, 2017).

The importance of providing colostrum for trophic feedings has been documented for many years (Mei, Zhang, Whang, Sangild, & Zu, 2006; Meier et al., 2010; Wagner, 2002), and has been incorporated into standard practice in many neonatal intensive care units (NICUs). As

a result, early exposure to colostrum and enteral feedings can significantly reduce the use of parenteral fluids, decrease the time to full enteral feedings, and lower the risk of infections for preterm infants (Hamilton, Massey, Ross, & Taylor, 2014; Moles et al., 2015; The SIFT Group, 2013).

However reports that demonstrate the safety, efficacy, and impact of providing colostrum for oropharyngeal care and as immune therapy (C-OIT) for preterm infants are more recent. In Table 1, recent reviews and studies demonstrate the safety and efficacy of colostrum as oral immune therapy (C-OIT). Many of the papers report mechanisms through which C-OIT work to protect preterm infants from NEC and NI. The purpose of this paper is twofold: 1) discuss the important properties of early C-OIT/oropharyngeal care and tropic feedings; and 2) provide examples of methods to collect viscous colostrum from hospitalized mothers who may not be able to sit up in a bed or chair to express colostrum by hand, or by breast pump during early hospitalization.

**Making the case for early colostrum collection**

Colostrum is rich in proteins, lipids, vitamins, minerals, growth factors, and immune cells (Hassiotou et al., 2013; Lee et al., 2015; Meier, et al, 2010). Colostrum and breast milk from mothers of preterm infants have higher levels of protein, fatty acids, nitrogen, immune factors, cells, immunoglobulins, and anti-inflammatory properties than mothers of term infants (Castellote et al., 2011; Fernandez, Langa, Martin, Jimenez, Martin, & Rodriguez, 2013; Moles et al., 2015). The highest concentration of protective factors is in the colostrum of mothers of VLBW infants; there is an inverse relationship between duration of pregnancy and gestational age of infant and the concentration of protective factors (Araujo et al. 2005; Dvorak, Fituch, Williams, Hurst, & Schanler, 2003; Koenig, de Alberquerque-Diniz, Barbosa, & Vaz, 2005).

There are also transitions in other components of intra- and extra uterine development as fetal and infant life progresses along a continuum.  For example, amniotic fluid and human milk share bioactivity and have the capacity to stimulate cell growth and enhance reparative processes. Amniotic fluid plays a significant role in gut maturation as it baths the primitive gut that later becomes the gastrointestinal tract.  Mother's Own Milk (MoM) provides nutrients as well as bioactive properties that stimulate neonatal cell growth and provide immune protection and facilitate immunocompetence (Wagner, 2002; Newburg, 2001 & Bernt & Walker, 2001). Ultimately providing MoM can decrease morbidity and mortality during the first 60 days of the infants life  (Corpelijn et al., 2012).

Human growth factors and hormones are also present in colostrum and mature breast milk and enhance growth of epithelial cells and gut maturation (Dvorek et al., 2003; Fernandez, et al., 2013).  As an example, stem cells have been identified in human mammary tissue (Cregan et al., 2007).  Human growth factors include polypeptides such as epidermal growth factor (EGF) that stimulate the proliferation of the intestinal mucosal epithelium and strengthen the mucosal barrier to antigens (Dvorek et al.; Fernandez, et al.).

Colostrum and mature breast milk contain bioactive cells highest in concentration just after birth (Balland & Morrow, 2013; Castellote, et al., 2011), and are impacted by the type of delivery (Cabrera-Rubio, Carmen Collado, Laitinen, Salminen, Isolauri, & Mira, 2012).  The two types of cells in colostrum that are highest in concentration after birth are phagocytes and lymphocytes, which provide significant protection against infection (Castellote, et al.; Fernandez, et al., 2013).  Lymphocytes are highest in breast milk just after birth and are activated into memory T-cells that can last for years in the infant and are critical to long-term immunity (Wirp, Adkins, Palkowetz, & Goldman, 1992).  Along with T-cells, lymphocytes contain β-cells, which

provide antiviral activity and cell-mediated immunity (Wirp et al.).  Phagocytes are also present in colostrum and release sIgA, important to absorb and engulf pathogens (Fernandez, et al.).

Although the collection of colostrum from mothers of preterm infants is important to provide the infants with immune therapy, nurses, lactation consultants and midwives who care for mothers in the early postpartum period face difficulties collecting colostrum.  Often mothers who deliver preterm infants may be unable to sit up, and must remain side lying or supine because of illness or birth complications.  During this time, colostrum may be present in small amounts and too viscous to collect via an electric hospital grade breast pump.  However, collecting colostrum and using it for oral care for their infants has been reported as a strong motivator for these mothers (Froh, Deatrick, Curley, & Spatz, 2015).  These researchers reported that for mothers of preterm infants, collecting colostrum for oral care for their infants was a strong motivator that encouraged them to continue to pump during their hospitalization.  These women described being able to express colostrum, as: “it motivates me,” “I’m a part of my baby getting better,” “doing it together,” “we’re getting somewhere” (Froh et al.)

**Collection of colostrum in the early postpartum period**

It is very important that hospitalized preterm infants to receive colostrum during the first few hours after birth in order to prime the infants’ gut.  Despite this recommendation, it may be difficult for some mothers of preterm infants to collect sufficient volumes during the first three days because of the variable viscosity and volumes of colostrum produced by pumping alone. Any delay in colostrum collection results in a delay in C-OIT and early trophic feedings.  If mothers are able to change positions they are encouraged to be upright, or side lying, If they need to remain supine in their hospital beds due to maternal illness, or birth complications, this position may present a barrier to using the pumping technique alone.

Although many researchers promote the collection of colostrum through pumping alone, many women are unable to express drops for C-OIT. Morton, Hall, Wong, Thauiru, Benitz, & Rhine (2009) demonstrated in their study of mothers with hospitalized infants that colostrum is more readily expressed by using hand expression and a hands-on pumping technique, than with pumping alone. Thus all nurses should be competent to help mothers collect colostrum soon after birth to provide tropic feedings and C-OIT and for their VLBW infants. Additionally, partners or significant others should be taught, and may participate in the collection process at the mother's discretion if she is temporarily unable to collect colostrum or breast milk on her own.

It is also important to begin early colostrum expression for other reasons. Morton, et al. (2009) reported that the mean daily volumes (MDV) of mothers who used hand expression and hands-on pumping techniques more than five times per day in the first three days postpartum had MDV of 820-955 milliliters per day by week eight. Additionally, Hill, Aldag, Zinnaman, and Chatterton (2007) reported that mothers who obtained volumes greater than 500 ml/day by week 6 predicted extended breast milk feedings of preterm infants. Thus practice contributes to attaining sufficient volumes to meet the infants' needs as well as positively impacting duration (Hill et al.; Morton et al.)

In order to facilitate collection of colostrum, the Medical University of South Carolina (MUSC), a tertiary medical center in southeastern United States with Level 2 and 3 nurseries, and a Baby Friendly Hospital designation first implemented the colostrum collection protocol in 2015. At MUSC, all women who are in preterm labor or at risk of delivering prematurely are counseled by neonatologists, neonatal nurse practitioners or neonatology fellows regarding the evidence to support the numerous benefits to provide their own colostrum and breast milk for

their infants. Lactation consultants with experience to support lactation of mothers of preterm and medically complex infants are available: 1) in the antepartum period for educational consults and assessments; 2) in the postpartum period and throughout their stay; and 3) throughout their infant's hospitalization. Mothers are educated about the value their own colostrum and breast milk has for their preterm infant, and about the medicinal and nutritional benefits. Mothers are supported in their choice to pump and transition to breastfeeding at the breast, or pump only and feed expressed breast milk by bottle. Next, all mothers who agree to provide breast milk, and who are separated from their infants are instructed in and assisted with pumping, hands-on pumping, and hand expression within 6 hours of birth as this is the official recommendation based on Baby Friendly Hospital recommendations (Parker, Sullivan, Krueger, & Mueller, 2015; WHO, 2009). While those mothers who are able to put their well infants to breast are encouraged to do so within one hour after delivery, mothers with ill infants are encouraged to begin pumping and hand expression within an hour or two after delivery and to continue doing so every three hours.

The colostrum collection techniques that are presented in this manuscript are used by MUSC lactation consultants and registered nurses. For women with a preterm or sick infant, we begin expression with a hospital grade pump AND expression by hand. For women who begin with the electric Medela Symphony® breast pump, they are instructed to use the Medela Initiation® mode (irregular breast pump suction pattern that mimics the suck pattern of a term newborn) with the Symphony® breast pump in the colostrum phase (until the mother achieves three consecutive pumping output measures of 20 ml or more, and no longer than up to five days postpartum) (Meier, Engstrom, Janes, Jegier, & Loera, 2012; Post, Stam, & Trom, 2015).

**Procedure to assist a mother with collection of colostrum.**

- If mother can tolerate it, she is encouraged to sit upright, or help her move to a side-lying position, which may facilitate flow of less viscous colostrum through the pump parts. Otherwise provide assistance to the mother in the supine position.
- Wash hands thoroughly and prepare the pump parts of the hospital grade breast pump.
- Describe the process prior to beginning pumping and request the mother's permission to perform the technique.
- Hold flanges in place on maternal breasts and proceed with pumping (Figure 1.1).
- Use hands-on pumping technique (massage in the direction of the nipple) to improve flow of colostrum.
- When colostrum is visible in the flange at the base of the nipple, hold flange in place at the breast, disconnect remaining pump parts, and draw the colostrum up with a sterile food grade BPA-free syringe without a needle (Figures 2.1-2.2).
- Label container according to specific hospital guidelines
- Colostrum in quantities of $\leq$ 3 ml may be transported in the capped syringe with appropriate labeling, by hand, to the neonatal nursery, because the cap comes off in the tube system.
- Amounts $\geq$ 5 ml can be transferred to a hospital-approved breast milk container.
- Repeat this process as necessary.
- At transport colostrum to the NICU, notify the infant's nurse that colostrum is available for oropharyngeal care and early trophic feedings for the infant.

**Hand expression technique**.

- Wash hands thoroughly.
- Describe the process prior to beginning the technique and obtain mother's permission to perform hand expression (Morton, et al., 2009).
- Perform the hand expression technique and collect colostrum in a hospital approved breast milk container, preferably a small container designed specifically for colostrum or small breast milk volumes, or a medicine cup with a narrow lip.
- Repeat the technique as needed during the expression session.
- If the mother is unable to hand express colostrum herself, the postpartum nurse, her partner, or significant other may hand express colostrum with her permission.
- If mother is not encumbered by intravenous and monitoring lines and is sufficiently alert, request a return demonstration of the hand expression and colostrum collection technique.

Insert figures 3.1-3.7 here.

- The hand expression technique can also be performed with a syringe used directly at the breast.
- The colostrum can be siphoned directly from the breast with a sterile BPA-free syringe and then transferred to a hospital-approved colostrum container or syringe.
- Amounts of one milliliter (mL) or greater are utilized for early trophic feedings.
- Amounts of $\leq$ one mL are used for oral care.

Insert figures 4.1-4.2 here.

**Conclusion**.

More than two decades ago, Wagner et al. (1995), reported that human milk plays a significant role in gut maturation through the presence of human milk macrophages that secrete

transforming growth factor-alpha. Due to colostrum's high content of molecular weight antibodies, anti-inflammatory factors, and protective components, early trophic feedings of colostrum are to be given in the early post-birth period, which is the ideal stage of absorption due to neonatal gut permeability (Wagner, et al., 1995). The evidence in this paper has reported the significance of C-OIT in addition to early trophic feedings to optimize health of the preterm and VLBW infant (Snyder et al., 2017).

When mothers of preterm and sick infants experience separation, the expression of colostrum can provide a sense of accomplishment and pride in being able to provide their own colostrum and breast milk for their vulnerable infant (Froh et al., 2015). Early, effective and frequent pumping combined with massage and hand expression of colostrum influences the onset of milk production and improves milk volumes (Morton et al., 2009; Parker, Sullivan, Krueger, Kelechi & Mueller, 2012; Parker et al., 2015). Mothers who express breast milk for early trophic feedings are more likely to continue to provide breast milk as the primary source of feeding for their preterm infants. Nurses, lactation consultants and midwives play a key role in facilitating collection of colostrum for the critical early trophic feedings of premature infants and the promotion of overall lactation success of their mothers.

**Implications for nurses at the bedside**

Implementation of the colostrum collection procedures presented in this paper may have a direct impact on the health of preterm infants. These procedures can be used for all hospitalized infants who are separated from their mothers at birth due to illness, congenital anomalies, or acute maternal illness. There are two key take away messages for nurses who support mothers who have recently delivered an extremely preterm infant, and may be working in in- and outpatient settings. For those nurses working at the bedside in birthing, mother-baby,

and neonatal intensive care units, colostrum for the preterm or ill infant in the NICU is as least as important as any medicine that the infant may receive. This paper has provided examples of how colostrum and MoM help protect infants from infections such as NEC or NI, and further serve as immune therapy which is important for vulnerable infants. Secondly, this paper provides nurses who are caring for women directly after delivery and during the postpartum period with techniques to help women collect and provide the smallest amounts of colostrum for their infants regardless of how sick they may be. Many women who give birth to a preterm infant have done so because delivery was required to save the woman's life. These are techniques that can be used by nurses and the woman's partner to assist with collection of colostrum.

**Implications for nursing research**

Many health care providers support women, their infants, and their families, in in- and outpatient settings to provide colostrum and breast milk for their infants. Although no one would disagree that for women to breastfeed and/or provide breast milk feedings via pumped milk are some of the most important postpartum events, many unanswered questions remain. For example, there continues to be debate about how timing to initiate pumping and hand expression may impact a woman's ability to provide drops of colostrum for her preterm/sick infant in the NICU. Yet, there are still individuals who encourage women to begin pump and hand expression within an hour of delivery at a time when they may be very sick after a preterm delivery was performed to save the mother's life. Parker et al (2015) reported that women who began expression within six hour of birth had larger volumes of milk at initial expression, and on days six, seven, and 42. When these same researchers removed women who began expression prior to one hour after birth from their analysis, there were no longer differences in the onset of lactogenesis II between two groups who began pumping: 1) within six hours, or 2) after six hours

Parker et al (2015). Although health care providers may recommend pumping with one hour as we recommend that newborns be put to the breast within one hour, we use the BFH guidelines (WHO, 2009) of beginning expression within six hours until there is other research to demonstrate otherwise. Also, let us remember to support and care for mothers who have just given birth to an extremely preterm or VLBW infant in ways that will optimize the health of their infant and themselves as they transition to being a mother of a VLBW infant.

*Figure 1.1* Placement of pump flange to breast

*Figure 2.1* Siphon Colostrum With Syringe While Holding Flange In Place At Breast

*Figure 2.2.* Siphon Colostrum From Flange Held At Breast

*Figure 2.3.* Siphon Colostrum From Pump Flange Held At Breast

*Figure 2.4.* Siphon Colostrum With Syringe While Holding Flange At The Breast


*Figure 3.1.* Hand Expression Technique Into Colostrum Container Or Medicine Cup

*Figure 3.2.* Hand Expression Of Colostrum Into Colostrum Container Or Medicine Cup

*Figure 3.3.* Hand Expression Of Colostrum Into A Container


*Figure 3.4.* Hand Expression Into Colostrum Container Or Medicine Cup

*Figure 3.5.* Hand Expression Into Colostrum Container Or Medicine Cup

*Figure 3.6.* Hand Expression Into Colostrum Container Or Medicine Cup

*Figure 3.7.* Hand Expression Into Colostrum Container Or Medicine Cup

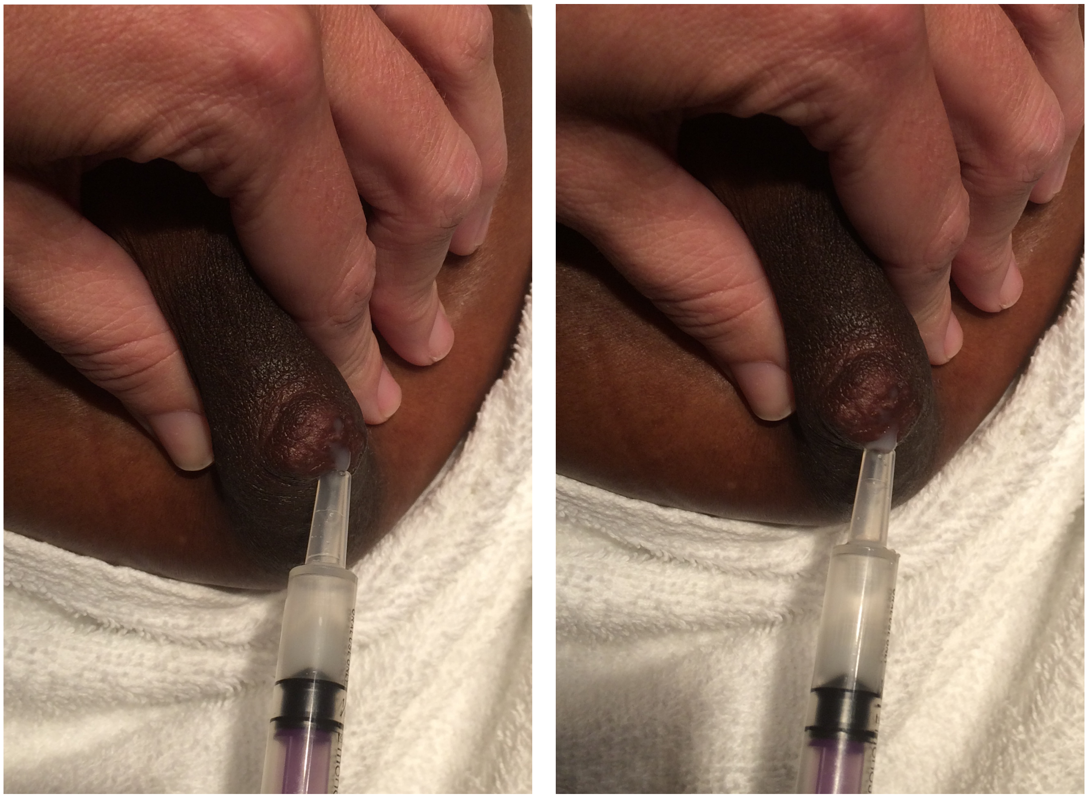

*Figure 4.1-4.2.* Hand Expression & Collection Of Colostrum Directly From Nipple

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Table 1

*Colostrum as oral immune therapy (C-OIT)*

| Reference(s) | Study Type | Summary/Results |
|---|---|---|
| ***OC for VLBW infants:*** Rodriguez et al., 2009 Bocci, 1991 Roitt, et al., 2001 | Systematic review of literature | Colostrum, as immune therapy, may compliment early trophic feedings in vulnerable, immunocompromised PTI who is exposed to prolonged NPO status, invasive life-saving procedures, & long (> 3-4 months) hospital stays & increased risk of iatrogenic infections. Gavage feeding bypasses the oropharyngeal-associated lymphoid tissue (OFALT) system consisting of the palatine tonsils & adenoids. OC is placing small amounts of colostrum directly onto the oral mucosa so the liquid & its bioactive components are absorbed by the mucus membranes. The gut-associated lymphoid tissue (GALT) may be stimulated from swallowing. Colostrum administered via the oropharyngeal route is associated with transfer of cytokines (Bocci, 1991), messenger molecules, which are synthesized & rapidly excreted by many cells in response to exogenous antigens or cytokine stimulus (Riott et al., 2001). |
| Ellis et al., 1997 | Review of literature | Numerous bioactive properties are present in MoM. Prolactin (23-24-kDa prolactin [PRL]) was initially recognized as having endocrine function & is recognized as potent immunomodulatory factor with cytokine-like action on neonatal immune cells. It promotes lymphocyte maturation & lymphocyte migration of activated immune cells administered via the OFALT/GALT systems from primary to secondary immune organs. |
| ***OC as oral immune therapy*** Gephart, & Weller, 2014 | Review of literature | Preliminary evidence demonstrates that colostrum as oral immune therapy (C-OIT) reduces time to full enteral feedings, is safe, & feasible. Future studies must be adequately powered to detect significant differences in outcomes of sepsis, NEC, & death. Procedures to measure |

| Reference(s) | Study Type | Summary/Results |
|---|---|---|
| | | the impact on the neonatal immune system must be determined. C-OIT poses a minimal risk to ELBW infants & a cost-effective intervention to improve beneficial neonatal outcomes. |
| ***Safety & feasibility of OC for ELBW infants:*** Rodriguez et al., 2010 | Pilot study: Safety & feasibility of C-OIT | Purpose to: 1) determine the safety & feasibility of oral administration of own mother's colostrum to ELBW infants; & 2) measure secretory immunoglobulin A & lactoferrin in tracheal aspirates & urine. 5 ELBW infants were administered 0.2 ml of own mother's colostrum every 2 hrs. beginning at 48 hrs. for 48 hrs. duration.<br>-Mean gestational age of infants 25.5 weeks<br>-$O_2$ saturation remained stable, no episodes of apnea, bradycardia or hypotension<br>-Wide variation in concentrations of secretory immunoglobulin A & lactoferrin in urine & tracheal aspirates among 5 infants<br>-all infants began to suck on the endotracheal tube when the colostrum was administered<br>Conclusion: Easy, inexpensive procedure, & well tolerated. Future research should examine optimal procedure to measure direct immune effects & clinical outcomes. |
| ***OC for VLBW infants:*** Rodriquez et al., 2015 | RCT protocol | Purpose of 5-year multi-center RCT to evaluate safety & efficacy of oropharyngeal administration of MoM to reduce late onset sepsis, NEC, & death in 622 infants. Group A received 0.2 ml of MoM via oropharyngeal administration every 2 hrs. for 48 hours, then every 3 hrs. to 32 weeks CGA. Group B received placebo (0.2 ml sterile water) with the same protocol. Milk, urine, mucosal swab, & stool samples will be collected at same time points before, during & after intervention. |
| Lee et al., 2015 | RCT | To determine immunologic effects of oropharyngeal colostrum administration, in this RCT among 48 PTI $\leq$ 28- |

| Reference(s) | Study Type | Summary/Results |
|---|---|---|
| | | weeks. Randomized to colostrum or sterile water oral care every 3 hrs. Lactoferrin assessed at 1 & 2 weeks. At 1 wk., lactoferrin in colostrum group higher (p=.01). At 2 wks, urine interleukin 1β level was lower in colostrum group. At 2 wks, salivary transforming growth factor β1 & interleukin-8 lower in colostrum group. There was reduction of clinical sepsis in colostrum group (p=.003).<br>Conclusion: Larger studies are needed to determine if OC with colostrum in PTI will decrease clinical sepsis, inhibit secretion of pro-inflammatory cytokines, & increase circulating immunoprotective factors. |
| ***Safety, feasibility,& impact of BM feedings through discharge:***<br>Snyder et al., 2017 | Observational longitudinal | To confirm safety & feasibility of OC & impact of OC in the first days with sustained breast milk feedings through discharge in preterm infants. N=218<br>(n=133) VLBW infants received OC compared to a pre-OC, age-matched control cohort in a previous study (n=85). No adverse events/changes in vitals during OC. There were associations between OC application & majority of enteral feedings as BM at 6 weeks of age (p=0.03) & at DC (p=0.007).<br>Conclusion: Infants who received OC were more likely to receive BM at DC. |

Abbreviations:
BM=Breast milk
C-OIT =colostrum as oral immune therapy
CGA=Corrected gestational age
DC=Discharge
ELBW=Extremely low birthweight infants
GA=Gestational age
MoM = Mothers own milk
NEC=Necrotizing enterocolitis
OC=oropharyngeal care
PTI=Premature infants
VLBW=Very low birthweight infants

CHAPTER 3

# CREAMATOCRIT MEASUREMENT OF FAT AND CALORIC CONTENT OF MOTHER'S OWN MILK IN THE NEONATAL INTENSIVE CARE UNIT: CLINICAL PEARLS TO ACHIEVE ACCURATE AND RELIABLE RESULTS

Abstract

Mother's own milk (MoM) is associated with numerous benefits for premature infants including improved neurodevelopment and decreased risk of necrotizing enterocolitis, late onset sepsis and retinopathy of prematurity. Human milk fat may comprise more than half of MoM, but is also the most variable macronutrient due to potential maternal individual differences, circadian or diurnal variations, and the impact of the interval between pumping sessions. There is also a risk of loss of human milk fat through gavage tubing and during storage and handling which places the premature hospitalized infant at further risk of inadequate growth velocity. Therefore, a feasible, rapid, and inexpensive bedside instrument for measurement of fat and calories of MoM in the clinical setting can provide one method of assessing and problem-solving inadequate growth velocity.

The current creamatocrit instrument (Creamatocrit Plus, Separation Technology, EKF, Inc.) available on the market is accompanied by an instruction manual that provides the clinician with instructions in the use, cleaning and maintenance of the instrument. This paper goes beyond the instruction manual to summarize the research evidence of the creamatocrit method and presents clinical pearls and recommendations to collect and

measure MoM to assist health care providers in the neonatal intensive care unit (NICU) setting to achieve accurate and reliable results.

**Background**

Mother's own milk (MoM), provides numerous nutritional benefits as well as bioactive properties including stem cells, growth hormones, pre- and probiotics, and anti-infective and immune-building properties (CDC, 2012; Patel, Meier & Engstrom, 2007). MoM for premature infant nutrition also promotes optimal brain growth, reduces the risk of necrotizing enterocolitis (NEC), chronic lung disease, retinopathy of prematurity, and late onset sepsis and improves neurodevelopmental outcomes (AAP, 2012; Belfort, et al., 2013; Lucas, Morley and Cole, 1992; March of Dimes, 2014; Patel, Meier & Engstrom, 2007).

Although MoM is the first choice for nutrition of the premature VLBW infant, it places the infant at risk of poor growth velocity due to the individual differences, variability of fat content and caloric density due to pumping procedures, diurnal differences in fat content throughout the day, storage and handling of breast milk and fat loss during gavage feedings (Atwood & Hartmann, 1992; Brenham-Behm, Carlson, Meier & Engstrom, 1994; Chang, Chen & Lin, 2012; Daley, Rosso, Owens & Hartmann, 1993; Gidrewicz & Fenton, 2014; Gunther, Camb & Stanler, 1949; Harzer, Haug, Dietrich & Genter, 1993; Hassitou et al., 2013; Jackson et al., 1988; Kociszewska-Najma, Borek-Dzieciol, Szpotanski-Sikorska, Wilkos, Pietzrak & Wilgos, 2012; Lucas, 1983). In order to maximize the fat and caloric content in MoM that is delivered to infants, it is important that health care providers have an understanding of the variability of the fat content. Specifically, individual maternal differences and exogenous risk factors such as

fat loss through gavage feedings and storage and handling of human milk may result in additional loss of fat and calories (Breham-Behm et al., 1994; Chang et al., 2010). The variability of fat content in human milk between mothers, within-mothers and at individual expression sessions throughout the day demonstrates the need for accurate, reliable, inexpensive and feasible testing of MoM in the neonatal intensive care unit.

**Importance of fat (lipids) for the VLBW infant**

Lipids in human milk have a diverse range of biological functions beyond the provision of calories to the growing infant (Belkind-Gerson, Carreon-Rodriguez, Contreras-Ochoa, Estrada-Mondaca & Parra-Cabrera, 2014; Delphanque, Gibson, Koletzo, Lapillone & Strandvik, 2015). The lipid profile and the synergistic properties of human milk are very complex and the physiologic roles are not well understood and require ongoing research (Delphanque et al., 2015).

Fat (lipids) in human milk contains long chain polyunsaturated fatty acids (LCPUFAs) such as doxyhexaeonoic acid (DHA), arachidonic acid (AA), and lipid soluble vitamins (A.D, E, and K) which are known to modulate gastrointestinal function, the immune response, cell membrane composition and function, lipoprotein metabolism, and the function of signaling pathways (Andreas-Kampmann & Mehring-LeDoare, 2013; Belkind-Gerson et al., 2014; Delplanque et al., 2015; Hsiao, Tsai, Chen & Lin, 2014).

LCPUFAs are essential to develop the brain and retina in utero, and AA and DHA are integral to develop~~ing~~ the cerebral cortex (Belkind-Gerson et al., 2014). Infants born prematurely are at greatest risk of DHA and AA deficiency as there is a high demand for the accretion of LCPUFAs such as DHA, and AA in the third trimester in the growing fetus (Al, van Hoeweling & Hornstra, 2003).

Human milk is rich in DHA and AA and is reported in higher concentrations in human milk than worldwide averages of infant formulas. Sabel, Strandvik, Petzold and Lunqvist-Persson (2012) reported a mean concentration of DHA in human milk of 0.44 +/- 0.14% vs. 0.32 +/- 0.22% in formula, and 0.52 +/- 0.09% of AA in human milk vs 0.47 +/- 0.3% in formula). DHA has demonstrated a critical role in neuron survival in animal and human models (Belkind-Gerson et al., 2008; Cao, Xue & Xu, 2005). DHA and AA are rapidly accreted in the brain and retina in the first year of life and are in high concentrations in human milk (Belkind-Gerson et al., 2008). The nervous system of the developing infant is rich in the polyunsaturated fatty acid (PUFA) DHA and is critical to develop the growing brain that undergoes rapid cell differentiation in the first year of life (Belkind-Gerson et al., 2008; Delphanque et al., 2015). The monounsaturated fat nervonic acid (which is important for myelination) has been reported to be 7-fold higher in the breast milk of mothers of premature infants (than in mothers of term infants or formula) and may play a role in brain growth and development (Ntoumani, Strandvik & Sabel, 2013).

DHA is also associated with down regulation of inflammatory responses, and DHA doses equivalent to human milk-fed infants have been associated with a decreased incidence of BPD (Manley, Makrides, Collins, McPhee et al., 2011). Human milk is high in saturated fatty acids that have structural and metabolic functions (Delplanque et al, 2015). Palmitic acid, a saturated fat that is present in human milk, is present in pulmonary surfactant (Schmidt et al., 2002).

Human milk (HM) contains bile-salt stimulated lipase (BSSL) that increases the bioavailability of human milk fat by enhancing lipolysis (Delphanque et al., 2015).

Human milk also contains 90-150 mg/liter (L) of cholesterol that is the substrate for the synthesis of bile acids, lipoproteins, vitamin D, and cell membranes (Belkind-Gerson et al., 2008; Delphanque et al., 2015). Cholesterol is incorporated into brain lipids in the first months of life and stabilizes cellular membranes (Delphanque et al., 2015). There is no cholesterol in vegetable-based formulas and 40 mg/L in dairy fat-based formulas (Delphanque et al., 2015).

Human milk is also rich in complex lipids such as phospholipid plasmalogens, glycoderophospholipids, and sphingolipids that modify cellular metabolism, enhance signal transmission and interact with the physiology of the brain, gut and skin (Belkind-Gerson et al., 2008; Delphanque et al., 2015). Complex lipids in the human milk fat globule also have anti-infective properties (Koletzko, Agostoni, Bergmann, Ritzenhaler & Shamir, 2011).

**Assessment of the caloric and fat content of breast milk in the clinical setting**

Health care providers in the NICU need a simple, inexpensive and user-friendly centrifuge to assess caloric and fat content in the NICU setting. This centrifuge is necessary to make recommendations for individualized lactation interventions to support the growth of hospitalized premature infants. The creamatocrit method for measurement of fat and calories in human milk was first introduced by Lucas, Gibbs and Lyster in 1978 and further described by Lemon, Schreiner and Gresham in 1980. It is similar to the hematocrit method and calculates the percentage of cream to total volume that is then substituted into an equation to yield energy or calories (kilocalories/ounces; kcal/oz).

**Creamatocrit Plus instrument**

The accuracy and validity of the creamatocrit method and the Creamatocrit Plus (Separation Technology, EKF, Inc.) instrument has been reported in six studies. The details and results of these studies are reported in Table 1 (Du et al., 2017; Gibbs, Lyster & Baum, 1978; Griffin, Meier, Bradford, Bigger & Engstrom, 2000; Meier, Engstrom, Murtaugh, Vasan, Meier & Schanler, 2002; Meier, Engstrom, Zuleger, Motykowski, Vasan, Meier, Hartmann & Williams, 2006; Lin, Hsieh, Chen, Chiu, Lin & Su, 2011; Wang, Chu, Mellen & Shenai, 1999).

Table 1

*Creamatocrit Method: Studies of Accuracy, Reliability, and Validity.*

| Reference | Study Design | Sample/Methods | Results |
|---|---|---|---|
| Gibbs, Lyster & Baum (1978) | Descriptive correlational<br><br>CRCT comparison to LA | N=60 MoM samples (n=37 composite, n=23 drip from opposite breast). Fresh MoM tested within 30 min of expression. CRCT measured in triplicate and compared to Gerber Method (requires 150X of amt that CRCT requires) (British gold standard) | CRCT accuracy (95% CI) is ±4.2 g/L; Gerber Method: ±0.05 g/L. Linear correlation between calculated (kcal/L) & CRCT (r=0.99). Accuracy of CRCT ±10% for fat and ±6% for kcal.<br><br>Conclusion: Kcal variation in MoM nearly all due to fat variation. Percent cream in MoM correlates well w/ fat & energy concentration. CRCT feasible, accurate & requires less volume of MoM |
| Wang, Chu, Mellen & | Descriptive correlational | MoM samples collected from N=17 mothers of PT | CRCT measure had strong correlation with fat & caloric |

| Reference | Study Design | Sample/Methods | Results |
|---|---|---|---|
| Shenai (1999) | CRCT comparison to LA | infants (fresh and frozen/thawed). Each sample divided between fresh and frozen. Frozen MoM thawed to 37$^{o}$ C. Samples centrifuged 15 min and tested in triplicate by CRCT method. Compared to lipids checked by gas chromatography. Kcal estimated from a conversion factor equation. | content of samples of fresh & frozen/thawed MoM. Fresh MoM CRCT (range 2.0-7.9%). Statistically sig correlation between CRCT & fat concentration (r=0.92%, p<0.001) & CRCT and caloric content (r=0.90%, p<0.001). Frozen/thawed MoM samples had a small but significant decrease in CRCT (2.0-7.3%). CRCT and fat concentration (r=0.82%, p<0.001) & CRT and kcal (r=0.86%, p<0.001).<br><br>Conclusion: CRCT method simple, accurate, feasible in clinical setting for real time analysis of MoM |
| Griffin, Meier, Bradford, Bigger & Engstrom (2000) | Blinded descriptive correlational w/ qualitative component<br><br>Reliability of CRCT method trial | N=26 mothers of PT hospitalized infants compared to N=4 APRNs. Phase One: Mothers taught CRCT method (mean education time=23 minutes). Phase Two: Mothers & APRNs performed CRCTs simultaneously & independently w/ same MoM sample. Mothers completed a reaction | Mothers CRCT values highly accurate compared to APRN results. Mean absolute difference between mothers & APRNs 0.69% with 50% & 84.6% of these differences respectively $\leq$ 0.5% & $\leq$ 1.0 % for CRCT.<br><br>96% of mothers reported that CRCT was easy to learn, felt comfortable w/ technique and they felt more involved in the care of their infant.<br><br>Conclusion: High inter-user |

| Reference | Study Design | Sample/Methods | Results |
|---|---|---|---|
| | | questionnaire | reliability, feasible, cost effective, and increases maternal involvement and satisfaction w/ their infant's care |
| Meier, Engstrom, Murtaugh, Vasan, Meier & Schanler (2002) | Blinded, descriptive correlational<br><br>Comparison of CRCT to LA | N=22 MoM samples from 17 mothers of PT infants. Compared CRCT w/ hematocrit reader method to gravimetric (GM) bomb calorimetry. Fresh and frozen/thawed samples tested. | Mean fat & caloric concentrations 50.87 g/L (28.3-86.5 g/L) and 703.97 kcal/L. Strong linear correlation between CRCT & total fat (r=0.94, p<0.001) than between CRCT & caloric density (r=0.76, p<0.001)<br><br>Conclusion: CRCT is an accurate, simple procedure for real-time testing of MoM in the clinical setting |
| Meier, Engstrom, Zuleger, Motykowksi, Vasan, Meier, Hartmann & Williams (2006) | Blinded, descriptive correlational<br><br>Comparison of CRCT to LA and colorimetric sprectroscopy, inter and intra-user reliability | N=36 MoM samples from 12 women at least 7 days PP. Comparison of intra-user and inter-user reliability & equivalence of CRCT values w/ 3 methods: standard lab centrifuge w/ a hematocrit reader & standard lab centrifuge w/ digital calipers. Investigator collected MoM samples. One sample tested fresh, one within 24-48 hrs (refrigerated), and one frozen/thawed. | Mean absolute inter- and intra-user differences were <1% (CRCT 93.5-98%) for all three methods.<br><br>Mean CRCT measures were nearly identical. Linear correlations between CRCT and lab measures: fat (r=0.95, p<0.001) and calories (r=0.95, p<0.001)<br><br>Conclusion: CRCT method accurate, reliable and feasible. Ideal for routine clinical management of MoM-fed infants w/ slow weight gain |

| Reference | Study Design | Sample/Methods | Results |
|---|---|---|---|
| Lin, Hsieg, Chen, Chiu, Lin & Su (2011) | Descriptive correlational<br><br>Comparison of CRCT to LA | N=98 MoM samples from 14 mothers of PT infants (27-36 weeks). Samples collected after 2 wks PP. MoM samples collected over 10 consecutive days, frozen & thawed w/ flowing warm water. Hand shaken 3 sec. CRCT method compared to bomb calorimetry | Mean kcal (0.67 kcal/L, CRCT (5.98 ±0.92). Linear correlations between CRCT & bomb calorimetry for kcal ($p<0.05$).<br><br>Conclusion: CRCT method convenient, accurate, feasible and requires minimal amounts of MoM for testing |
| O'Neill, Radmacher, Sparks & Adamkin, 2013 | Descriptive correlation<br><br>Comparison of CRCT to LA & HMA | N=50 previously frozen and thawed to 40°C analyzed by Calais (Metron Instruments, Solon, OH) human milk analyzer (HMA: mid-infrared spectroscopy) and CRCT method compared to lab analysis | Mean fat content by CRCT (5.8±1.9 g/dL) was significantly higher than HMA (3.2 ±-1 g/dL) compared to lab analysis ($p<0.001$). Lab assays showed no statistically significant difference with fat and caloric content w/ HMA.<br>Limitation: Previously frozen samples, collection and expression techniques not described.<br>Conclusion: CRCT method overestimates fat & caloric content compared to HMA |

| Reference | Study Design | Sample/Methods | Results |
|---|---|---|---|
| Du, Gay, Lai, Trengrove, Hartmann & Geddes (2017) | Descriptive correlational<br><br>Comparison of CRCT to LA | N=60 samples of MoM from term mothers divided into low, medium & high fat samples. Samples frozen/thawed and warmed to 37 C for 30 minutes. Same samples compared using 3 methods: Gold standard GM method, EFA & CRCT | Excellent correlation found between all 3 methods ($r^2$>0.99). Fat content measured by GM was higher than EFA & CRCT.<br><br>Comparison of 3 methods w/ largest mean difference observed w/ GM & EFA. Smallest difference between GM & CRCT.<br><br>Conclusion: Correlation between gold standard GM & CRCT ($r^2$>0.995). CRCT method feasible, reagent-free and accurate. Ideal for use in clinical setting for real-time analysis of MoM. |

Abbreviations:
APRNs: advanced practice registered nurses
BM=breast milk
$^{o}$C=degrees Celcius
CI=confidence interval
CRCT=creamatocrit
EFA=esterified fatty acid method
GA=Gestational age
GM=gravimetric method
HMA=human milk analysis (mid-infrared spectroscopy)
Kcal=kilocalories
L=liter
LA=laboratory assays
MoM = Mothers own milk
PT=Premature
PP=postpartum

All six studies compared the creamatocrit method to standard lab analysis measures including gravimetric, bomb calorimetry and gas chromatography. Linear correlations between the CRCT method and lab analysis ranged from $r^2$=0.92 to 0.995 with statistical significance of < 0.05 to 0.001 reported in five of the studies with the exception of the O'Neill et al. (2013).

O'Neill et al. (2013) hypothesized that the human milk analyzer (HMA) (Calais, Metron Instruments, Solon, OH) provided more accurate results for fat and energy content of human milk than analysis by creamatocrit (Creamatocrit Plus, Medela, McHenry, IL). The Calais HMA is a mid-infrared spectroscopy instrument used for the analysis of macronutrients in human milk. Infrared energy with specific wavelengths radiate from an incandescent source lamp and passes through six filters mounted on a disk. Readings are done at six wavelengths (three for fat, one for protein, one for lactose and one for solids) and results are based upon the absorption of infrared energy by specific chemical bonds. A detector than converts infrared energy into an electrical signal processed by a computer to generate values for each macronutrient (O'Neill et al., 2013; Calais HMA, Metron Instruments, Solon, OH).

Mean fat content by CRCT method (5.8 +/- 1.9 grams/dl) was significantly higher than HMA (3.2 +/- 1 gram/dl, p<0.001). Mean energy by CRCT (21.8 +/- 3.4 kcal/ounce) was significantly higher than HMA (17.1 +/- 2.9, p<0.001). Laboratory assays (LA) showed no statistically significant difference with fat and energy with the HMA. The CRCT results for fat were significantly higher than LA or HMA (p<0.001), up to 74-76% higher. Energy results for CRCT were also significantly higher than LA or HMA (p=0.002). A limitation of this study is that previously frozen samples and thawed

samples of human milk were tested and specific expression techniques were not described.

Two studies have been conducted on intra- and inter-user reliability of the creamatocrit method (Griffin et al., 2000; Meier, Engstrom, Zuleger, et al., 2006). Meier, Engstrom, Zuleger et al. (2006) compared the intra-user and inter-user reliability of three measurement techniques for the fat and caloric content of breast milk including the Creamatocrit Plus, the hematocrit reader with calipers and standard lab centrifuge. Mean creamatocrit (CRCT) measures were found to be nearly identical for all three techniques with mean absolute intra-user and inter-user differences of less than 1% CRCT.

Griffin et al. (2006) demonstrated inter-rater reliability between advanced practice nurses (APRN's) and mothers performing creamatocrit measures on maternal breast milk using the Creamatocrit Plus instrument. Each mother had an infant in the neonatal intensive care unit at the study site. The mean absolute differences between APRNs and mothers CRCTs was 0.69%, with 50% and 84.6% of these differences $\leq$ 0.5% and $\leq$ 1.0% CRCT.

In a more recent publication, Du et al. (2017) compared the esterified fatty acid method (EFA) and the creamatocrit method (CRCT) to the gravimetric method that is considered the gold standard of fat content measurement for human milk. Although the gravimetric method is considered the gold standard, the authors reported that it is labor intensive and requires the use of chemicals and large volumes of human milk to perform which not feasible in the clinical setting. Du et al. found that the correlation between the three methods was high ($r^2$=0.99), statistical differences ($P<0.001$) were observed in the overall fat measurements and within each group (low, medium, and high fat content in

the human milk samples). They concluded that there was a stronger correlation with the lower mean (4.73 g/L) and percentage differences (5.16%) with the creamatocrit method than with the EFA when compared to the gold standard gravimetric method. The authors concluded that the creamatocrit method was the most feasible instrument for real-time analysis of fat content due to ease of operation, rapid results, cost effectiveness, and minimal amounts of human milk required for testing.

A limitation of all six CRCT studies is the variability of the sampling methods including whether or not fresh or frozen breast milk samples were tested, and the specific collection techniques from the maternal breast. Lin et al. (2007) reported the preparation of their samples (temperature and rewarming) for their study but did not report how the samples were collected (via breast pump or hand expression or both) nor if samples were collected from one breast or from both. Meier, Engstrom, Zuleger et al. (2006) specified their breast milk collection procedures and their sampling technique with the use of fresh breast milk samples. Griffin, et al. (2006) used fresh breast milk samples. Breast milk collection procedures in this study were not reported. Du et al. (2017) used previously frozen samples of human milk that were warmed to 37$^{o}$C.

**Instructions for the measurement of fat and calories of MoM with the Creamatocrit Plus: Manufacturer recommendations and clinical experience**

The Creamatocrit Plus instruction manual (Separation Technology, Inc., EKF, 2015) provides general instructions for the use, cleaning and maintenance of the instrument. It indicates that the breast milk for sampling should be mixed for five seconds but does not provide evidence-based and clinically useful techniques to optimize the accuracy of the results in the clinical setting. The following instructions summarize

the techniques recommended by the manufacturer (Separation Technology, Inc., EKF) and provides information to the user how to handle, homogenize, and measure MoM samples to achieve accurate and reliable results which are critical to the nutritional management of premature hospitalized infants.

In a study of breast-pump dependent mothers of hospitalized premature infants (MUSC IRB PRO00056491: 2017-2018) that examined the fat and caloric content of breast milk, a total of 288 creamatocrits were tested by the primary investigator (BH) with the Creamatocrit Plus (Separation Technology, EKF, Inc.) on individual breast milk samples collected at three points in the day over a three-day period. Each fresh breast milk sample that was refrigerated and never frozen, was warmed with the Medela (Medela, Inc., McHenry, IL) waterless warmer to 37$^{o}$C, drawn into two creamatocrit tubes, and crossed checked for accuracy. Of the 288 creamatocrit measures of which each sample was checked twice and spun at the same time, the PI achieved 48.95% of the samples with 100% agreement between the two samples of the same MoM sample and 51.04% agreement within one kilocalorie per ounce of the two samples. The PI's creamatocrit testing experience with this study and over ten years of experience with the Creamatocrit Plus instrument for testing MoM at the Medical University of South Carolina (MUSC) in the NICU has lead to clinical pearls and methods to achieve reliable and accurate readings in the NICU setting.

**Creamatocrit measurement instructions: Guidelines specific to the NICU setting**

Testing MoM by the creamatocrit method is a common practice at MUSC, and we have tested thousands of breast milk samples over a period of ten years. We ask that the

mothers collect their breast milk after fully emptying their breast with a breast pump and then immediately pour 5 mL into a small hospital-approved breast milk container provided for them. The mother is instructed to write the time of the pumping session and the date on the label of the MoM sample and refrigerate the sample(s) and bring them to the NICU the next day for testing. Mothers are specifically instructed not to freeze their samples as we have found this can affect the accuracy of the creamatocrit results. Silprasert, Dejsarai, Keawvichit, and Amatayakul (1987) reported a significant reduction in the fat content measurement tested by the creamatocrit method of human milk after freezing (-20$^{o}$C) and thawing ($p<0.001$) compared to samples checked at room temperature (24-25$^{o}$C) and at 4$^{o}$C.

We have found that in order to achieve the most reliable and accurate results, lactation consultants and dieticians at the Medical University of South Carolina (MUSC) cross check each MoM sample twice and compared with each other. Samples are collected and tested based on individual circumstances of the mother and infant. For example, in order to assess an overall average of the fat and calories of the MoM in a 24-hour period, we ask the mothers to collect samples in early morning, mid-afternoon and evening. These instructions are based upon the research of possible diurnal differences in MoM, impact of intervals between pumping sessions, and individual variation of fat and caloric content of mothers throughout a 24-hour period (Moran-Lev et al., 2015; Gidrewicz & Fenton, 2014; Kozizewski-Najman et al., 2012; Weber, Jochum, Buhrer & Obladen, 2001; Lubetsky, Mimouni, Dollberg & Solomon, 2007).

If a mother, for example, is producing a high volume of breast milk at her early AM pumping session, we may spot check one sample at that time. Otherwise, we

generally test three samples collected within the three time points in the course of a 24-hour period. It is critical not to assume that a single sample result can be generalized to the overall mean fat caloric content of the mother's breast milk. A recent systematic review and meta-synthesis of 41 studies that examined the nutrient content of preterm and term human milk concluded that caloric and fat content of human milk varies within a breastfeeding or expression session and had diurnal differences in fat content throughout the day (Gidrewicz & Fenton, 2014).

We have also found that using fresh MoM that may have been refrigerated but never frozen leads to more reliable readings. Warming the milk to body temperature ($37^{o}$C) using a warmer such as the Medela® Waterless Warmer (Medela®, Inc., McHenry, IL) yields a more homogenized sample and tighter readings when checking two samples of the same MoM sample (Figure 1.1). Gently swirling the MoM sample several times (5-8 seconds) after warming has also improved the reliability and accuracy of creamatocrit measures at MUSC. Creamatocrit readings that do not fall within the range of one kilocalorie of each other of the same MoM sample are retested.

Creamatocrit tubes are to be used only to measure the MoM sample. Use of other tubes, such as hematocrit tubing has lead to spurious results at MUSC. The manufacturer of the Creamatocrit Plus (Separation Technologies, Inc. EKF) indicates that for smooth operation and extended life of the centrifuge, two, four or six tube holders are to be inserted into the rotor to assure balance.

After balancing the tube holder rotor and securing and warming the MoM sample, the breast milk should be siphoned into the creamatocrit tube by gently placing the tip of the tube into the breast milk and slightly tipping the sample container to allow the milk to

flow until 2/3 to ¾ of the tube is filled (Figures 1.1-1.2). Separation Technology, Inc. (2015) recommend that the tube be filled to ½ to ¾ full. We have found that 2/3 to ¾ full is the best amount to achieve reliability of the results between two tubes of the same MoM sample. Use a gloved finger to stop the flow of milk at the dry end of the tube and wipe the tube off.

After the MoM sample is drawn into the creamatocrit tube to 2/3 to ¾ full, invert the tube while securing the wet end with a gloved finger and place the dry end of the tube vertically into the sealant twice to prevent blow-out of the sealant during the centrifugation. We have also found that tapping the sealant end of the tube on the surface of a table several times secures the two sealant plugs and prevents blow-out (Separation Technology, Inc., 2015; personal communication, Paula Meier, PhD, RN, November 2007). The tubes should be wiped off and both tubes of the same MoM sample should be placed across from each other in the tube holders. Document the numbers of each sample per the number of the tube holder in the rotor of which the CRCT tube was placed. Up to three MoM samples (two tubes of each sample or a total of six tubes in the rotor) can be checked per centrifugation. We document the date and time of the sample collected and the creamatocrit readings in the infant electronic health record (EHR) as well the maternal daily milk supply.

Once the tubes with the MoM are placed in the centrifuge, tightly close the lid and start the spin cycle by pressing the RUN button. An audible alarm indicates that the spin cycle is complete. Total centrifuge time is 180 +/- 3 seconds (Creamatocrit Plus Operator's Manual, Separation Technology, Inc., 2015). Separation Technology, Inc. recommend that each tube should be taken out individually and read within one minute.

The tube should be placed in the groove of the reader tray and held securely against the left edge of the reader groove throughout the three-step reading (Figure 1.2).

The tube should be rotated in the groove so that the full slanted interfaces of the MILK/CREAM and CREAM/AIR can be easily seen. Separation Technology recommends that the interfaces between the MILK/CREAM and CREAM/AIR can slant in either direction for the reading. Assure that you do not move the tube during the reading and press it securely against the left edge of the reader tray (Figure 1.2).

While holding the creamatocrit tube in the reader tray, press ENT and follow the prompts for each step in the measurement process (Figures 1.3-1.4). The first step after pressing ENT instructs the user to move the transparent slider along the tube to the interface of the tube where the sealant and milk meet Position the vertical black line at the point of that interface and press ENT. The user is then instructed to move the slider to the slant of the MILK/CREAM interface and bisect that angle or position the black line directly in the middle of slant and press ENT again. Repeat this step by sliding the reader to the CREAM/AIR interface and position the black line on the transparent reader to bisect that slant (place the black line in the middle of the angle) and press ENT again (Figures 1.3-1.4).

When you press the ENT button after this step, the LCD will then display the creamatocrit result (Figure 1.5). When the user presses ENT again, the kilocalories per ounce are displayed. When the ENT button is pressed again, the kilocalories per 100 ml is displayed and the final reading is kilocalories per liter (Figure 1.5). At MUSC, we then read the corresponding tube from the same sample immediately after and cross check the results. If we do not get a reading within one kilocalorie of the first reading, the MoM

sample is then retested. The results are then reported to the neonatology team and documented in the infant's EHR. The mother is also informed of the results and provided an explanation of their meaning. On numerous occasions, mothers are invited to observe their milk being tested by the creamatocrit method performed by an experienced lactation consultant. Meier, Engstrom, Zuleger et al. (2006) reported that mothers testing their own breast milk by creamatocrit method achieved the level of accuracy of APRN's and reported that the procedure was easy to perform and they felt more involved in the care of their infant.

**Conclusion and implications for practice**

The caloric and fat content of breast milk received by the premature VLBW infant can impact their growth and development. Kociszewski-Najman et al. (2012) reported that the nutrition required of the preterm neonate to achieve satisfactory growth consists of breast milk that supports 100-120 kilocalories per kilogram in 24 hours. Identification of mothers at risk for production of breast milk low in caloric and fat content can inform lactation-based nursing and dietary interventions to prevent and improve inadequate growth in VLBW infants.

New technology is available to test human milk for fat and caloric content, including mid-infrared spectroscopy (Cassadio et al., 2010; Corvaglia, Basstini, Paeletto, et al., 2008; Michaelson, Peterson, Skafte, Jaeger & Peiterssen, 1988; O'Neill et al., 2013), but is still beyond the reach of NICUs in the United States due to pending FDA approval for clinical use. To date, the FDA has not endorsed the use of human milk analyzers (mid-infrared spectroscopy) to use in the clinical management of infants, but

research is ongoing, and approval of these instruments may be achieved in the future (Insoft, Newburg & Bar-Yam, 2016).

Six studies demonstrated the validity of the creamatocrit instrument and two studies reported reliability for the measurement of caloric and fat content in maternal breast milk in the clinical setting (Du, et al., 2017, Griffin et al., 2006; Meier, Engstrom, Zuleger et al, 2006; Lin et al., 2011), and one study demonstrated the mothers' abilities to perform the creamatocrit procedure in a hospital setting (Meier, Engstrom, Zuleger et al., 2006). As clinicians, we must also consider that infants are already at risk of inadequate growth velocity due to additional iatrogenic factors including fat loss during freezing, thawing and preparation of human milk (Chang et al., 2012: Jackson et al., 1988; Veira et al. 2004; Lucas et al., 1978, 1983) and fat loss as a result of gavage feedings (Brennan-Behm et al., 1994). In order to develop clinically useful lactation-based interventions to promote the growth of VLBW infants, a useful, user-friendly and inexpensive measurement instrument must be available to clinicians. Until more sophisticated, reliable, accurate, valid and affordable instruments are available to NICUs worldwide, the creamatocrit method remains a feasible option to assist clinicians to problem-solve inadequate growth velocity in very premature infants.

**Figures**


*Figure 1.1* Warm MoM and siphon into creamatocrit tube

*Figure 1.2* Step Two: Fill creamatocrit tube 2/3 to ¾  full. Insert dry end into putty twice

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*Figure 1.3* Follow instructions on digital display using transparent reader

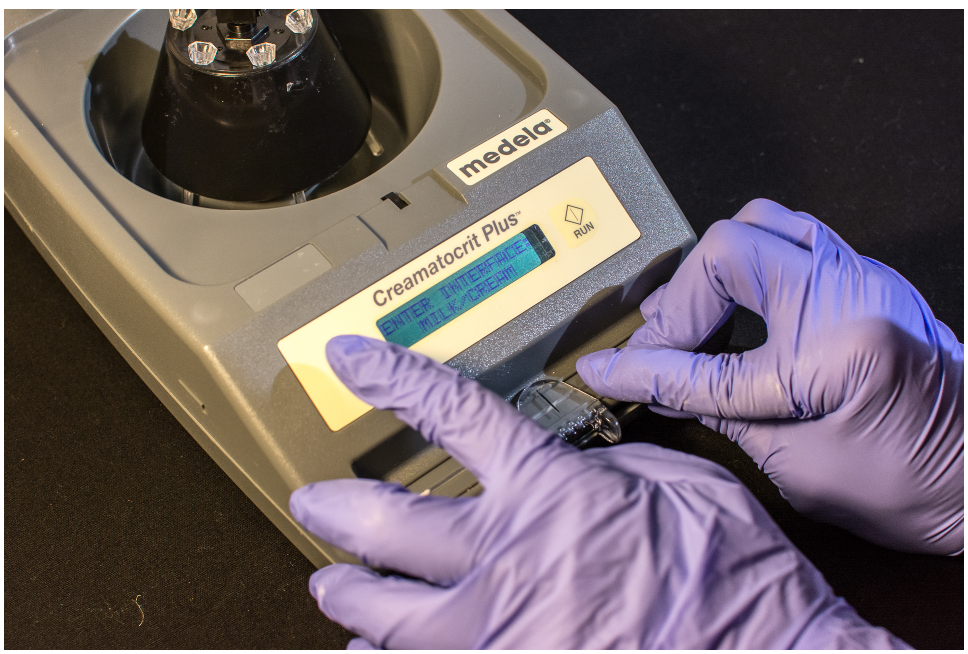


*Figure 1.4* Press ENTER after each digital display instruction is performed

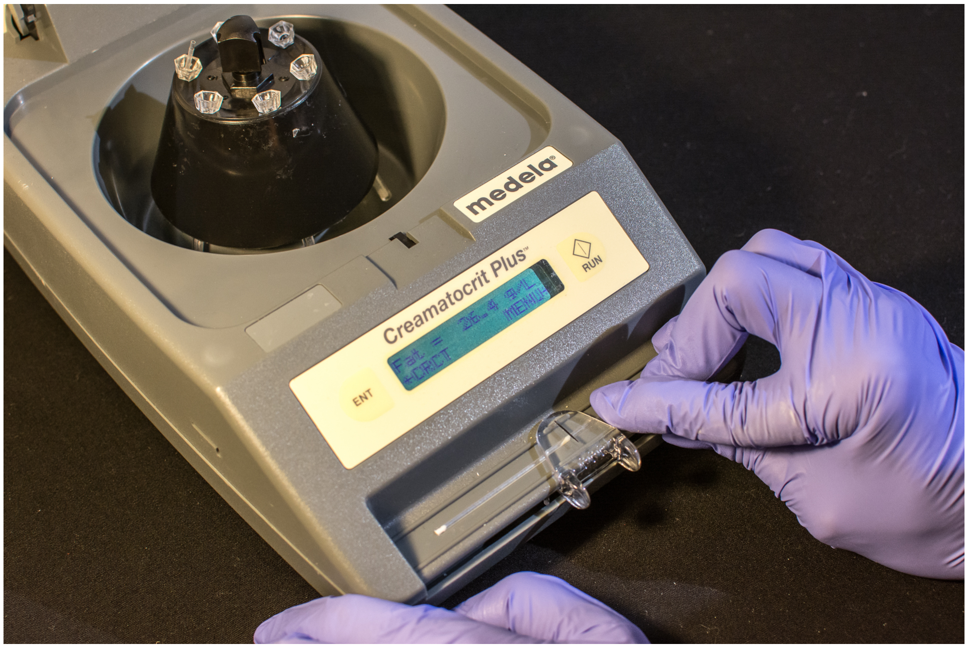

*Figure 1.5* Press ENTER for each reading (CRCT, Kcal/ounce, Kcal/liter & fat g/L)

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# CHAPTER 4

# FAT AND CALORIC CONTENT OF BREAST MILK OF PUMP-DEPENDENT MOTHERS OF PREMATURE HOSPITALIZED INFANTS: COMPARISON OF HIGH VS. AVERAGE TO LOW VOLUME PRODUCERS, THE IMPACT OF THE VOLUME IN THE BREAST AT A SINGLE PUMPING SESSIONS AND THE INTERVAL BETWEEN PUMPING SESSIONS

## Abstract

**Background:** Although mother's own milk (MoM) provides numerous advantages to premature infants, it can place infants at risk of inadequate growth velocity. Despite standard fortification guidelines for human milk, the premature infant remains at risk of meeting sufficient growth parameters due to individual maternal differences in fat and caloric content of their breast milk. Additionally, there is a risk of loss of human milk fat with storage and handling procedures and loss via gavage feedings.

**Objective:** This study aimed to perform creamatocrit analysis of the fat and caloric content of the breast milk of pump-dependent mothers of hospitalized premature infants in a neonatal intensive care unit (NICU) and compared maternal differences between average to low volume producers ($\leq$500 ml/day) and high volume producers ($\geq$900 ml/day). It also examined the impact of fat and caloric content of the interval between pumping sessions and the volume in the breast at single pumping sessions.

**Methods**: Nine human milk samples (198 fresh samples) were obtained from breast pump-dependent mothers with a premature infant (23-36 weeks at birth) in a NICU

(N=22 women; n=6 mothers who produced 500 ml/day or less, n=11 who produced 900 ml/day or greater, and n=5 who produced 501-899 ml/day) [MUSC IRB PRO00056491). All mothers were at least 14 days postpartum at the initial point of sample collection. The mothers documented their pumping sessions and volumes produced over three consecutive days and collected breast milk samples during specific timeframes (early morning after their night sleep stretch, between 9 AM – 12 noon, and between 9 PM to 12 AM). All samples were measured using the creamatocrit method by the primary investigator and were tested for fat and caloric content.

**Results:** There was not a statistically significant difference in fat grams per liter and kilocalories per ounce between average to low producing mothers of premature infants compared to high producing mothers ($\geq$900 ml/day) (P=0.4067 and P=0.6054).

Both fat and caloric content were significantly positively associated with milk volume at a single pumping session (p=0.015 and p=0.014, respectively).

Sleep stretch (interval between pumping session on early AM sample) was not significantly associated with either fat or energy content of the pumping session though both fat and energy decreased with increasing time interval. For each additional hour slept, fat decreased by 1.2 g/L, and for each additional hour slept, the kilocalories decrease by 0.36. Marital/cohabitation status of the mother was statistically significant for milk volumes of $\geq$ 900 ml/day ($p<0.03$).

**Conclusion:** Findings of this study provide additional information of the variability of fat and caloric content of MoM at different points in the day and the impact of volume in the breast at single pumping sessions. It reinforces the need for testing

MoM for fat and caloric content in the clinical setting to problem solve and individualize feeding plans to optimize postnatal growth.

Keywords: breast milk, breast pump-dependent, creamatocrit, caloric content, fat, MoM (mother's own milk), high volume producers, average to low volume producers, intervals between pumping sessions, neonatal intensive care unit, premature infants, volume in the breast

## Background

Premature birth (infants born at less than 37 weeks gestation) affects 8-18% of all infants worldwide with the premature birth rate rising in the last two years to 10% of all births in the United States (US) (CDC, 2017). Prematurity is the leading cause of death in children less than the age of 5 years and the leading cause of neurodevelopmental problems in children (CDC, 2017; March of Dimes, 2014). Premature infants are also at high risk of serious infections such as necrotizing enterocolitis (NEC) and sepsis and chronic lung disease (AAP, 2012; CDC, 2017). Mother's own milk (MoM) provides numerous bioactive and nutritional benefits for the premature infant and is associated with decreased rates of chronic lung disease, necrotizing enterocolitis (NEC), late onset sepsis and retinopathy of prematurity, and improves neurodevelopmental outcomes (AAP, 2012; Corpelijn et al., 2012; Belfort et al., 2013; Hsiao, Tsai, Chen & Lin, 2014; Lucas, Morley & Cole, 1992; Patel, Meier & Engstrom, 2007).

Although MoM is well established as superior nutrition for the premature infant, it also places the hospitalized premature infant at risk of inadequate postnatal growth due to insufficient protein, calcium, phosphorus and iron (Brown, Embleton, Harding & McGuire, 2016). This is managed with current fortification guidelines of human milk in NICUs in the United States and Europe which is endorsed by the American Academy of Pediatrics (AAP) and the North American and European Societies of Gastroenterology, Hepatic and Nutrition (NASPGHAN, ESPGHAN) (Brown et al, 2016; Moro et al., 2015; Schanler, Abrams & Hoppin, 2018).

Fat contributes up to 55% of the caloric content of human milk (Andreas, Kampmann & Mehring-Le-Doare, 2015). Fat is the major source of energy in human

milk and provides lipid soluble vitamins, essential fatty acids, and bioactive components such as immunoglobulins, acetyhydrolase, and oligosaccharides (Belkind-Gerson, Carreon-Rodriguez, Contreras-Ochoa, Estrada-Mondaca & Parra-Cabrera, 2008; Delplanque, Gibson, Koletzo, Lapillone & Strandvik, 2015; Koletzo, Agostini, Bergmann, Rtizenhaler & Shamir, 2011). Premature infants who are fed MoM are also at further risk of inadequate growth due to individual maternal variability of fat content and caloric density, diurnal variation, and fat loss via gavage feedings and during the storage and handling of human milk (Brenham-Behm, Carlson, Meier & Engstrom, 2013; Chang, Chen & Lin, 2012; Daley, Rosso, Owens & Hartmann, 1993; Gidrewicz & Fenton, 2014; Harzer, Haug, Dietrich & Genter, 1993: Hassitou et al., 2013; Veira-Mora, Rocha, Pimenta & Lucena, 2004).

Kociszewska-Najman et al. (2012) reported that the nutrition required of the premature infant to achieve satisfactory growth and optimize growth and development should consist of human milk that supports 100-120 kilocalories (kcal) per kilogram in 24 hours. Given the variability in the fat content and caloric density of MoM and the risk of fat loss via gavage feedings and the storage and handling processes of human milk, neonatal providers are faced with the challenge of the provision of MoM with sufficient lipid content and calories to optimize postnatal growth in this at-risk and vulnerable population.

## Purpose of the Study and Research Questions

This study examined the measurement of fat content and caloric density of breast pump-dependent mothers of premature hospitalized infants of mothers. The two groups studied were women who produced high volumes of breast milk ($\geq$900 ml/day) (Hassitou

et al., 2013) and those who produced average to low volumes (≤500 ml/day) (Hassitou et al., 2013; Lin, Hsieh, Chen, Chu & Si, 2011). This study also examined the impact of the volume of breast milk expressed at a single pumping session and the time interval between pumping sessions on the fat and caloric content of MoM that to our knowledge has not been studied in this population.

**1: Primary research question**: Is there a difference in the fat (fat grams/liter) and caloric content (kcal/oz) of the breast milk of mothers of premature infants (23-37 weeks gestation) who produce average to low volumes (≤500 ml/day) and those who produce high volumes (≥900 ml/day)?

**2a and 2b: Secondary research questions**: Does the volume in the breast at a single pumping session impact the fat and caloric content of breast milk? Does the time interval between pumping sessions (period of time from last pumping session of the day throughout the nighttime sleep stretch) impact the fat and caloric content of breast milk (early AM MoM collected after nighttime sleep stretch)?

In addition to current standard fortification guidelines of human milk (Moro et al., 2015; Schanler, Abrams & Hoppins, 2018), identification of women at-risk of producing breast milk that is low in fat and caloric density, and understanding the impact of interval between pumping sessions and the volume in the breast at single expression session may inform individualized lactation and nutritional interventions specific to the needs of premature infants to optimize postnatal growth and development.

## Methods

**Inclusion/exclusion criteria and sampling**. The study design is descriptive cross-sectional with purposive sampling. Mothers of infants who were pumping with a hospital grade breast pump to provide breast milk for a hospitalized premature infant (born at 25-37 weeks gestation) with a daily production of $\leq$ 500 ml or $\geq$ 900 ml at 14 days postpartum or greater were included (Bishara, Dunn, Merko & Darling, 2009; Meier, Engstrom, Patel, Jeiger & Bruns, 2010). Participants were recruited from the Medical University of South Carolina (MUSC) neonatal nurseries. Mothers needed to have sufficient transportation to bring the study samples directly back to the PI.

The women produced a daily volume of breast milk that exceeded >100 ml above the needs of the infant to assure that the nutritional needs for breast milk of their infant were met. Women who were taking a galactogogue (a medication or herb that may increase milk supply) of any kind were excluded from the study. Women of all ethnicities were recruited into this study, although they did need to be able to read and write in English.

**Procedures.** All women were 18 years of age or older and consented via MUSC IRB procedures ((MUSC IRB PRO00056491). All demographic and breast milk sample data were de-identified and entered into a fire wall and password-protected RedCap data file. The mother's age, BMI at the time of collection, marital status, infant's gestational age and birth weight at birth, and maternal insurance status were recorded to describe the study sample.

- All participants in the study were provided with written and verbal instructions by the PI. They were also provided access to call the PI via a cell phone at any point

in the study to respond to questions or review instructions. Each mother was instructed to keep a pumping log for a period of three days and record the time of each pumping session and the volume of breast milk produced at each pumping session. MoM samples were tested fresh within 24 to 48 hours to maintain the freshness and integrity of each sample. All samples were measured fresh, refrigerated and never frozen. The rationale for testing milk in the fresh state was based upon the work of Chang et al. (2012) and Veira, Moreira, Rocha, Pimenta and Lucena (2004) who demonstrated a reduction in fat after freezing and rewarming breast milk samples. Chang et al. (2012) and Veira et al. (2004) demonstrated a reduction in fat after freezing and rewarming breast milk samples.

- Mothers were provided with de-identified study labels and hospital-approved BPA-free breast milk storage containers (Medela®, Inc., McHenry, IL) and instructed to collect three 10 milliliter (ml) samples at three separate points in the day (just after the longest sleep stretch in the AM, between 9 AM -12 noon, and again between 9 PM-12 midnight) by emptying both breasts, combining the breast milk from both breasts and then pouring 10 ml into the study containers. Each mother collected nine samples of 10 ml each for a total of 90 ml over a period of three days. Study samples collected at three points in the day provided nine representative samples with respect to the diurnal differences in breast milk ((Manley et al., 2011; Moran-Lev, Mimouni, Ovental, Mangel, Mandel & Lubetsky, 2015; Kocizewski-Najman et al., 2012; Weber, Jochum, Buhrer & Obladen, 2001, Lubetsky, Mimouni, Dollberg & Solomon, 2007) and to capture the early AM sample for the sleep stretch interval. All samples were labeled with

a study number and mothers indicated the time and date of the pumping session associated with the sample.

- Mothers were instructed to keep the samples fresh and refrigerated and not to freeze them. The samples were brought by the mother either the day-of or day-after sample collection in a study-provided insulated cooler with gel freezer packs. All samples were tested by the PI within 24-48 hours with the previously validated Creamatocrit Plus instrument (Separation Technologies, Inc., EKF) (Du et al., 2017; Griffin, Meier, Bradford, Bigger & Engstrom, 2000; Meier, Engstrom, Murtaugh, Vasan, Meier & Schanler, 2002; Meier, Engstrom, Zuleger, et al., 2006; Lin et al., 2011). Studies that reported the validation, accuracy and reliability of the instrument are presented in Chapter 3. This instrument measures percent fat, fat grams per liter, fat grams per deciliter and kilocalories per ounce and per liter.
- All breast milk samples were warmed with the Medela® Waterless Warmer (Medela, Inc., McHenry, IL) to 37$^{o}$C and the sample was gently swirled for 5-8 seconds just prior to drawing it into the creamatocrit tubes. Each sample was tested immediately after warming. All creamatocrit testing of the breast milk samples were performed by the PI (BH) with greater than 10 years of experience in the use of the Creamatocrit Plus (Separation Technologies, EKF, Inc.). Each sample was cross-checked twice for accuracy. The specific method of testing the MoM samples is presented in Chapter 3.

**Data analysis**

Data were analyzed using the latest version of SAS® (SAS Analytics, Inc.). A Fischer's exact test was performed in the demographic analysis to test the probability between two groups having different proportions. For continuous variables, t-tests were used for single measures. A student's t-test was used from the primary research question to compare average to low and high volume producers due to multiple measures per participant. Mixed model linear regression was used to test the secondary research questions of the impact of the time interval between pumping sessions and the volume in the breast at a single pumping session on the fat and caloric content of MoM. Each participant had two to nine samples per question (n=44 measures for samples collected after the nighttime sleep stretch interval and n=198 measures for volume in the breast).

## Results

There were twenty-two (N=22) women who were breast pump-dependent mothers of hospitalized premature infants that participated in this study. A total of 198 composite fresh, refrigerated breast milk samples (nine samples per mother) were collected. Eleven women (n=11) produced high volumes ($\geq$900 ml/day) of breast milk per day (or greater), six (n=6) produced average to low volumes per day ($\leq$500 ml/day) and the remainder of the mothers (n=5) crossed over into the mid-range during the study period (501-899 ml). The demographic characteristics of this sample are displayed in Table 1.

The sample of mothers (N=22) consisted of approximately half with Medicaid and half with private insurance status. The sample was primarily of black and white race and non-hispanic. There was a statistical significance in regard to marital/cohabitation status for mothers who produced high volumes of breast milk each day (> 900 ml/day).

One hundred percent of mothers who reported being married or cohabiting with a significant other produced high volumes of breast milk each day (p=.003).

Table 1

*Maternal and Infant Demographics*

| **Variable** | **Total** | **Midrange Producers** | **Low-Avg Producers** | **High Producers** | **p-value (Low v High)** |
|---|---|---|---|---|---|
| Insurance | | | | | 0.64 |
| Medicaid | 10 (46) | 3 (60) | 3 (50) | 4 (36) | |
| Private | 12 (55) | 2 (40) | 3 (50) | 7 (64) | |
| Race | | | | | 1.00 |
| Black | 10 (46) | 5 (100) | 2 (33) | 3 (27) | |
| Other | 2 (9) | 1 (17) | 1 (9) | 1 (9) | |
| White | 10 (45) | 0 (0) | 3 (50) | 7 (64) | |
| Marital Status | | | | | 0.03* |
| Single | 6 (27) | 3 (60) | 3 (50) | 0 (0) | |
| Married/ Cohabitation | 16 (73) | 2 (40) | 3 (50) | 11 (100) | |
| Ethnicity | | | | | 1.00 |
| Hispanic | 2 (9) | 0 (0) | 1 (17) | 1 (9) | |
| Non-Hispanic | 20 (91) | 5 (100) | 5 (83) | 10 (91%) | |
| | Mean (SD) | Mean (SD) | Mean (SD) | Mean (SD) | |
| Mom's Age | 30.0 (5.4) | 32.0 (5.9) | 26.2 (5.8) | 31.3 (4.3) | 0.06 |
| Mom's BMI | 28.2 (8.1) | 30.9 (10) | 30.0 (5.9) | 26.1 (8.3) | 0.33 |

Note. Percentages appear in parentheses; tests used: Fishers exact test for insurance, race, marital status, & ethnicity, t-test for age, BMI; *p<0.05

1. **Primary null hypothesis**: There is no difference in the fat (fat grams/liter) and caloric content (kcal/oz) of the breast milk of mothers who produce average to low volumes (≤500 ml/day) and those who produce high volumes (≥900 ml/day)?

The results for the primary research question are displayed in Tables 2 and 3 and Figures 1 and 2. The primary null hypothesis is supported and there was no statistical significance between groups for fat grams per liter and kilocalories per ounces (P=0.6203 and P=0.6255). Figures 1 and 3 show the box plots that illustrate the range of results and differences between average to low producing mothers (≤500 mL/day) and high producing mothers (≥900 ml/day) for fat grams per liter and kilocalories per ounce.

Table 2

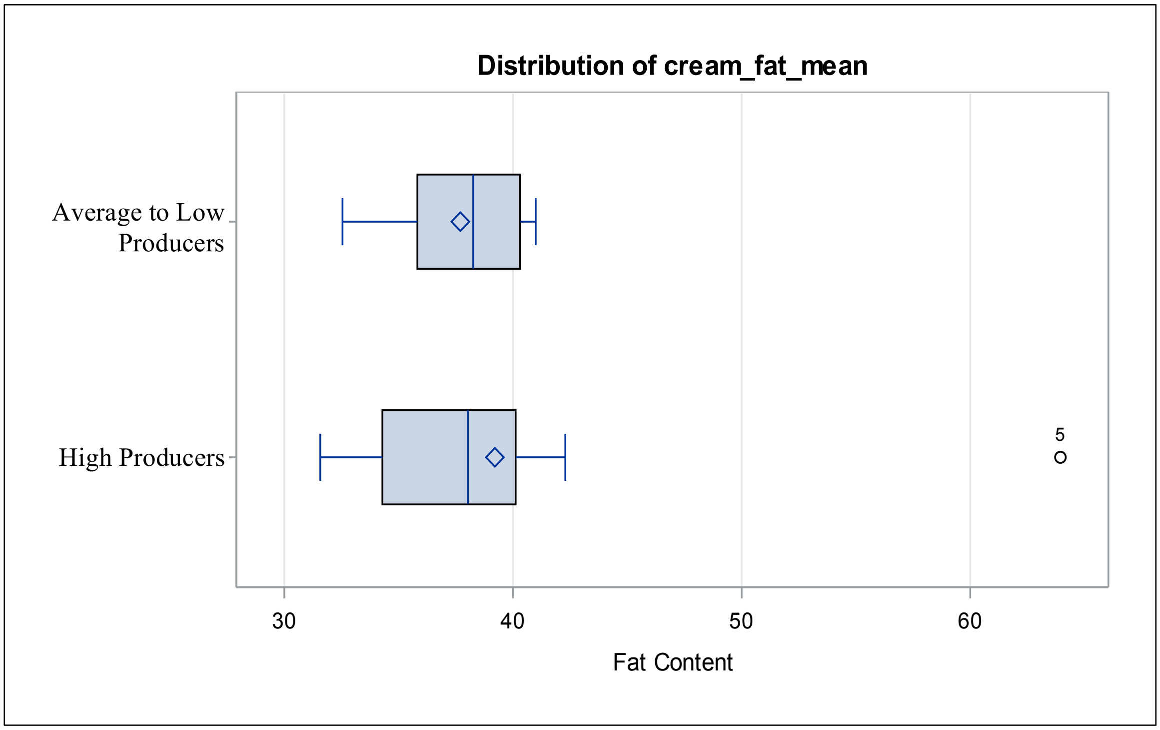
*Difference in Fat Grams/Liter Between Average to Low Producers and High Producers*

| Cohorts | N | Mean | Standard Deviation |
|---|---|---|---|
| Average to Low Producers | 6 | 37.6976 | 3.2297 |
| High Producers | 11 | 39.2081 | 8.8662 |

p=0.62

Figure 1

*Difference in Fat Grams/Liter Between Average to Low Producers and High Producers.*




Lines mark median and minimum/maximum values. Box marks interquartile range. Diamond marks mean.

*Legend for Figures*

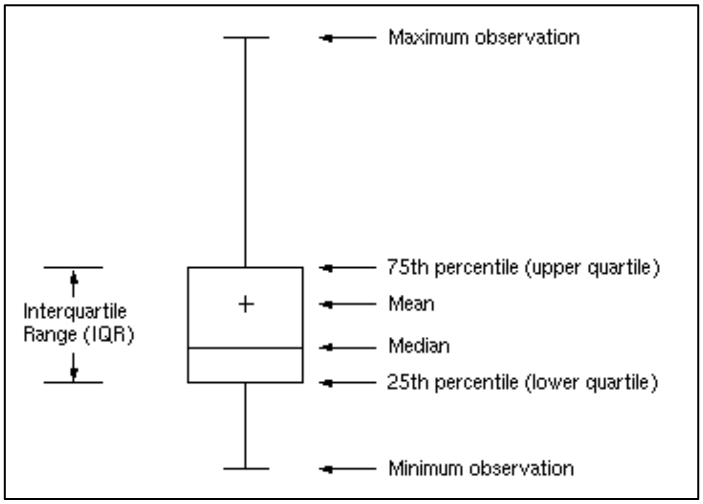


Table 3

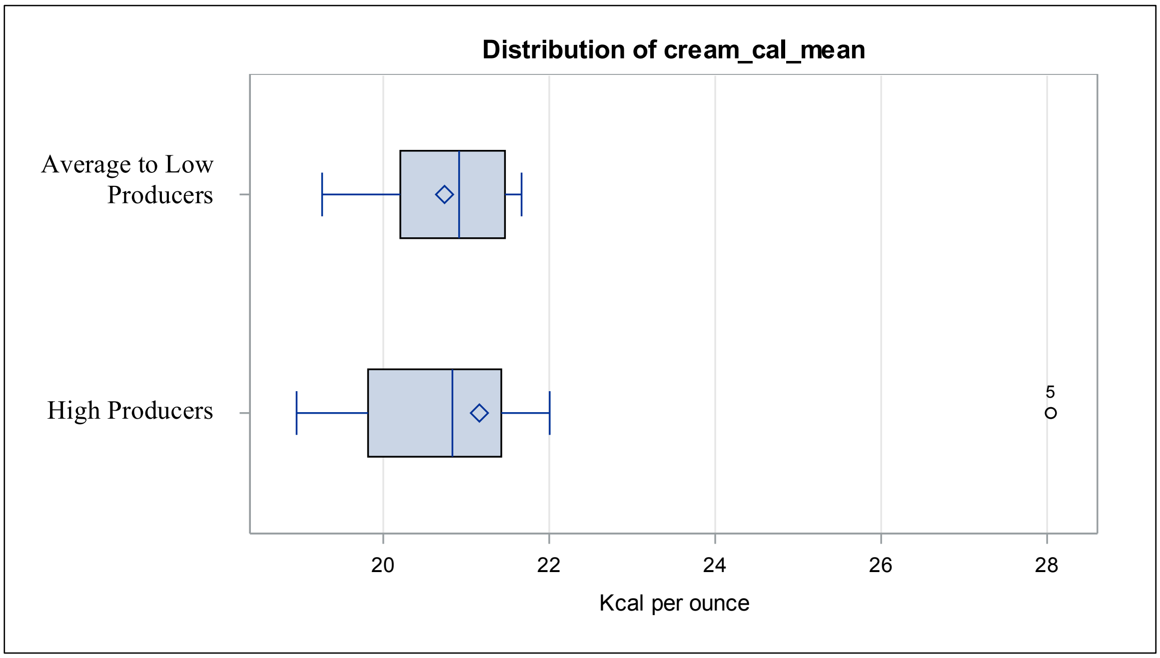
*Difference in Kilocalories/Ounce Between Average to Low and High Producers*

| Cohorts | N | Mean | Std Dev |
|---|---|---|---|
| Average to Low Producers | 6 | 20.7383 | 0.9168 |
| High Producers | 11 | 21.1583 | 2.4717 |

p=0.63

Figure 2

*Difference in Kilocalories per Ounce Between Average to Low and High Producers*




**2a. Secondary null hypothesis: The volume in the breast at a single pumping session does not predict the fat and caloric content of the breast milk.**

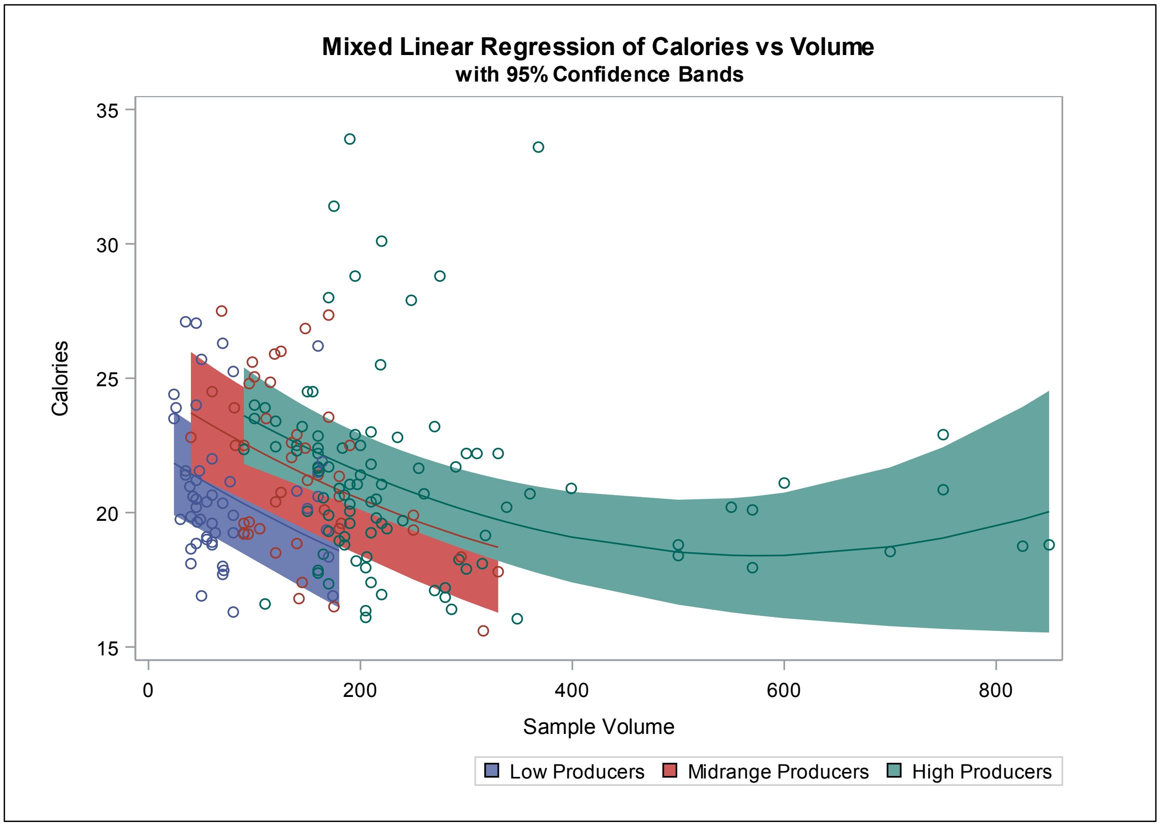
The amount of breast milk in the breast at a single pumping session ranged from 23 ml to 850 ml for all mothers in the study (N=22), including average to low, midrange and high producers. All samples (n=198) were included in the data analysis for this hypothesis. The fat and energy at a single pumping session ranged from 19.3-85 g/L and

15.6-33.9 kcal/oz. Both fat and energy were significantly positively associated with milk volume at a single pumping session ($p=0.015$ and $p=0.014$, respectively).

To further evaluate this association of higher volume in a pumping session with higher energy and fat, we assessed how this differs between low, intermediate, and high producers (Figure 3). As expected, high producers demonstrated higher average pumping volumes per session. In addition, the high producers also demonstrated higher average energy and fat content for a given pumped milk volume. For example, for a pumping session of 100 ml, the average energy was 23.4 kcal/oz for high producer, 22.3 kcal/oz for midrange producers, and 20.1 kcal/oz for low producers.

Figure 3

*Volume of Breast Milk at a Single Pumping Session and Kilocalories/Ounce Between Average to Low, Middle Range, and High Producing Mothers*




Type 3 Tests of Fixed Effects: Group p=0.0606 p-value NOT significant

**2b. Secondary null hypothesis: The interval between pumping sessions (maternal sleep stretch at night) does not predict the fat and caloric content of breast milk (MoM sample collected after the nighttime sleep stretch)?**

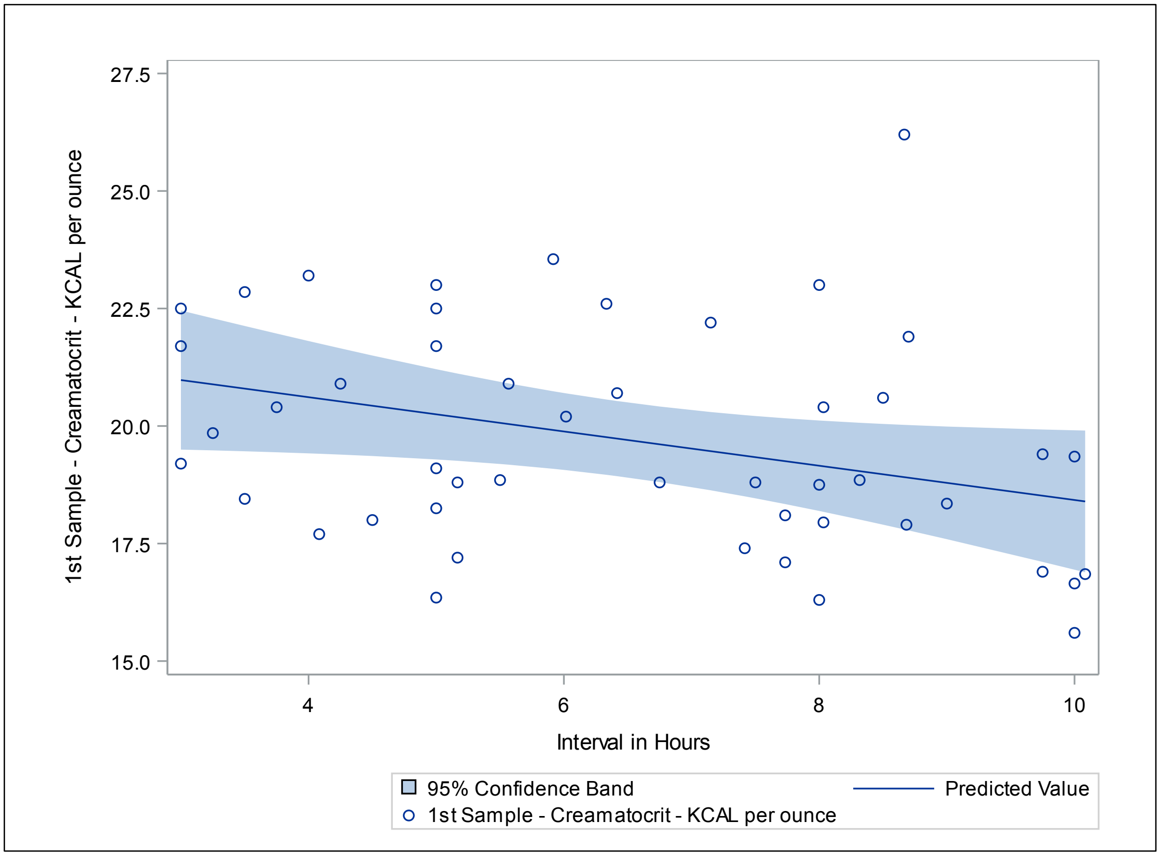
Figure 4 displays the results for the hypothesis of the impact of the sleep stretch (interval between pumping sessions). Each mother provided two measures of the sleep stretch time interval during her three-day collection period (n=44 MoM samples collected after

the nighttime sleep stretch). The period of time from the last nighttime pumping session to the earliest AM pumping session ranged from 3 hours to 10 hours in this study sample. This is the period of time of which the mother did not pump her breasts.

Sleep stretch was not significantly associated with either fat or energy content of the pumping session though both fat and energy decreased with increasing time interval. For each addition hour slept, fat decreased by 1.2 g/L. For each additional hour slept, the kilocalories decrease by 0.36 (14%). This value (0.36) is a beta-coefficient or estimate that resulted from the regression analysis that was standardized so that the variance of the outcome variable (fat and caloric content of MoM) and the independent variable (time interval or nighttime sleep stretch) are one measure of how strongly the independent variable influences the outcome variable. For example, extending the sleep interval from 4 to 10 hours was associated with a decreased average energy from 21 to 18 kcal/oz. However, neither of these associations was statistically significant in this study.

Figure 4

*Impact of the Sleep Stretch (Time Interval) on Kilocalories per Ounce*




**Discussion**

After a comprehensive review of the literature, there were no studies identified in the literature that reported the comparison of fat and caloric content between high versus average to low volume producers and the impact of the interval between pumping sessions and the volume in the breast at a single pumping session on fat and caloric content in the breast pump-dependent populations of mothers of premature infants. The maternal inter- and intra-individual variability of fat and caloric content of MoM found in this study is supported by recent studies (Koletzko et al., 2011; Sauer, Boutin & Kim, 2016) including the systematic review and meta-analysis (N=41 studies with 843

mothers) conducted by Gidrewicz and Fenton (2014). It was an unexpected finding in this study that high-producing mothers expressed breast milk higher in fat and caloric content than low-producing mothers.

Mothers of premature infants who are dependent on a breast pump to sustain lactation are instructed at MUSC that they may have a five-hour sleep stretch at night before resuming pumping sessions every three hours as long as they meet six to eight pumping sessions per day to sustain lactation. The range of pumping frequency amongst mothers in this study was four to nine times per day. In this study, sleep intervals between pumping sessions ranged from three to ten hours. In a recent review of literature, the interval or time lapsed between pumping sessions and the potential impact on the fat and caloric content of MoM in this population of women has not been studied.

Another interesting finding in this study is that of the marital/cohabitation status of the mothers. We found a statistically significant result for mothers who reported to be married or cohabiting with a significant other and maternal breast milk supply $\geq$ 900 ml/day (n=0.03). Morag, Leibovitch, Simchen, Maayen-Metzger & Strauss (2016) reported-that term infants born to single mothers and those mothers with less than 14 years of education were less likely to be providing breast milk for their infant at two weeks postpartum. There is a paucity of literature available that explores the partner's role or experience with sustaining the breast milk supply of mothers of premature infants. In a systematic review of parents' views of factors that help or hinder breast milk supply of mothers in NICUs, only one study was reported that involved the role of the father or significant other (Alves, Rodriques, Fraga, Barros & Silva, 2013). The father or significant other in this study was identified as a main facilitator to breastfeeding in the

NICU. Hill, Aldag, Zinnamon and Chatterton (2007) reported that premature infants who were no longer receiving breast milk from previously lactating mothers upon discharge from the NICU had a significantly smaller proportion of mothers who supported that the father or significant other was "their biggest supporter to continue to lactate" (p. 35).

**Limitations of the study**. A limitation of this study was the use of convenience sampling, which was used due to feasibility and limited funding. Convenience sampling requires less time to recruit research participants and allows for the study of a topic that could not be examined with the use of probability sampling. Convenience purposive sampling is considered a weak approach to sampling as multiple biases may occur (Burns, Grove & Gray, 2013). Due to the small sample size (N=22), there is a risk of a Type 2 error in the comparison between average to low and high volume producers. The interpretation of the findings of this study should be approached with caution and presented with limited generalizability.

Measurement error was also a risk to this study. Measurement error was minimized by having all breast milk samples delivered directly to and tested by the primary investigator with greater than 10 years of experience testing MoM samples with the previously validated Creamatocrit Plus instrument (Du, et al., 2017; Griffin, Meier, Bradford, Bigger & Engstrom, 2000; Lin, Hsieh, Chen, Chiu, Lin & Su, 2011; Meier, Engstrom, Zuleger, Motykowski, et al., 2006). The PI (BH) was available to the mothers throughout the study by cell phone to respond to questions. Milk volumes at each pumping session and throughout the three-day collection period were self-reported and documented by the mothers and pumping sessions were not directly observed. All mothers in the study received initial and ongoing lactation support and education in the

pumping technique and collection method of their breast milk from IBCLCs experienced in the care of lactating women with premature and medically complex infants. All of the mothers were pumping with hospital-grade double electric breast pumps.

A weakness of previous studies that have reported data on the fat and caloric content of human milk is variability in the collection and sampling methods that have varied from testing in the fresh state to previously frozen (Corvaglia et al., 2008; Daley, Rosso, Owens & Hartmann, 1993; Jackson et al, 1988; Sauer, Bauten & Kim, 2016). This study tested all of the samples in the fresh, refrigerated state and the MoM was then warmed to body temperature (37$^{o}$C) to maximize homogenization of the sample and thus improve accuracy of the creamatocrit results (Engstrom, Meier, Motykowski & Meier, 2008).

## Conclusion and Implications for Practice and Research

Results of this study may provide evidence-based knowledge about the composition of breast milk of women with a hospitalized premature infant specifically fat and caloric content. Ongoing studies are critical to define best practices in neonatal nurseries of the breast milk collection, handling and measurement techniques. A common practice in NICUs in the US is to fractionate or separate the initial lower fat portion of the pumping session of high producing mothers and feed the higher fat hind milk to premature infants. Some NICUs may also request that mothers fractionate their breast milk by prioritizing to certain points of time in the course of a day leading to potential wastage of MoM. Although the feeding of higher fat hind milk may improve weight gain in the premature infant (Amali-Adekwu, Ogala & Bode-Thomas, 2007; Slusher, Hampton, Bode-Thomas, Pam, Akor & Meier; 2003; Valentine, Hurst &

Schanler, 1994), the findings of this study do not support these practices unless MoM is tested for fat and caloric content in the clinical setting prior to and during hind milk feeding interventions.

With knowledge of the variability of fat and caloric content of breast milk and use of a validated measurement instrument to quantify the individual differences of mothers, the findings may also provide information that could be critical to ensure neonatal nurses, dieticians, lactation consultants and neonatologists can develop individualized feeding plans to promote the growth of hospitalized premature infants. In addition to the provision of evidence-based fortification guidelines (Brown, Embleton, Harding & McGuire, 2016) of breast milk for premature infants, a greater understanding of caloric and fat content in MOM can lead to lactation interventions specific to the needs of the mother/infant dyad. Measurement of caloric and fat content of MoM and the feeding plans can be implemented by nurses, lactation consultants, neonatal dieticians, neonatologists and mothers to improve the growth and development of hospitalized vulnerable premature infants.

There is also opportunity to conduct ongoing research in the role of the father or significant other in the support of their partner's role in optimizing and sustaining lactation for mothers of premature infants. This may include nursing educational interventions to promote and support lactation, and ongoing involvement and support of the father or significant other in the NICU and outpatient settings.

This study provides information for nurses, neonatologists and neonatal dieticians critical data to identify breast pump-dependent mothers of premature infants who are at-risk of producing breast milk low in fat and calories. This study provided preliminary

data of the fat and caloric content of MoM from pump-dependent mothers of premature infants at certain periods of time in the course of the day, between-group differences (average to low vs. high producers) and the impact of intervals between pumping sessions but more studies are needed with larger sample sizes. Knowledge of the mother's fat content and caloric density of her breast milk can also inform standard fortification plans and feeding interventions specific to the needs of her infant. This may result in in decreased morbidities associated with poor postnatal growth, earlier discharges and significant health care savings.

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# CHAPTER 5

# CONCLUSION: SYNTHESIS, IMPLICATIONS FOR PRACTICE, FUTURE RESEARCH AND POLICY

## Synthesis and Summary of Dissertation

Premature infants (infants born at less than 37 weeks gestation) comprise 10% of all births in the US (CDC, 2017). Infants born at less than 32 weeks and weigh ≤ 1500 grams have a 23.9% risk of mortality and have a high rate of co-morbidities including necrotizing enterocolitis (NEC), late onset sepsis, retinopathy of prematurity (ROP), cerebral palsy, chronic lung disease (BPD) and neurodevelopmental impairments. They also have high rates of re-hospitalizations and usage of healthcare specialty services (CDC, 2017; Meier, Engstrom, Patel, Jeiger & Bruns, 2010). Mother's own milk (MoM) reduces the incidence and severity of NEC, ROP, late onset sepsis, and BPD, and improves neurodevelopmental outcomes (Furman, Taylor, Minich & Hack, 2016; Hylander, Strobino & Dhanireddy, 1998; Johnson, Patel, Bigger, Engstrom & Meier, 2014; Lucas & Cole, 1990; Patel et al., 2013; Schanler, 2005; Schanler, Shulman & Lau, 1999).

In a synthesis of a review of literature, Meier, Engstrom, Patel, Jeiger and Bruns (2010) reported that the first two weeks of a premature infant's life is a critical window of time for the development and maturation of the neonatal immune system and gut maturation. The importance of early colostrum collection techniques from mothers of premature infants that can be used by nurses (and designated caregivers) for

oropharyngeal care and early trophic feedings to support early immune development and gut maturation was summarized in Chapter 2. In addition, the colostrum collection techniques presented may have a direct impact on the mother's ability to establish and maintain an adequate breast milk supply for her infant (Morton et al., 2009; Parker, Sullivan, Kelechi & Mueller, 2013).

The colostrum collection techniques presented in Chapter 2 may also motivate mothers to continue to provide breast milk for their infant, increase their confidence with caring for their infant, and improve their satisfaction by being an integral part of their infant's care (Ikonen, Paavilainen & Kaunonen, 2015; Fugate, Hernandez, Ashmeade, Miladonovic & Spatz, 2014).

When health care providers provide evidence-based education and breastfeeding support to mothers, it can contribute to future long-term health benefits for both the mother and her infant. In the landmark Women's Health Initiative Study of 139,681 women, a lifetime history of more than 12 months of breastfeeding was associated with a reduction in hypertension (OR=0.88), diabetes (OR=0.80), hyperlipidemia (OR=0.81) and cardiovascular disease (OR=0.91) (Schwarz et al., 2010). In a systematic review of the protective effects for infants of the chronic non-communicable diseases of adulthood, Kelishadi and Farajian (2014) reported a growing body of evidence that breastfeeding has a protective role in the development of obesity, dyslipidemia, diabetes, hypertension, cardiovascular disease, and Type 2 diabetes in later adulthood.

Improving clinical support for the breastfeeding mother of a hospitalized premature infant is a critical first step in the ongoing provision of MoM. In a systematic implementation of the Spatz Ten Steps to Promote and Protect Breastfeeding of

Vulnerable Infants (Fugate et al., 2015), the authors reported a 3.1 fold increase in infants receiving MoM at discharge. The ten steps include informed consent of mothers, establishment and maintenance of milk supply, human milk management, early oral care and feeding, skin-to-skin contact, non-nutritive sucking and transitioning to the breast, measurement of milk transfer, preparation for discharge and adequate follow up. Patient satisfaction scores rose specifically regarding the perception of their support of breastfeeding by hospital staff. But despite the increase in the use of MoM in this study, there was not a significant increase of VLBW infants discharged at less then the 3$^{rd}$ percentile for growth.

This finding supports the data reported by Harbar et al. (2015) that very low birth weight infants often gain weight poorly and demonstrate growth failure during the course of their initial hospitalization. In their study, 362,833 premature infants in the Vermont Oxford Network were followed between 2000-2013 with birth weights ranging from 501-1500 grams (without major birth defects). Harbar and his colleagues defined post-natal growth failure as a discharge weight less than the 10$^{th}$ percentile and 3$^{rd}$ percentiles for postmenstrual age. Average growth velocity (GV) was defined as grams per kilograms per day (g/kg/day) and was computed by using a 2-point exponential model on the basis of birth weight and discharge weight. During the study period (N=362,833 premature infants), the average GV increased from 11.8 to 12.9 g/kg/day. Although the average GV decreased in the study population, in 2013, half of the infants still demonstrated postnatal growth failure (Harbar et al., 2015).

The findings of Harbar et al. (2015) emphasize the importance of addressing the specific properties and characteristics of MoM to optimize the growth and development

of the premature infant. Given that fat comprises up to 50% or more of the caloric content of MoM (Meier, Engstrom, Murtaugh, Meier & Schanler, 2002; Hassiotou et al., 2013; Lucas, Gibbs, Lyster & Baum, 1978), it is our responsibility as health care providers to understand how to measure, manage and deliver MoM to optimize the nutrition of premature infants. Meier, Engstrom, Murtaugh et al. (2002) wrote more than 16 years ago, that “concerns have been raised in the scientific literature regarding the quality control of mother’s milk that is used for infant feedings in the NICU” (p. 649). The authors proposed that quality control of MoM, including establishing and maintaining the maternal milk supply with a hospital grade breast pump, and the storage, handling, delivery and measurement of the components of MoM should continue to be evidence-based. The measurement of MoM for individual milk samples for clinical nutrition management should be conducted with an accurate, feasible and inexpensive instrument that uses the least amount of MoM to avoid wastage and any compromise to the volume provided to the infant.

The standardization of the collection of MoM and the measurement technique with an affordable and accurate instrument that is accessible to NICUs for real-time analysis of fat and caloric content for clinical nutrition management of VLBW infants was covered in Chapter 3. New and preliminary data about the differences in the fat and caloric content of MoM of average to low and high volume producers was presented in Chapter 4. The maternal inter- and intra-variability of the fat and caloric content of MoM data supports the findings of current literature (Koletzko, Agostoni,Bergmann, Ritzenhaler & Shamir, 2011; Sauer, Boutin & Kim, 2016). This is also the first study (after a thorough review of the literature) that examines at the impact of fat and caloric

content on the interval between pumping sessions in this population of mothers. Data from this study supports the need to implement evidence-based nutrition management of premature infants fed MoM.

Current fortification of human milk (primarily with protein, calcium, and phosphorus) is based upon the assumption that human milk is 20 kcal/ounce (Sauer & Kim, 2011; Weber et al, 2001). In a recent meta-analysis of the literature, Brown, Embleton, Harding and McGuire (2016) reported the results of 14 clinical trials (N=1071 infants) of multi-nutrient fortification of human milk of hospitalized premature infants. Trials were determined to be small, methodologically weak and of low quality. Multi-nutrient fortification of breast milk increased rates of growth (MD 1.81 g/kg/day, 95% CI 1.23-2.40), length (MD 0.12 cm/week, 95% CI 0.07-0.17), head circumference (MD 0.08 cm/week, 95% CI 0.04-0.12) (Brown et al., 2016). Brown et al. concluded that that current evidence of multi-nutrient fortification demonstrates a slight improvement in in-hospital growth rates.

In addition to current fortification guidelines, we must continue to consider the importance of human milk fat to the infant. In addition to the summary of the components of human milk fat (Chapter 2) and its contribution to 50% or more of the caloric content of MoM, Hassitou et al. (2013) reported a higher cell count in human milk expressed at the end of a feeding or expression session which may have implications in the clinical management and health outcomes for premature infants.

## Implications for Clinical Practice

In our experience at MUSC, VLBW infants who are fed their own mother's milk and are slow-to-gain weight despite fortification may qualify for hind milk feedings if

their mother produces 30% or more breast milk than her infant requires each day. Fractionating or separating the MoM----which involves pumping for a few minutes, collecting and keeping the low fat breast milk at home, continuing the pumping session and providing the later portion of the pumping session (higher fat hind milk) to the infant---has demonstrated improved growth velocity in this population of infants (Ogechi, William & Bode-Thomas, 2007; Saarela, Kokkonen & Koivisto, 2005, Slusher, Hampton, Bode-Thomas, Pam et al., 2003; Valentine, Hurst & Schanler, 1994). This high fat breast milk feeding intervention cannot be implemented in NICUs without understanding the evidence behind the hind milk feeding technique. This includes when in the course of a day to collect the whole or composite MoM samples and the collection of a sufficient amount of representative MoM samples to provide adequate baseline information. Clinicians must understand how to achieve the most accurate results when measuring fat and caloric content with a validated instrument, and how to monitor the feeding intervention with respect to comparing the baseline composite (whole breast milk) samples and subsequent fractionated higher fat MoM samples. In addition, it is critical for nurses to support lactating mothers to maintain their breast milk supply which is important to sustaining the hind milk feeding intervention and for continued breast milk feedings beyond the period of time that the intervention takes place.

Feeding hind milk to VLBW infants is not without its flaws, which was one of the primary reasons the study (Chapter 4) presented in this dissertation was undertaken. Hind milk separation is an accessible option only to infants whose mothers produce an excess of their daily MoM volume needs. If a mother has an insufficient milk supply or just meets the MoM volumes of her infant each day, she should not participate in hind

milk separation as this will decrease the total amount of her breast milk the infant has access to and requires. If research data demonstrates that the MoM expressed at certain periods of time in the day yields a greater risk of lower fat and caloric content, clinicians can problem-solve and test MoM samples at these points in the day to prevent slow weight gain before it occurs.

Nurses and other health care providers involved in the care of mothers and their premature infants should support, educate and promote breastfeeding in the prenatal phase, throughout the infants' hospitalization and through the post-discharge period with the central caveat that MoM provides numerous short and long-term health benefits for both the infant and the mother. Evidence-based practices should be implemented, such as early and frequent pumping and hand expression techniques, use of a hospital grade breast pump, and continued encouragement and support to meet the mother's feeding goals.

Nurses play a key role in the implementation of these practices as well as the use of an accurate measurement instrument to analyze the fat and caloric content of MoM. They can facilitate multi-disciplinary decision-making to optimize the nutrition of hospitalized premature infants.

Nurses should partner with mothers to participate in all aspects of providing breast milk to their infant. In addition to pumping, hand expression and collection and storage of their milk, mothers (and family members) should participate in oropharyngeal care, testing of the caloric and fat content of their breast milk, and be engaged in daily rounds with the multi-disciplinary team to have a clear understanding of the management of their infant's nutrition plan and progress with growth.

## Implications for Research

As we continue in our understanding of the properties of human milk and work with the maternal individual differences in composition (specifically the fat and caloric content), the greatest challenge to ongoing research is a strict standardization of breast milk collection procedures, storage and handling of breast milk samples, and testing in either fresh, fresh/refrigerated or frozen/thawed states.

In Chapter 3, Table 1, the sampling and testing procedures of the breast milk in each of the studies was presented. Five of the six studies presented tested fresh breast milk (Lucas, Gibbs & Lyster, 1978; Wang et al., 1999; Griffin et al., 2000; Meier, Engstrom, Murtaugh et al., 2002; Meier, Engstrom, Zuleger et al., 2006). In the classic Lucas study of 1978, breast milk was tested 30 minutes after expression. Several of the studies used a combination of fresh and frozen/thawed samples (Wang et al., 1999; Meier, Engstrom, Murtaugh, et al., 2002; Meier, Engstrom, Zuleger et al., 2006). Thawing and warming procedures of previously frozen or refrigerated samples were not reported in all studies. Centrifuge time of samples varied from three minutes with the CRCT method to 15-30 minutes in other studies. Du et al (2017) stated that centrifugation of human milk raises methodological concerns. For example, samples centrifuged at the 15 minutes at 1315g versus the standard 15 minutes at 12,000g may result in a lower compaction of the cream layer and may lead to higher creamatocrit values.

With respect to reported diurnal differences in human milk (Chapter 1), the timing of the breast milk collection is also critical as well as the volume of the mother produced

each day and at single pumping sessions. As an example, in a recently published study by Sauer, Boutin and Kim (2016), the mothers were asked to collect milk samples via pumping three hours after the last expression or breastfeeding session in the AM and PM. The daily milk volumes of the mothers were not reported other than identified in the limitation section as "super producers." Laboratory analysis was used to test for caloric and fat content, and the average caloric content of the samples was 17.9 kcal/ounce. They concluded that the assumption that human milk standardized at 20 kcal/ounce was not supported. The results of this dissertation study do not support that assumption either. The authors did conclude that individual nutrition management of premature hospitalized infants, specifically VLBW infants, is critical.

Sample size determination to achieve adequate power is also important to future studies. A power analysis for sample size determination was not reported in any of the six studies presented in Chapter 3 (Table 1). The question lies in whether sample size should be determined by the number of mothers in a study or the number of MoM samples tested. An ideal method for assessment of caloric and fat content of MoM may be to pool a 24-hour sample. If 24-hour pooling is feasible, one would then calculate power based on the number of mothers in the sample. This may be a technique to improve research practices, but this is not how most infants are fed in NICUs. The mothers pump and provide breast milk in individual containers at each pumping session. Pooling 24 hours of breast milk may not be feasible and frequent handling of breast milk may also alter breast milk fat and caloric content.

The research conducted in the properties of human milk is varied in methods and sampling, but we must continue our efforts to understand and harness its bioactive,

synergistic and nutritional qualities to optimize the growth and health outcomes for premature infants. We must continue to consider the importance of human milk fat to the infant. In addition to the summary of the components of human milk fat (Chapter 2) and its contribution to 50% or more of the caloric content of MoM, Hassitou et al. (2013) found a higher cell count in human milk expressed at the end of a feeding or expression session which may have implications in the clinical management and health outcomes for premature infants.

## Implications for Policy

Breast pump-dependent mothers of premature hospitalized infants are at high risk of delayed lactogenesis and insufficient milk supply to meet the needs of their infant as they face considerable stressors which include separation from their infant, coping with their infant's medical condition and prolonged hospital course, and dependence on an adequate hospital-grade breast pump to sustain lactation (Chatterton, Hill, Aldag, Hodges, Belknap & Zinnaman, 2000; Hill & Aldag, 2004; Meier, Engstrom, Janes, Jeigier & Loera, 2012; Parker, Sullivan, Krueger, Kalechi & Mueller, 2013, Post, Stam & Tromp, 2015).

The evidence of the protective effects of MoM are clear for the premature infant, but MoM remains a precious commodity which requires multiple layers of support by healthcare providers, support persons in the mother's life, and society to sustain lactation. Policy makers also play a critical role in supporting women to provide breast milk to their infants.

The US Surgeon General's Call to Action to Support Breastfeeding (2011) reported that returning to work is a significant barrier to sustaining lactation and mothers

who return to work are at risk of a shorter duration of breastfeeding. The report acknowledged that breastfeeding support in the workplace is critical. The Patient Protection and Affordable Care Act (ACA) offers some support for breastfeeding women but remains inconsistent between insurance providers and regulations to protect mothers who must express breast milk in the workplace (Sherburne-Hawkins & Doe-Fleisner, 2015). Income disparities among women persist, as socioeconomic status is one of the strongest predictors of whether or not women choose to breastfeed and the length of time mothers breastfeed (Kimbro, 2006). In two studies of low income women, Kimbro (2007) and Kimbro, Griffitths, Dezateau and Law (2006) reported that the highest risk of quitting breastfeeding occurred within the first four weeks postpartum followed by the period of return to work.

Legislation can play a role in increasing breastfeeding rates and reducing barriers for working mothers. Sherburne-Hawkins and Doe-Fleisner (2015) described the current ACA legislation for breastfeeding support to be a patchwork of regulations. For example, only a small minority of states in the US address the needs for pumping breaks in the workplace which places the mother at risk of low milk supply and early weaning (Kimbro, 2006; Kimbro, Griffiths, Dezateau & Law, 2007). Also, some insurance companies provide manual pumps which are insufficient to sustain an adequate milk supply (Meier, Engstrom, Janes, Jeigier & Loera, 2012; Parker, Sullivan, Krueger, Kalechi & Mueller, 2013; Post, Stam & Tromp, 2015) while other insurance companies provide personal pumps or cover the rental costs of hospital grade pumps. Mothers of lower socioeconomic levels who qualify for the Women's, Infant's and Children's (WIC) Supplemental Nutrition Program may have access to a hospital grade pump if their state

has sufficient allocated funds to do so. If not, they may be provided a substandard breast pump (Sherburne-Hawkins & Doe-Fleisner, 2015). Sherburne-Hawkins and Doe-Fleisner (2015) reported that implementing federal laws (versus allowing each state to interpret the ACA individually) would strengthen breastfeeding support for women in the workplace and improve access to evidence-based breastfeeding supplies and provision of expert lactation support regardless of the mother's insurance status.

Legislation at the state and federal levels can make a large impact on the support of breastfeeding mothers especially in the areas of extended postpartum leave, workplace support, and provision of breastfeeding supplies via insurance companies and the WIC program. Policy and procedure development for the clinical care of breastfeeding mothers and premature infants in the inpatient and outpatient settings benefit from collaborative global networks lead by clinical experts. Currently, more than 1,000 NICUs representing 2.2 million infants participate in the VON (2018). The Vermont Oxford Network (VON, 2018) consists of teams of health care professionals representing Level 1, 2 and 3 nurseries throughout the world. VON maintains data on premature infants and consistently remains abreast of current literature and implications for clinical practice. They also provide neutral independent data analysis to form voluntary benchmarks for NICUs worldwide. VON also provides quality improvement collaboratives for interdisciplinary teams with in-person and online educational programs and conducts clinical trials to respond to practical clinical issues to improve direct clinical care of premature infants.

In summary, MoM is critical to the care of premature hospitalized infants. The management of its unique properties to optimize their growth, development and health

outcomes is integral to nursing care of this mother/infant dyad. With an understanding of the variability of fat and caloric content within and between breast pump-dependent mothers of premature infants and new data regarding the impact of the interval between pumping sessions in this population of mothers, nurses, neonatologists, and neonatal dieticians can provide evidence-based and individualized plans of care to optimize postnatal growth. This data may allow NICUs to identify certain points in the day that mothers may be at risk of producing breast milk insufficient in fat and caloric content to individualize mothers' pumping practices and their infant's nutrition plan. Nurses play a critical role in educating and supporting mothers to provide breast milk for their premature infant and how to handle, measure and deliver human milk to infants to optimize their nutrition. With emerging knowledge about the properties of MoM, nurses, dieticians, and neonatologists can collaborate and minimize erroneous estimation of fat and caloric content of MoM to improve upon current nutrition management plans for premature infants.

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**CURRICULUM VITAE**

Barbara Haase

Place of Birth: Milwaukee, WI

EDUCATION

University of Wisconsin-Milwaukee
Nursing PhD Program (PhD candidate)
"Fat and Caloric Content of Breast Milk of Mothers of Premature Infants: Comparison of High vs Average to Low Volume Producers and Impact of Intervals Between Pumping Sessions"

University of Wisconsin-Madison
Madison, Wisconsin, USA 1992
**MASTER OF SCIENCE IN NURSING** (High Honors)

Specialty Training: Pediatric Nurse Practitioner Program with emphasis on primary health care delivery of children, and Pediatric Clinical Nurse Specialist Program with focus on the care of chronically ill children.
Master's Thesis: "A Qualitative Study of Mothers' First Feeding Experiences in the Neonatal Intensive Care Unit with Infants with Bronchopulmonary Dysplasia."

University of Wisconsin-Milwaukee
Milwaukee, Wisconsin, USA 1987
**BACHELOR OF SCIENCE IN NURSING** (Magna cum Laude)

CURRENT ACTIVE LICENSES
Nationally Certified Pediatric Nurse Practitioner (1992-present)
Internationally Certified Lactation Consultant (1999-present)
Advanced Practice Nursing License (State of South Carolina, 2004-present)

PROFESSIONAL EXPERIENCE

**ADVANCED PRACTICE LACTATION CONSULTANT**
May 2004-present, Medical University of South Carolina, Charleston, SC
Assessment and management of lactation problems for postpartum, Levels 1-3 nurseries, and lactation outpatient clinic. Responsible for development of lactation protocols, staff and physician education, as well as community and conference lectures. Lecturer for the MUSC College of Medicine Human Lactation Course. Active in multiple committees, including High Risk Lactation, Discharge Nutrition Planning, Multidisciplinary Feeding Team, and Best Fed Beginnings Project (which led to current Baby Friendly designation). Special interest in preterm infants and infants with complex medical needs. Actively participates in research studies related to high risk lactation topics. Current research responsibilities include study of

domperidone use for treatment of mothers with premature hospitalized infants and breast milk fat and calories study

**INDEPENDENT LACTATION CONSULTANT**

September 2000-2004 Belgium, Europe

Independent breastfeeding consultations, education, and support in home settings in Belgium, Europe. Provided education and breastfeeding resources to professionals involved in the care of breastfeeding mothers. Consultant lecturer for Belgian Department of Children's Public Health. Assisted first group of IBCLC candidates to take the first IBCLE Examination offered in this country

**PEDIATRIC NURSE PRACTITIONER, LACTATION CONSULTANT AND CLINICAL INSTRUCTOR**

University of Illinois at Chicago Jan. 1993-May 2000

Chicago, Illinois, USA

-Management of a 300+ pediatric practice serving their primary health care needs, including well child visits, routine sick visits (diagnosis and treatment of common illnesses), referrals to specialty physicians and case management of complex cases.

-Breastfeeding consultation for the patient needs of physicians, residents, medical and nursing students, and nursing staff. Referral source for mothers with routine and complex breastfeeding problems.

-Newborn nursery responsibilities included admission and discharge examinations of newborns, breastfeeding consultations, and training of nursing, midwifery, and medical students and nursing staff in physical assessment, medical management, and lactation of the newborn.

-Clinical instructor duties included training of graduate and undergraduate nursing students, lecturing at the university on topics such as breastfeeding, physical assessment, child development, and child abuse.

-Member of the University of Illinois Multidisciplinary Breastfeeding Task Force which developed policies, training programs, conferences, and educational materials for all breastfeeding related areas

**RESEARCH TEAM LEADER**

University of Wisconsin-Madison 1990-1992

Madison, Wisconsin, USA

Chief Investigator: Karen Pridham, RN, Ph.D.

-Collection and analysis of quantitative and qualitative research data for a large, longitudinal study looking at premature infant feeding experiences

**PEDIATRIC HOME HEALTH CARE NURSE/EDUCATOR**

New Horizons Home Health Care 1989-1992

-Case management and nursing care of chronically ill children and training of home health care nurses. Development of lectures and training videos for the care of medically complex children in the home setting.

**PROGRAM COORDINATOR AND MEDICAL NURSING DIRECTOR**

Holiday Home Camp
Lake Geneva, Wisconsin 1989-1991
-Direction and development of programs and nursing care for underprivileged children in a summer camp setting. Devised the camp's health policies and trained international nurses each summer session. Developed educational parenting and health programs for neglectful and abusive mothers, and adolescent mothers and their children

**PEDIATRIC NURSE**
Children's Hospital of Wisconsin
Milwaukee, Wisconsin 1987-1989
-Nursing care of acute and chronically ill children with emphasis on the care and education of children and their families experiencing metabolic diseases and renal failure and renal transplant

AWARDS AND HONORS

**University of Wisconsin-Milwaukee Chancellor's Award** (2013-2016)

**University of Wisconsin-Milwaukee College of Nursing Dean's Scholarship: PhD Program** (2013-2016)

**South Carolina Palmetto Gold Nursing Award** (2013): Selected as one of top 100 nurses in South Carolina for nursing excellence for promoting and advancing nursing care of mothers and infants

**University of Wisconsin-Madison School of Nursing Graduate Fellowship for Full Time Study** 1990-1992

**University of Wisconsin School of Nursing Fellowship for Graduate Study of Rural and Native American Health**
AHEC PROJECT/Ashland, Wisconsin (1992)

Master of Science in Nursing, University of Wisconsin-Madison, High Academic Honors

Bachelor of Science in Nursing, University of Wisconsin-Milwaukee, Magna cum Laude

MEMBERSHIPS

National Association of Pediatric Nurse Practitioners and Nurse Associates (1992-present)
International Board of Certified Lactation Consultants (1999-present)
European Board of Internationally Certified Lactation Consultants (2000-2004)
South Carolina Breastfeeding Coalition (2004-present)

**PUBLICATIONS**

**Haase, B.,** Barreira, J., Murphy, P., Mueller, M. & Rhodes, J. (2009). The development of an accurate test weighing technique for preterm and high-risk hospitalized infants. *Journal of Breastfeeding Medicine*, 4(3), 151-156.

**Haase, B**., Taylor, S.N., Mauldin, J., Johnson, T. & Wagner, C.L. (2016). Domperidone for treatment of low milk supply in breast pump-dependent mothers of hospitalized premature infants. *Journal of Human Lactation*. 32(2), 373-381

**Haase, B**., Taylor, S.N, Morella, K., Johnson, T. & Wagner, C.L. (2016) Abstract P-11: Domperidone for the treatment of low milk supply in mothers of hospitalized premature infants: A multidisciplinary development of safe prescribing practices and a retrospective chart review (2010-2014) of maternal response to treatment. *Journal of Breastfeeding Medicine*, Abstracts of the International Society of Researchers of Human Milk and Lactation, March 2016.

ABSTRACTS

Taylor, S., Finch, C**., Haase, B.,** Barreira, J. & Annibale, D. (2013). Are very low birth weight infants able to avoid growth failure with predominantly human milk feedings? Annual International Meeting of the Academy of Breastfeeding Medicine, 2013.

Wagner, C.L., Murphy, P.K**., Haase, B**. & Barreira, J. Domperidone in the treatment of low milk supply in mothers of critically ill infants. Annual International Meeting of the Academy of Breastfeeding Medicine, 2011.

Taylor, S., Barreira, J., **Haase, B**., Murphy, P., Mauldin, J., Browning, D., Hulsey, T., Ebeling, M. & Wagner, C.L. Multidisciplinary improvement of hospital lactation support process: Impact on mother's milk delivery to infants. Annual International Meeting of the Academy of Breastfeeding Medicine, 2011

White, K., West, D., Sharpe, M., Murphy, P, **Haase, B**., Barreira, J. BRAVO! Breastfeeding: An education performance, Medical University of South Carolina, Vermont Oxford Fall Conference, 2008.

LECTURES, EDUCATION PROGRAMS AND PROJECTS

***LOCAL AND INTERNATIONAL INVITED SPEAKING ENGAGEMENTS AND PROJECTS**, 2000-present:*

**MANAGEMENT OF COMMON LACTATION PROBLEMS IN THE OB OUTPATIENT SETTING**, MUSC OB Grand Rounds Presentation, February 2017

**TREATMENT OF LOW MILK SUPPLY IN MOTHERS OF PREMATURE INFANTS: BENEFITS/RISKS OF DOMPERIDONE AND SAFE PRESCRIBING PRACTICES**, October 6, 2016. Department of MUSC OB/GYN, Grand Rounds Presentation

**HINDMILK INTERVENTION IN THE HIGH RISK NURSERY: OPTIMIZING WEIGHT GAIN OF AT RISK INFANTS**: delivered at "The Lactation Conference", April and September, 2012

**LACTATION SUPPORT OF A DIABETIC MOTHER,** MUSC Perinatal Update, 2011 (also delivered as a webinar in February 2012 for the International Lactation Consultant Association)

**SUPPORTING BREASTFEEDING IN THE HIGH RISK NURSERY:** Neonatal Nutrition Conference, University of Minneapolis Children's Hospital (October 2008)

**TRANSITIONING PRETERM INFANTS TO THE BREAST AND CUE BASED FEEDINGS**: University of Minnesota Neonatal Nutrition Conference (October 2008)

**IMPLEMENTATION OF EVIDENCE-BASED BREASTFEEDING PRACTICES FOR HOSPITALIZED INFANTS IN A HIGH RISK NURSERY: A MULTIDISCIPLINARY TEAM APPROACH**, 2006-2007, presented at the ILCA conference 2007 and the Wolf and Glass Sucking and Swallowing Disorders Conference, Columbia, SC. **This lecture was elected by ILCA to be a CEU module**.

**BREASTFEEDING TEAM LEADER PROGRAM (2004-2008) (MUSC):** development and implementation of an in-depth training program for bedside nursing leaders in evidence based breastfeeding practices for high risk nurseries

**RESIDENT PHYSICIAN TRAINING PROGRAM ( MUSC):** development of lectures for resident physicians in breastfeeding practices including anatomy/physiology and management of breastfeeding problems

**BREASTFEEDING IN HIGH RISK NURSERIES,** member of expert panel discussion with Paula Meier, PhD at SC Breastfeeding Coalition Conference 2005

**INTERNATIONAL EVIDENCE BASED GUIDELINES FOR THE MANAGEMENT OF BREASTFEEDING IN THE FIRST 14 DAYS:** presented at three Belgian Child and Family Health (Kind en Gezin) conferences (2000-2004)

**PROJECT LEADER:**

**HINDMILK INTERVENTION PROJECT:** included comprehensive review of literature, development of protocol for high risk infants with inadequate growth, and

presentation to MUSC Evidence and Values Committee, and staff education (2006-2017)

**MUSC BRAVO BREASTFEEDING COURSE**: Developed and implemented large scale breastfeeding education program for perinatal nursing division in consort with MUSC Nursing Education team (4 hour course with one hour didactic demonstration module) (2009-present)

**HIGH RISK LACTATION (BRAVO) COURSE:** Developed and implemented a lactation education program for all high risk neonatal nurses at MUSC (7 hour course) (2011-2015)

**TRANSITIONING THE PRETERM INFANT TO THE BREAST PROTOCOL-**comprehensive review of literature, development of protocol, and implementation and education for high risk nurseries at MUSC (2004-present)

**DEVELOPMENT OF ACCURATE TEST WEIGHT METHOD FOR HIGH RISK BREASTFED INFANTS WITH LEADS –**written and developed this instructional video based upon our 2009 research results on test weights for hospitalized high risk breastfeeding infants (Video available in ILCA Bookstore and on the MUSC Intranet for staff education) (2007-2015)

**LACTATION POSITION STATEMENT AND MANAGEMENT OF CLEFT LIP AND CLEFT LIP AND PALATE INFANTS-** developed with MUSC Department of Speech and Language Pathology, presented to MUSC pediatric attending physicians, neonatologists and nursing staff (2007-2017)

**University of Illinois at Chicago (UIC)**, Chicago, Illinois (1992-2000)

*LECTURES GIVEN FOR MASTERS AND BACHELORS NURSING STUDENTS AT UIC*

**CHILD ABUSE: An Overview**: Comprehensive lecture covering the identification and treatment of victims of child abuse

**BREASTFEEDING MANAGEMENT IN THE POST PARTUM PATIENT:** Included a variety of lectures on assessment, practice, and pumping and storage

**NEONATAL PHYSICAL ASSESSMENT:** Comprehensive overview of physical assessment of the term newborn

**PEDIATRIC PHYSICAL ASSESSMENT:** Overview of the examination of the pediatric patient

*HOSPITAL AND CLINIC BASED EDUCATION:*

**BREASTFEEDING ASSESSMENT, MANAGEMENT, PUMPING AND STORAGE:** Ongoing education of nursing and medical staff, as well as university students

**NEONATAL PHYSICAL ASSESSMENT AND WELL BABY MANAGEMENT:** Education of midwifery, medical, and advanced practice

nursing students in the examination and management of the newborn, including medical management of neonatal jaundice, hypoglycemia, and referrals to specialist physicians.
**CLINICAL NURSING INSTRUTOR: ADVANCED PRACTICE NURSING EDUCATOR:** Preceptor and mentor to advanced nursing students in the newborn nursing and pediatric clinical settings. Included all aspects of clinical education

*COMMUNITY BASED LECTURES*

**GROWTH AND DEVELOPMENT OF THE INFANT AND TODDLER**
Lecture prepared and given to public health workers in Chicago. Audience included public health nurses and aides, social workers, and case managers

**BREASTFEEDING: ASSESSMENT OF APPROPRIATE FEEDING and BREASTFEEDING AND THE WORKING MOTHER**
These workshops were prepared and given at the UIC Annual Breastfeeding Conference for nurses, physicians, and students.

**CARE OF THE INFANT WITH A BLIND PARENT**
Developed in consort with blind parents of infants for the Chicago Center for the Blind

*HOME HEALTH CARE BASED EDUCATION/LECTURES (1989-1992)*

**CARE OF THE CHRONICALLY ILL HOME CARE PATIENT**
Development of workshops and a video for care of the medically complex pediatric patient. This included training of pediatric home care nurses

**UNIVERSITY OF WISCONSIN-MADISON SCHOOL OF NURSING, MADISON, WISCONSIN (1990-1992)**

**QUALITATIVE AND QUANTITIVE DATA COLLECTION**
Training of staff members in research data collection for a large, longitudinal neonatal research study

**WOMEN, INFANTS AND CHILDREN NUTRITION PROJECT (WIC), (THE US NATIONAL PUBLIC MATERNAL/CHILD SUPPLEMENTAL NUTRITION PROGRAM), MILWAUKEE, WISCONSIN (1988-1990)**

**INFANT AND CHILDHOOD NUTRITION EDUCATION**
Development of education related to the feeding and nutrition of infants and children for a large minority impoverished population. Also included development of written educational materials

**COORDINATOR OF WIC BREASTFEEDING PROMOTION PROGRAM**
Coordination of verbal and written materials promoting breastfeeding in the WIC project. Included training of mother breastfeeding supporters and WIC staff members.

**PRENATAL EDUCATION COORDINATOR**
Developed the project's prenatal verbal and written education, including prenatal nutrition and health and preparation for childbirth.

**PARENTING CHILDREN FROM INFANCY TO AGE 5**
Staff and parent training of parenting and discipline in young children. Included principles of positive reinforcement, alternatives to physical punishment, time-ins and time-outs, and daily reading with children

**HOLIDAY HOME CAMP (CHILDREN'S CAMP SPECIALIZING IN THE MINORITY UNDERPRIVILEGED POPULATION OF CHICAGO, IL), LAKE GENEVA, WISCONSIN (1988-1992)**

**ADOLESCENT MOTHERS EDUCATION COORDINATOR**
Development of a 6 day program for adolescent mothers and their children, including nutrition, growth and development and parenting education

**ABUSIVE MOTHERS PROGRAM EDUCATION COORDINATOR**
Development of educational materials and workshops for mothers who were re-establishing custody of their children after child abuse or neglect. Education program included understanding of growth and development of children and parenting principles. Also included education of staff members as mentors to mothers.

**CHILDREN'S HOSPITAL OF WISCONSIN, MILWAUKEE, WISCONSIN (1987-1989)**

**PEDIATRIC JUVENILE DIABETES EDUCATOR**
Education of staff members, children and families affected by Type 1 diabetes. Included management of the disease, nutrition, and insulin administration

**PEDIATRIC TRANSPLANT EDUCATOR**
Education of staff members and parents and families with renal or liver transplants. Included pre- and post-operative care, administration of medications, and signs and symptoms of transplant rejection