Modeling adverse outcomes in very low birth weight infants based on an infant diet of mother's breast milk and donor breast milk

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Abstract

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Objective: This study evaluated the dose and threshold effects of mother's breast milk (MBM), donor breast milk (DBM), and preterm formula (PTF) fed to very low birth weight (VLBW) infants on specific adverse health outcomes measured from birth to discharge from the hospital. This study contributes to the understanding of how much MBM is necessary during a premature infant' hospitalization to improve health outcomes. It also provides information on the effects of DBM and PTF upon premature infant health outcomes.

Methods: The current study is a retrospective analysis of VLBW infants who received MBM and/or DBM from birth to 1,800 grams. When MBM was not available, infants were fed PTF after 1,800 grams until discharge from the hospital. Any VLBW infant born at the University of Washington Medical Center Seattle, WA, after the initiation of the center's DBM program was included in this study. Infants were excluded if any major congenital anomalies existed. The DBM program included infants born at 32 weeks or less gestational age or weighing less than or equal to 1,800 grams. VLBW infants were eligible for DBM until 33 weeks gestational age and weight was greater than 1,800 grams. Once an infant was no longer eligible for DBM, the infant was transitioned to preterm formula (PTF) if there was not enough MBM.

Descriptive statistics were used to summarize quantitative variables. Dosage and threshold effects of MBM, DBM, and PTF for VLBW infants were evaluated using receiver operating characteristic curve (ROC) analysis. Bronchopulmonary dysplasia, necrotizing enterocolitis, late-onset sepsis, and mortality were analyzed using logistic regression models. Predictor variables included the percentage of MBM, DBM, and PTF in the infants' total diet of all milk types from birth to discharge. Length of stay, weight gain, and total days on ventilator were regressed upon the percentage of MBM, DBM, and PTF in the infant's total diet. Covariates included birthweight, gestational age, gender, Apgar scores at both 1 and 5 minutes, exposure to prenatal and postnatal steroids.

Outcomes: The outcomes for this study included the incidence of bronchopulmonary dysplasia, necrotizing enterocolitis, late-onset sepsis, mortality, length of hospital stay, weight gain, and total days on ventilator for VLBW infants who were fed MBM, DMB, and PTF.

Results: A total of 302 low birth weight infants were included in this study. The mean gestational age was 28.65 ± 3.08 weeks and mean birthweight is 1.02 kg ± 0.30 kg. The sample is 53.3% male and 46.7% female infants. The mean length of stay is 67.37 ± 37.01 days with the mean discharge weight of 2.92 kg ± 1.09 kg. Among the participants, 55.6% had bronchopulmonary dysplasia, 27.2% experienced medical necrotizing enterocolitis, 27.2% had late-onset sepsis, and 18.5% died. Logistic regression analyses showed that the percentage of MBM significantly predicted a decrease in BPD but not medical necrotizing enterocolitis, late-onset sepsis, or mortality. The increased percentage of MBM contributed to a reduction in length of stay and total days ventilated. The percentage of DBM yielded comparable results for these outcomes. ROC analysis showed that a diet composed of greater than 65.5% of MBM (odds ratio = 0.56, $p = 0.050$) was associated with survival, whereas a diet with greater than 31.5% of PTF (odds ratio = 1.94, $p = 0.029$) was associated with mortality.

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DEDICATION

To my loving one-eyed pirate, George,

to papa CHIPS, spunky mama, wheeling ‘lil bro, Dr. Jonnie,

and all my extended family and friends

Thank you all for your loving support!

“Only struggle a little more. Carry your cross without complaining. Don’t think you are anything special. Don’t justify your sins and weaknesses, but see yourself as you really are. And, especially, love one another.” ~Father Seraphima Rose~

CHAPTER 1

Introduction

Prematurity and very low birth weight (VLBW) are the most common reasons for infant mortality worldwide (Ganapathy, Hay, & Kim, 2012). Infants born prematurely are also at an increased risk for necrotizing enterocolitis (NEC), intestinal perforation, and bronchopulmonary dysplasia (BPD). An infant's diet is one contributing factor to preventing NEC and reducing BPD (Assad, Elliott, & Abraham, 2016), and human breast milk is the optimum diet for infants (Eidelman, 2012; Organization, 2011; Ramani & Ambalavanan, 2013; Wojcik, Rechtman, Lee, Montoya, & Medo, 2009). Studies indicate that high doses of human milk reduce morbidities and are linked to beneficial structural and functional changes in the gastrointestinal tract (Hanson, 2004; Meier, Patel, & Esquerra-Zwiers, 2016). Whereas human milk promotes beneficial gut microflora (Hanson, Lyden, Furtado, Van Ormer, & Anderson-Berry, 2016; Hanson, 2004). Human milk contains probiotics, prebiotics, and human milk oligosaccharides (HMO) (Bode, 2012; Hanson et al., 2016; Hanson, 2004; Jantscher-Krenn & Bode, 2012), all of which promote healthy gut microflora. To avoid feeding VLBW infants preterm formula (PTF), which appears to have a detrimental effect on gut microflora (Hanson et al., 2016; Hanson, 2004), neonatal intensive care units use donor breast milk (DBM) when the mother's breast milk (MBM) is not available. However, the pasteurization process of DBM can reduce the benefits of the human milk, and adding nutrients such as probiotics, liquid protein, and human milk fortifiers to DBM is recommended (Bertino et al., 2012; Bertino et al., 2009; Hanson et al., 2016; O'Connor, Ewaschuk, & Unger, 2015).

MBM is best for infants, but DBM is a better volume filler than formula when there is not enough MBM available. What is unknown is whether there is an optimum percentage or volume of MBM necessary for improved health outcomes. There are current studies examining exclusively human milk diets—meaning diets in which the infants are not given any bovine-based products—but they do

not evaluate the dosage and threshold of various milk types in the infants' diet. This study evaluates whether there is a minimum percentage of MBM and DBM with a bovine-based human milk fortifier and PTF that will affect outcomes for the following: bronchopulmonary dysplasia, necrotizing enterocolitis, late-onset sepsis, mortality, length of stay, weight gain, and total days on ventilator for VLBW infants.

Statement of the Study Purpose

Approach:

This study had one primary aim and one hypothesis:

Primary Aim

Aim: This study evaluated dosage and threshold effects of MBM, DBM, and PTF on the following outcomes: BPD, NEC, late-onset sepsis, mortality, length of stay, weight gain, and total days on ventilator for very low birth weight infants.

Hypothesis: There will be a lower incidence of BPD, necrotizing enterocolitis, late-onset sepsis, mortality, a reduced length of stay, and fewer days on mechanical ventilation for VLBW infants who are fed a larger proportion of MBM compared to both DBM and preterm formula between birth and hospital discharge. Greater weight gain is expected in infants fed MBM or PTF compared to a diet of DBM.

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CHAPTER 2

Modeling adverse outcomes in very low birth weight infants based on an infant diet of mother's breast milk and donor breast milk

Significance of the problem

Infants born prematurely are at an increased risk for NEC and intestinal perforation because of an immature gastrointestinal system. The incidence of NEC in VLBW infants is between 6% and 10% in the United States (Ramani & Ambalavanan, 2013). The most common form of preterm infant NEC—a function of an immature gastrointestinal tract with inflammation or abnormal gut microflora—is an acute inflammatory condition affecting the bowels, and it typically occurs in the first few weeks after birth (Neu, 2015). Depending on the severity of the disease, it can be either surgically or pharmacologically treated. The pathophysiology of NEC is not well understood (Ramani & Ambalavanan, 2013); however, prematurity, diet, delayed enteral feedings, intestinal microbes, use of antibiotics, use of antenatal steroids, Cesarean birth, and bronchopulmonary dysplasia have been identified as contributing factors (Bode, 2012; Hanson et al., 2016; Hanson, 2004; Jantscher-Krenn & Bode, 2012; Neu, 2015).

Prebiotics, probiotics, and human milk oligosaccharides (HMO) are the specific human milk components that protect infants from inflammation (Hanson, 2004). Prebiotics are non-digestible carbohydrates that combine with probiotics promoting healthy beneficial gut bacterial. Several studies indicate that premature infants fed an exclusively human milk diet (meaning any supplemental nutrients are also human milk based) have reduced feeding intolerance, lower incidence of NEC (both surgical and medical), greater prevention of late-onset sepsis and mortality, and improved neurodevelopmental outcomes (Assad et al., 2016; Bertino et al., 2009; Bloom, 2016; Chan, 2003; Delfosse et al., 2013; Edwards & Spatz, 2012; Eidelman, 2012; Ganapathy et al., 2012; Hair et al., 2014; Hair, Hawthorne, Chetta, & Abrams, 2013; Hair et al., 2016; Herrmann & Carroll, 2014; Huston et al., 2014; Meier et al.,

2016; Patel et al., 2013; Schanler, 2015; Sullivan et al., 2010). This is important because Schanler and colleagues (2005) found that infants receiving DBM plus bovine-based fortifier or PTF had the same rate of NEC and sepsis (Heiman & Schanler, 2006; Schanler, Lau, Hurst, & Smith, 2005). In contrast, infants receiving MBM plus bovine-based fortifier had a 50% reduction in NEC and sepsis (Heiman & Schanler, 2006; Schanler et al., 2005). This difference may be due to the use of MBM vs. DBM. MBM provides an antibacterial protection that is reduced in DBM by the pasteurization process; therefore, the combination of DBM plus bovine-based fortifier may not be adequate to provide protection from NEC and sepsis (Chan, Lee, & Rechtman, 2007; Heiman & Schanler, 2006; O'Connor et al., 2015; Schanler, 2015; Schanler et al., 2005). Chan and colleagues (2007) found a bovine-based fortifier (Enfamil Human Milk Fortifier) with supplemental iron diminished the antibacterial activity of MBM, whereas the human milk-based fortifier (Prolact +4) did not. Findings noted an increase of organisms E.coli, Enterobacter sakazakii (ES), Staphylococcus, and Group B Streptococcus in the bovine-based fortifier with supplemented iron. These four organisms are the leading cause of NEC and sepsis in neonates (Chan et al., 2007). The addition of a bovine-based milk fortifier and supplemental iron prevents human milk's antibacterial protection from suppressing these organisms (Chan, 2003). Limiting exposure to bovine proteins in formulas with added iron and bovine-based fortifiers with supplemented iron may lead to the reduction of NEC and late on-set sepsis in neonates.

Studies have also reported that an infants' diet of human breast milk will reduce the incidence of BPD, whereas PTF may increase BPD (Assad et al., 2016; Eidelman, 2012; Ramani & Ambalavanan, 2013; Wojcik et al., 2009). The development of BPD has not been well studied and descriptive reports are inconsistent (Schanler, Lau, Hurst, & Smith, 2005; Spiegler et al., 2016). What is known, however, is that environmental factors such as infection, hyperoxia and ventilator-induced lung injury (Spiegler et al., 2016) are contributing factors to the development of BPD. Breast milk contains antioxidant properties that potentially protect VLBW infants from oxidative stress (Spiegler et al., 2016), which may

be a result of high oxygen concentration sometimes needed to maintain arterial oxygen saturation in VLBW infants. Nutrition with high caloric and protein count, fluid restriction, and early enteral feeding are also associated with a decreased incidence of BPD (Spiegler et al., 2016).

Studies have led the American Academy of Pediatrics (AAP) (Eidelman, 2012) and the World Health Organization (WHO) ("WHO Guidelines Approved by the Guidelines Review Committee," 2011) to recommend the use of human milk for all infants, especially those born prematurely (Bloom, 2016; Carroll & Herrmann, 2013; Chan, 2003; Delfosse et al., 2013; Edwards & Spatz, 2012; Eidelman, 2012; Gartner et al., 2005; Hair et al., 2016). The use of pasteurized donor human breast milk is recommended when the mother's breast milk is not available (Eidelman, 2012; C. Hanson et al., 2016; Meier et al., 2016). Neonatal intensive care units (NICUs) use DBM frequently when MBM is not available in an effort to reduce the occurrence of NEC. NICUs also strive to avoid the use of preterm bovine-based formula (Colaizy, 2015; O'Connor et al., 2015). However, evidence that DBM reduces NEC is extrapolated from studies that indicate that maternal breast milk is associated with lower rates of NEC compared to PTF (Colaizy, 2015; Meier et al., 2016; Schanler, Shulman, & Lau, 1999). Therefore, the NICUs are operating on best practice rather than established scientific fact.

Feeding a VLBW infant a human milk-based diet is also more beneficial than any bovine-based diet in reducing morbidity and mortality. Studies indicate that the amount of MBM compared to the amount of DBM an infant receives can affect the outcomes of NEC and late-onset sepsis (Carroll & Herrmann, 2013; Cristofalo et al., 2013; Heiman & Schanler, 2006; Johnson, Patel, Jegier, Engstrom, & Meier, 2013; Schanler, 2015; Sullivan et al., 2010)**,** but no current study evaluates this dosage relationship. This study aims to evaluate the volume relationship. MBM is best for infants; however, what is still unknown is how much of an infant's diet needs to be MBM in order to achieve optimum health benefits and how much PTF can be given before infants begin to experience poor outcomes. This study also examines DBM, which is known to have fewer beneficial properties than MBM, though it is

still better for infants than PTF. The study evaluates the total percentage and total volume of an infants' diet of DBM during hospitalization necessary to achieve optimum health benefits.

Maternal Characteristics

The etiology of preterm deliveries is complex and multifactorial. Preterm birth may be related to individual-level behavioral and psychosocial factors, as well as environmental exposures, medical conditions, infertility treatments, and genetics. Many of these factors occur in combination. For example, the socioeconomically disadvantaged and members of ethnic minority groups tend to have poor prenatal care. Early and regular prenatal care reduces complications such as preterm birth, small for gestational age infants, and perinatal death. Prenatal care can also control existing conditions such as high blood pressure, diabetes, and obesity.

Controlling these conditions can prevent more serious conditions such as preeclampsia and abnormal microbiota development. Preeclampsia is associated with fetal growth restriction, preterm birth, and placental abruption. Low birth weight is the main risk factor for a poor intrauterine nutritional environment (Aaltonen et al., 2011). A mother's body mass index (BMI), weight gain during pregnancy and mode of delivery can influence the composition and activity of microbiota development in infants (Cabrera-Rubio et al., 2012). For example, colostrum samples from obese mothers showed a lower diversity than normal-weight mothers (Cabrera-Rubio et al., 2012). Poor metabolic development may result if abnormal gut microflora associated with obese pregnant women is transferred to their infants (Cabrera-Rubio et al., 2012). Mode of delivery can also affect microbiota found in the newborn infant. Studies suggest that mother-infant transmission of microbiota occurs during vaginal delivery (Cabrera-Rubio et al., 2012). The microbiota composition in infants delivered by cesarean delivery are different from vaginally born infants. Studies suggest that physiologic (for example hormonal) changes produced during labor influence the composition of microbiota.

The health of a mother during pregnancy affects the infant both during the antenatal and postnatal stages. Other factors including a history of multiple pregnancies, smoking, use of alcohol and drugs of abuse increase the risk of having a preterm infant and are considered to be obstetric complications (Varga et al., 2017). Maternal smoking is associated with intrauterine growth restriction (IUGR), a condition when an infant is not growing at a normal rate inside the womb (Varga et al., 2017). Alcohol and drug use during pregnancy can lead to low birth weight infants, low Apgar scores, breathing problems and long term developmental disabilities. Still, many times premature births occur from unknown reasons.

Breast Milk

Infants in the NICU are typically fed either MBM or DBM at birth. Most NICUs treat DBM as equivalent to MBM, assuming that it provides the same nutritional support and shapes the immune system to provide protective properties to infants. Dr. L. Hanson (2004) describes in "The Immunobiology of Human Milk" that MBM has a key role in providing immunological defense for the infant when their own immune defense are immature. Breast milk contains functional and protective nutrients that support the microenvironment for gut development and maturation. MBM modifies infant gut microflora and is an important postpartum element to modulating metabolic and immunologic development for an infant's health (Aaltonen et al., 2011; Cabrera-Rubio et al., 2012). It is also a rich supply of microbes, growth factors, and components that regulate host-microbe interactions when breast milk is the sole nutrition for the infant (Cabrera-Rubio et al., 2012). Regulatory cytokines, lactoferrin, and HMO assist in preventing infections and supporting growth of beneficial bacteria (Bode, 2012, Cabrera-Rubio et al., 2012, Neu, 2015; Neu & Bernstein, 2002). HMOs, found in high concentration in the colostrum, are very diverse, complex, and unique to the individual. They function as a prebiotic for the infant (Cabrera-Rubio et al., 2012; Jantscher-Krenn & Bode, 2012; Neu, 2015; Neu & Bernstein, 2002; Sherman et al., 2009) and protect the infant from inflammatory diseases such as NEC (Bode,

2012; Jantscher- Krenn & Bode, 2012; Meier et al., 2016). Lactoferrin, also found in high concentration in colostrum of mothers who deliver preterm, is a potent prebiotic (Meier et al., 2016). However, donor breast milk is not equal to mother's breast milk due to the pasteurization process (Carroll & Herrmann, 2013; Hair et al., 2014; Hanson, 2004; Meier et al., 2016; O'Connor et al., 2015), which can affect the immunological properties of breast milk (Bertino et al., 2012; Hanson et al., 2016). For example, pasteurization reduces lactoferrin by as much as 88% (Meier et al., 2016), and DBM does not include the nutrient-rich colostrum that MBM can provide.

MBM and DBM may also differ in their caloric, protein, and fat content (Table 2.1). Fat content of DBM ranged from 0.9 to 3.2 g/dl, fat ranged from 1.8 to 5.5 g/dl, and calories ranged from 48 to 85 kcal/dl (de Halleux, Pieltain, Senterre, & Rigo, 2017). A study examining banked donor milk's macronutrient content showed that donor milk differed from a newly lactating mother's breast milk (Wojcik et al., 2009). Protein content decreases in late stages of lactation. Many NICUs assume that human milk has approximately 0.67 kcal/ml of protein however protein is likely much lower in DBM (Underwood, 2013). The NICU assumes that MBM and DBM has 20 calories per ounce, studies indicate that DBM has fewer calories per ounce than MBM (Hanson et al., 2016; Underwood, 2013; Wojcik et al., 2009). Generally, DBM is obtained from women delivering term infants and mothers late in their lactation phase (Heiman & Schanler, 2006; Meier et al., 2016), which can affect protein, fat, and nutrient content. Milk produced in the late phase of lactation has a lower nutrient composition since an infant's diet is supplemented by other forms of food as it develops and the nutritional content of the mother's milk reflects this (Heiman & Schanler, 2006; Meier et al., 2016; Wojcik et al., 2009). Many micronutrients vary in human breast milk depending on maternal diet and body stores making a detailed review of micronutrient composition of human milk challenging (Ballard & Morrow, 2013). These micronutrients include vitamins A, B1, B2, B12, D, and iodine. Another study found that DBM had only 18%-53% of the nutritional antioxidants such as α-carotene, β-carotene, β-cryptoxanthin, lycopene,

lutein + zeaxanthin, retinol, and α-tocopherol, compared to MBM (Hanson et al., 2016). Mothers delivering prematurely produce milk that contains more protein and fat than the breast milk of mothers delivering at term (Heiman & Schanler, 2006; Underwood, 2013). Due to these differences between MBM and DBM, studies caution against the exclusive use of DBM without additional nutrient supplementation for all infants, regardless of whether they were born preterm (Bertino et al., 2012; Bertino et al., 2009; Hanson et al., 2016; O'Connor et al., 2015). These additional nutrients may include adding human milk fortifiers, liquid protein, and medium-chain triglyceride, even if the infant is born at term. However, all premature infants at birth, even when provided MBM, will be given nutrient supplements because of the need for additional calories and protein to promote adequate growth and healing.

MBM stimulates functional changes in the gastrointestinal tract, resulting in healthy gut microflora development (Jantscher-Krenn & Bode, 2012). This development of healthy microflora is important, because approximately 70% of an individual's immune system is in the gut (Sherman et al., 2009). Studies indicate that breastfed infants experience less NEC, gastroenteritis, infections, and other inflammatory bowel diseases compared to formula-fed infants (Eidelman, 2012; Meier et al., 2016; Sherman et al., 2009). However, there are no current studies that evaluate DBM's effect on preterm infants' gut microflora development.

Stages of Lactation

The first stage of lactation occurs after delivery. Colostrum is produced in low quantities in the first few of postpartum, rich in immunologic components. Immunologic components include secretory IgA, lactoferrin, leukocytes and epidermal growth factor. Small concentrations of lactose, sodium, chloride, magnesium, potassium, and calcium are found in colostrum (Ballard & Morrow, 2013). The next stage is the production of transitional milk. Transitional milk shares some of the same characteristics of colostrum and typical occurs from day 5 to two weeks postpartum (Ballard & Morrow,

2013). Human milk is considered fully mature by four to six weeks postpartum. During the course of lactation, the composition of the breast milk will have subtle changes (Ballard & Morrow, 2013).

Pasteurization

To prevent the potential transmission of viral infections to infants, pasteurization of donor breast milk is necessary. The Human Milk Banking Association of North America recommends using the Holder Pasteurization process (O'Connor et al., 2015). The Holder pasteurization process requires the breast milk to be heated to 62.5 degrees Celsius for 30 minutes (Hanson et al., 2016; O'Connor et al., 2015), thereby altering the chemical and nutritional content of the milk. Another pasteurization under investigation is to use high temperature for a short time (72 – 75 degrees Celsius for 15 – 16 seconds) eliminates bacteria with less protein loss, but greater loss of antimicrobial activity (Underwood, 2013). Further research is needed to determine the optimal pasteurization method that will minimize transmission of bacterial and virus while maximizing bioactivity. Pasteurization significant decreases in lactoferrin, lysozyme, insulin-like growth factors, hepatocyte growth factor, water-soluble vitamins, bile salt-stimulated lipase, lipoprotein lipase, and anti-oxidant activity (Underwood, 2013). Any processing of breast milk—including container changes, additional freeze-thaw cycles, and especially pasteurization—will reduce some of the nutrients (Meier et al., 2016; O'Connor et al., 2015) as many of the immune cells are inactivated, and proteins, minerals, and antibacterial microbes are reduced (Bertino et al., 2012; Chan et al., 2007; O'Connor et al., 2015; Schanler, 2015; Wojcik et al., 2009). Microbiomes are necessary to provide barriers against pathogen colonization, promote antibody development, and lower intraluminal pH (Sherman et al., 2009).

Human Milk Fortifiers

There are many benefits of an exclusive human milk diet for premature infants; however, it is also known that breast milk alone is not sufficient to meet the higher nutritional needs of extremely preterm and VLBW infants (Bertino et al., 2012; Wojcik et al., 2009). Feeding breast milk alone to

VLBW infants is associated with slower growth rates in the early postnatal period (Carroll & Herrmann, 2013; Chan et al., 2007; Heiman & Schanler, 2006; O'Connor et al., 2015). This slower growth rate is due to premature infants' need for a higher protein content and increased caloric intake while also controlling fluid intake. To boost the nutritional content of breast milk fed to premature infants, human milk fortifiers (HMF) are used (Bertino et al., 2012; Chan et al., 2007; Heiman & Schanler, 2006). Bovine-based HMF key nutrients are protein, calcium, phosphorous, and vitamin D (Table 2.2). It is common to fortify breast milk so that it reaches 24 calories per ounce. However, if weight gain is slow, fortification can be increased to as much as 30 calories per ounce. All preterm infants in this study had bovine-based fortified breast milk; however, the need for calories varied depending on the growth rate of the infant. There are different two forms of bovine-based human milk fortifiers, liquid and powered.

Formula

Infant formula has changed over time with the goal of nourishing formula-fed infants such that their growth will be similar to that of breastfed infants (Lonnerdal, 2014), see Table 2.2 for nutrient composition. However, formula-fed infants typically gain more body fat and weight over a shorter period of time than breastfed infants. There is also a significant difference in the type and amount of gut microflora (Bode, 2012; Lonnerdal, 2014; Sherman et al., 2009). The beneficial bacteria found in the gut microflora of breastfed infants is likely due to complex individualized HMOs present in breast milk (Jantscher-Krenn & Bode, 2012; Sherman et al., 2009). Formula-fed infants, on the other hand, have gut microflora that more closely resemble adult gut microflora (Sherman et al., 2009). HMOs, which are typically plant-based, are added to infant formula; however, they differ structurally, are less complex, and are not individualized; therefore, HMOs found in formula are not functionally equivalent to the HMOs in breast milk (Jantscher-Krenn & Bode, 2012). Formulas have had limited success in providing health outcomes similar to those of breastfed infants, such as the reduction of BPD, NEC, and late-onset sepsis (Lonnerdal, 2014).

Breast Milk in the NICU

Breast milk is the gold standard for premature infants (Ikonen, Paavilainen, & Kaunonen, 2015). To encourage breast feeding, hospitals that have a Baby-Friendly designation support the culture of providing breast milk. Lactation support is available and nursing staff are educated to improve consistency of breastfeeding advice and counseling to mothers. Nevertheless, there are many challenges and concerns to expressing (breast pumping to provide MBM to infant) and breastfeeding in the NICU. When a VLBW infant is born they typically are too ill or born at a gestational age that is not appropriate for breastfeeding. However, mothers are encouraged to express their breast milk for their infant. Expressing breast milk can be difficult for some mothers when their infant is in the NICU because the separation of mother and infant, maternal stress, anxiety, and the uncertainty of the infant's' prognosis are all barriers affecting the ability to express breast milk (Ikonen et al., 2015). Additionally, some mothers are ill themselves and may have extended hospitalization after birth, which may impede the expression of their breast milk.

Even with good support and regular pumping, some mothers have low breast milk supply. For example, medications such as pseudoephedrine, diuretics, and blood pressure medication may inhibit a mother's ability to produce an adequate breast milk supply. Other medication is contraindicated for nursing. Medication such as ergot alkaloids, radiopharmaceutical, cyclosporine, methrotrexate, some anticonvulsants, and drugs of abuse (for example, cocaine and heroin) can be harmful to the infant and should be avoided if nursing (Ressel, 2002). Moderate to heavy amounts of alcohol can also decrease milk production. Previous breast surgery can affect milk production, as well as maternal obesity, pregnancy induced high blood pressure, and poorly controlled insulin-dependent diabetes. There are also social factors affecting mothers' ability and desire to nurse, including previous breastfeeding experience, family support, and cultural beliefs (Ikonen et al., 2015).

How Breast Mild Influences Health Outcomes for VLBW Infants

BPD is associated with immature lungs, infection, and hyperoxia, ventilated-induced lung injury (Spiegler et al., 2016). Hyperoxia occurs when infants are exposed to an excessive supply of oxygen or high partial pressure of oxygen when mechanically ventilated. Infants developing BPD typically have longer hospital stays, poor weight gain and increased total ventilated days. Human breast milk contains antioxidant properties protecting infants from oxidative stress typically associated with extended ventilated days. Antioxidants such as, α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein + zeaxanthin, retinol, and α-tocopherol can protect and delay cell damage (Hanson et al., 2016). Therefore, it is possible that breast milk can prevent morbidity like BPD associated with immature lungs.

Medical NEC and late-onset sepsis usually are associated with one another. Medical NEC is a function of an immature gastrointestinal tract with abnormal gut microflora. The walls of the intestine are invaded with bacteria leading to a local infection and inflammation typically occurring a few weeks after birth. Late-onset sepsis is any infection at least 7 days after birth. Human milk prebiotics, probiotics, and HMO protects infants from inflammation associated with NEC and late-onset sepsis (Hanson, 2004). These components along with regulatory cytokines and lactoferrin support the microenvironment for gut development by preventing infections, growth of beneficial bacteria, and maturation (Bode, 2012, Cabrera-Rubio et al., 2012, Neu, 2015; Neu & Bernstein, 2002). In contrast, iron containing formula is associated with an increase of organisms E.coli, Enterobacter sakazakii (ES), Staphylococcus, and Group B Streptococcus, all linked with NEC and sepsis in neonates (Chan et al., 2007). Therefore, it is possible that breast milk can prevent infections associated with immature gut such as NEC.

Breast milk actively protects VLBW infants from a variety of diseases and infections because of its abundant immune cells and close association with illness progression. Infections can increase length of hospital stay, lead to poor weight gain in infants, and increase total ventilated days. BPD, medical NEC, and late-onset sepsis are all associated with increase mortality.

Current Clinical Practice

Currently, NICUs treat DBM as equivalent to MBM, on the assumption that they both provide the same protective properties and calories to infants. Studies have shown that they are not equal, though, and that there are sizeable differences in the relative compositions of MBM and DBM (Meier et al., 2016). This study provides knowledge on the dose and threshold effects of milk type on VLBW infant health outcomes. This study will inform clinicians if there is an optimum percentage of MBM in an infant's diet that is necessary to prevent BPD, NEC, late-onset sepsis, and mortality. Currently, it is unknown whether even a small exposure to MBM is enough to reduce the occurrence of certain health outcomes or if a certain level of exposure over time is necessary.

CHAPTER 3
Methods

This is a retrospective study of VLBW infants born at the University of Washington Medical Center (UWMC) Seattle, Washington. The UWMC is a large regional teaching hospital serving the states of Washington, Alaska, Montana, Idaho, and Wyoming, as well as a diverse population of ethnicities including Black, White, Native American, Alaska Native, Asian, Hispanic, and Native Hawaiian (see Table 4.1 for complete details). UWMC has the region's first level IIIB neonatal intensive care unit. Level IIIBs are designated to care for infants of extreme prematurity (28 weeks' gestation or less) or extremely low birth weight (1,000 grams or less). These hospitals can also care for severe and complex illness needing very specialized care.

Inclusion Criteria

Infants were included in this study if they were born at UWMC between October 2012 and January 2017. October 2012 was selected as the start date because this is when the UWMC initiated their donor milk program for preterm infants. Only VLBW infants weighing 1,500 grams or less at birth were eligible to participant in this study. Only VLBW infants who received MBM and/or DBM at birth and infants who received PTF only if MBM was not available once infants weighed above 1,800 grams and 33 weeks corrected gestational age were included in this study.

Exclusion Criteria

Infants were excluded from the study if any major congenital anomalies existed. Originally, infants that experienced any NEC or gastrointestinal perforation prior to day 9 of life were also excluded. Typically, infants will experience signs and symptoms of NEC at full feeds. Based on the UWMC feeding advancement protocol, infants are at full feeds around day 7-8 of life. However, this exclusion criterion was modified because it eliminated all but one infant experiencing NEC in this

population. (The VLBW infants in this sample displayed signs and symptoms of NEC around day 6-8 of life, right at full feeds.) Infants were also excluded if parents declined to participate in the DBM program, if the infant received any formula prior to weighing 1,800 grams and 33 weeks corrected gestational age, or if the infant received any human milk fortifier other than Enfamil Human Milk Fortifier powder or Similac Human Milk Fortifier concentrated liquid. UWMC has a protocol that once an infant reaches 1,800 grams and 33 weeks corrected gestational age, the infant is no longer eligible for DBM. At this point, the infant would receive preterm formula if there is not enough MBM for the feedings.

Participant Characteristics

There were 358 infants in the dataset extracted from UWMC but only 302 VLBW infants met the criteria for this study (Figure 3.1). Fifty-four infants were removed for not meeting the inclusion criteria. One infant was removed due to discharge weight being missing from the record. Another infant was removed for conflicting information. This infant's weight was listed as 0.500 kg, the discharge weight was listed as 0.500 kg, and the infant's length of hospital stay was 5 days. The dataset states that the infant survived and was discharged home, not transferred to another facility. This is not a reasonable outcome for an infant weighting 0.500kg; this infant mostly likely did not survive. Another infant's weight was listed as 0.1420 kg, which is not viable; however, it was determined that, since the infant survived and was discharged after 62 days of hospitalization, the weight was misreported and was corrected to 1.420 kg. Finally, another infant had a discharge weight of 33,100 kg, which is not a reasonable discharge weight for an infant. It was determined that the weight was misreported, and it was corrected to 3.310 kg. A total of 302 VLBW infants were included in this study. Table 4.1 shows the descriptive statistics for VLBW infants in the study. The sample was 53% males and 47% females. A little over half of the sample was White (57%), and the rest of the sample included a mix of Black, Native American or Alaska Native, Asian, Hispanic, and Native Hawaiian, or Pacific Islander.

Institutional Review Board

This study met the institutional review board (IRB) human subject division exempt review policy because the study is a chart review involving data already collected for non-research purposes. All requested data were de-identified and extracted by the Institute of Translational Health Sciences (ITHS) in Seattle, WA. The author worked closely with ITHS to define the exclusion and inclusion criteria.

Variables

This study includes three independent variables: the percent of total feedings represented by each of MBM, DBM, and PTF, from birth to discharge. Many of the infants received a combination of MBM, DBM and PTF; therefore, the dosage, measured by milliliters (ml), of MBM, DBM, and PTF was calculated. Some infants received formula when the infant became ineligible for DBM and there was not enough MBM available. The total percentages of each milk type were calculated using the following formulas:

$$\% \text{ MBM} = 100 * \text{MBM} / (\text{MBM} + \text{DBM} + \text{PTF})$$

$$\% \text{ DBM} = 100 * \text{DBM} / (\text{MBM} + \text{DBM} + \text{PTF})$$

$$\% \text{ PTF} = 100 * \text{PTF} / (\text{MBM} + \text{DBM} + \text{PTF})$$

The dependent variables for this study were the incidence of BPD, medical NEC, late-onset sepsis, and mortality. A medical NEC diagnosis is defined as the presence of pneumatosis intestinalis as determined by a pediatric radiologist reading abdominal exams. Late-onset sepsis is defined as at least one positive microbial infection from a blood culture and/or cerebrospinal fluid after 7 days of life, along with antibiotic therapy for treatment. The study also includes the continuous categorical variables of length of hospital stay, weight gain, and total days on ventilator.

Covariates

Although many variables may impact VLBW infants' health outcomes, the current study focuses on the following covariates: birthweight, gestational age, gender, Apgar scores, and the use of antenatal or postnatal steroids. Typically, infants born with higher Apgar scores at 1 minute and 5 minutes have a higher survival rate (Varga et al., 2017), but birthweight and gestational age are considered the strongest predictors of morbidity and mortality. They are therefore important variables to consider (Afjeh, Sabzehei, Khoshnood Shariati, Shamshiri, & Esmaili, 2017; Carlo et al., 2011; Varga et al., 2017). There is a direct correlation between birthweight and gestational age: the earlier the gestational age, the lower the birthweight. Infants at the earliest gestational age are at the greatest risk for morbidities such as respiratory distress and the need for mechanical ventilation (Afjeh et al., 2017; Stoll et al., 2010; Varga et al., 2017). Late-onset sepsis and NEC are also more frequent at lower gestational age, and there is a greater risk of BPD at younger gestational ages (Stoll et al., 2010).

Gender is an important variable because males face up to double the risk of developing severe BPD compared to females (Collaco, Aherrera, & McGrath-Morrow, 2017; Stoll et al., 2010). Studies indicate that the differences in hormones and surfactant may be the underlying gender difference for the increase of chronic lung disease, despite males typically weighing more at birth than females (Collaco et al., 2017; Stoll et al., 2010).

Antenatal corticosteroids are given to women at risk of having a preterm infant to assist the infants' lungs maturation and reduce respiratory distress at birth (August & Kandasamy, 2017; Roberts, Brown, Medley, & Dalziel, 2017; Varga et al., 2017). Postnatal treatment of surfactant has been the standard of care since 1990 to assist with reducing respiratory distress and BPD in premature infants (Afjeh et al., 2017). Extremely low birth weight infants benefit from the appropriate dose of surfactant to help prevent chronic respiratory complications and to improve survival (Varga et al., 2017). These

covariates all play an important role in premature infants' survival rate and both short-term and long-term morbidities.

Logistic Regression Analysis

The binary dependent variables BPD, medical NEC, late-onset sepsis, and mortality were analyzed using logistic regression models (Lomax & Vaughn, 2012; Tabachnick & Fidell, 2013). Logistic regression was used to understand the association of each milk type with the four primary outcomes. The main purpose of the analysis was to determine whether the model significantly improved after adding the set of milk types. This study does not examine the total model fit; therefore, only the odds ratio, confidence intervals, and *p*-values for the milk types were included in the results. The analyses were conducted twice: unadjusted and adjusted for covariates. The covariates included birthweight, gestational age, gender, Apgar scores, and the use of antenatal or postnatal steroids, and all were standardized.

Linear Regression Analysis

The continuous dependent variables included length of hospital stay, weight gain, and total days on ventilator, and these variables were analyzed using linear regression (Lomax & Vaughn, 2012; Tabachnick & Fidell, 2013). Linear regression models were conducted both with and without adjusting for covariates; univariate linear regressions were used to understand the relationship of the percentage of each milk type to each outcome. Covariates were standardized then added to model for ease of interpretation.

Receiver Operating Characteristic Curve Analysis

Receiver operating characteristic curve (ROC) analysis is a descriptive tool often used when medical test results are not simply positive or negative, but are measured on continuous or ordinal scales (Pepe, 2003). The notion Y is used to define a dichotomous decision rule. The decision rule is based on whether some transformation of the results exceeds a threshold value (Pepe, 2003). The ROC curve can

describe the range of trade-offs that can be achieved. All possible operating characteristics are displayed with the ROC curve analysis. Selecting only a single threshold can be limiting but the ROC curve can also be used to describe a separation between two distributions.

In this study the threshold or the minimum or maximum percentage of MBM, DBM, and PTF necessary to affect adverse outcomes was explored graphically. Dichotomous outcomes were analyzed using receiver operating characteristic curve (ROC) analysis (Pepe, 2003, Tabachnick & Fidell, 2013). The ROC is used to test the performance of binary variables, as its discrimination threshold—the point where there is noted distinction between the sensitivity and specificity is varied (Pepe, 2003, Tabachnick & Fidell, 2013). The plot shows sensitivity versus (1-specificity). The binary variables were plotted ensuring that the effect (Y) was in the positive direction. The greater the area under the curve, the more predicative the variable at that point (Pepe, 2003, Tabachnick & Fidell, 2013). The maximum point of difference, at the greatest distance of the curve, was selected as the cut point. Once the cut point was selected, the corresponding sensitivity and specificity were identified, indicating the percentage of threshold for each binary variable. All analyses were completed using SPSS Software Version 24.

Study Power Disclosure Plan

This was retrospective study with a fixed number of participants. Power was examined using Power 2 software assuming one independent variable and alpha equal to 0.05, for a power of 80%. The 302 participants in this study should be enough to detect a minimal correlation of $r = 0.16$, a small to medium effect size (Cohen, 1977). The sample size is sufficient to interpret both significant and non-significant findings.

Dataset

Once data extraction was completed, the dataset was explored and cleaned, checking for outliers, normality, independence, homogeneity of covariance matrices, and linearity using boxplots, histograms and descriptive statistics.

The dataset was expected to have only one milk type per line entry, with a total volume per child, but many times there were two milk types per line entry with just one volume total. There were never three milk types on a line entry, since no infant would have received MBM, DBM, and PFT at the same time. For the analysis, it was necessary to separate the milk types into separate entries. In some cases, the nurse included a comment about how much of each milk type the infant received for the feeding, whereas at other times there would be only one total for both milk types. When this occurred, the author divided the total amount into equal parts for consistency across the dataset.

Assumptions of Linearity

The scatterplots of the independent and dependent variables indicate that the assumption of linearity was reasonable with no serious violations observed. The boxplots and histograms suggest a relatively normal distribution, and therefore normality may be assumed. A relatively random display of points in the scatterplot of residuals against values of independent variables suggests that the assumption of independent errors has been met. A relatively random display of points, where the spread of residuals appears constant over the range of values of the independent variable, suggest homogeneity of variance.

CHAPTER 4
Results

Descriptive Statistics

The analyses focus on the various percentages of MBM, DBM, and PFT in VLBW infant diets and their relationship to adverse outcomes. Table 4.3 is the description of the total percentage of MBM, DBM, and PTF given to the infants in this study. The infants in this study received an average of 64 % MBM (± 37% *SD*) ranging from 0% to 100% and an average of 9% DBM (± 17% *SD*), ranging from 0 % to 91 %. The percentage of PTF in the infants' diets averaged 24 % (± 17% *SD*), ranging from 0% to 88 %.

The average birthweight was 1.02 kg (± 0.30 kg *SD*), ranging from 0.44 kg to 1.49 kg (Table 4.1). The infant born at 0.44 kg did not survive. The average gestational age at birth was 28.65 weeks (± 3.08 weeks *SD*), ranging from 21.71 weeks to 40.00 weeks. The infant born at 40 weeks was small for gestational age weighing only 1.49 kg. Table 4.2 shows the percentage of participants experiencing BPD (56%), medical NEC (27%), late-onset sepsis (27%), and mortality (18.5%), as well as the average length of hospital stay, discharge weight, and total ventilated days. The average length of hospital stay for the VLBW infants in this study was 67 days, with an average discharge weight of 2.92 kg (Table 4.2). The standardized odds ratios for covariates for the primary outcomes are displayed in Table 4.4. Birthweight, gestational age, and Apgar scores are significantly associated with BPD, medical NEC, late-onset sepsis, and mortality. As birthweight, gestational age, and Apgar scores increased, the occurrence of BPD, NEC, late-onset sepsis, and mortality decreased.

Bronchopulmonary Dysplasia: Standardized Percentages

Table 4.4 and Figures 4.1 through 4.4 show the results of univariate standardized logistic regression for BPD, controlling for birthweight, gestational age, gender, Apgar scores at both 1 and 5 minutes, prenatal and postnatal steroids. When the covariates are included in the model, they play a

major role in the incidence of BPD. Birthweight and gestational age are known strong predictors for BPD (August & Kandasamy, 2017; Collaco et al., 2017; Stoll et al., 2010; Varga et al., 2017), and the milk type becomes less significant when birthweight and gestational age are added. However, the percentage of DBM is significantly associated with a decrease in the odds of experiencing BPD, unadjusted (odds ratio = 0.78, p = .037), and this relationship was slightly attenuated with the addition of control variables in the adjusted models (odds ratio = 0.71, p = .028) (Table 4.5). The standardized percentage of PTF was significantly associated with experiencing BPD in the unadjusted (odds ratio =1.65, p <.001) and adjusted (odds ratio = 1.50, p <.001) models, respectively.

NEC, Late-onset Sepsis, and Mortality: Standardized Percentages

This study found that the percentage of milk type was not associated with for NEC, late-onset sepsis, and mortality (Table 4.5).

Length of Hospital Stay, Weight Gain, and Ventilation

As the percentage of MBM (β = -*.096; p* = .071) and DBM (β = -*.188; p* < .001) increased, hospital stay decreased by .10 and .19 days, respectively (Table 4.6). On the other hand, PTF was significantly associated with increased hospitalization. The percentage increase of MBM (β = -*.095; p* = .089) and DBM (β = -*.223; p* <.001) were associated with a decrease in weight gain of .10 kg and .22 kg, respectively. An increase in the percentage of PTF was associated with an increase in weight gain (β= *.342; p* <.001). PTF (β = *.5.8; p* <.001) was significantly associated with increased total ventilated days of .34 days and 5.8 days, respectively.

Receiver Operating Characteristic Curve

Table 4.7 shows the results for the ROC analysis. An infant diet high in MBM was associated with a decrease in BDP, medical NEC, and late-onset sepsis. The ROC analysis revealed that only a very small amount of DBM is needed to be statistically significant for decreasing BPD, medical NEC, and late-onset sepsis. Only 5.5% of PTF in an infant's diet was associated with an increase in BPD and 7.3%

of PTF was associated with an increase in medical NEC and late-onset sepsis. However, since none of the variables analyzed had an area under the curve greater than .8 for any milk type, these results, while some are statistically significant, do not indicate a strong association, and further investigation is necessary.

CHAPTER 5

Post Hoc Analysis

Introduction

It is clinical practice to feed infants their mother's milk before DBM or PTF, the rationale being that infants should receive as much of their own mother's milk as possible. However, this practice sometimes leaves infants with several feedings in a row of DBM or PTF if there is a low supply of MBM. Some mothers are unable to visit daily and will not have enough of their own milk to supply all feedings between visits; therefore, an infant may receive MBM one day but on subsequent days receive only DBM or PFT. In practice, some infants experience an increase in feeding intolerance when there is no MBM. When this feeding intolerance is noted, clinicians may recommend spreading out the available MBM by either mixing MBM with DBM or PTF, or feeding the infant MBM every other feeding. The author has observed that this practice increases feeding tolerance.

The post hoc analysis of the dataset will examine infants' mean volume per day of each milk type. It may be that daily exposure to MBM over time is more important than the total percentage of MBM an infant receives. MBM has many protective benefits, and it may be that infants need to have daily exposure to prevent adverse outcomes.

There are recent studies examining exclusively human milk diets, but they do not evaluate the daily dosage and threshold of MBM versus DBM. More research is needed to examine and understand whether and how a daily exposure to MBM affects the outcomes of BPD, NEC, late-onset sepsis and mortality, regardless of whether the human milk is mixed with a bovine-based human milk fortifier. This analysis begins to address the issue of daily exposure to MBM rather than only considering total volume.

Variables

This post hoc analysis includes three independent variables: the mean total volume of MBM, DBM, and PTF per day in the infants' diet from birth to discharge. The mean total volume of each milk type, measured in milliliters, was calculated using the following: First, the sums of each milk type were calculated for each infant during their hospital stay. Next, the sum of each milk type was divided by the length of hospital stay to yield the mean per day measured in milliliters.

Analysis

The main purpose of this post hoc analysis is to examine the relationship between the total volumes of each milk type per day fed to VLBW infants during their NICU stay with four main outcomes. The binary dependent variables BPD, medical NEC, late-onset sepsis, and mortality were analyzed using logistic regression models (Lomax & Vaughn, 2012; Tabachnick & Fidell, 2013). The continuous dependent variables length of hospital stay, weight gain, and total days on ventilator were analyzed using linear regression (Lomax & Vaughn, 2012; Tabachnick & Fidell, 2013). Separate models were estimated with and without covariates. Univariate linear and logistic regressions were used to understand the relationship of the total volume of each milk type per day with each outcome. Covariates were standardized for ease of interpretation. In the final analysis, dichotomous outcomes were evaluated using receiver operating characteristic curve (ROC) analysis to evaluate the maximum amount of milliliters needed to elicit a change in outcome (Tabachnick & Fidell, 2013). All analyses were completed using SPSS Software Version 24.

Results

Table 5.1 is the description of the total volume per day of MBM, DBM, and PTF given to the infants in this study. The infants in this study received a mean of 140 ml MBM (± 116 ml *SD*) ranging from 0 ml to 1013 ml and a mean of 17 ml DBM (± 30 ml *SD*), ranging from 0 ml to 179 ml. The mean volume of PTF in the infants' diets averaged 55 ml (± 72 ml *SD*), ranging from 0 ml to 350 ml.

Bronchopulmonary Dysplasia: Volume of Milk per Day

Table 5.2 show the results of logistic regressions with standardized odds ratios for volume of milk per day, controlling for birthweight, gestational age, gender, Apgar scores at both 1 and 5 minutes, prenatal and postnatal steroids. DBM is significantly associated with a decrease in the odds of experiencing BPD, unadjusted (odds ratio = 0.99, $p = .006$), and in the adjusted models (odds ratio = 0.99, $p = .012$). The volume per day of PTF was significantly associated with experiencing BPD in the unadjusted (odds ratio = 1.01, $p = .002$) and adjusted (odds ratio = 1.01, $p = .022$) models respectively.

NEC: Volume of Milk per Day

Table 5.2 shows that the volume of MBM per day is significantly associated with a decrease of medical NEC in the unadjusted model (odds ratio = 0.99, $p < .001$). DBM was also associated with a decrease in medical NEC in the unadjusted models **(**odds ratio = 0.99, $p = .037$). The volume of milk per day of PTF in the adjusted models show that PTF is associated with an increase in medical NEC (odds ratio = 1.00, $p = .030$).

Late-onset Sepsis: Volume of Milk per Day

Table 5.2 shows that, in the unadjusted model, MBM was significantly associated with a decrease of late-onset sepsis (odds ratio = 0.99, $p < .001$). The volume per day of DBM was associated with a decrease in late-onset sepsis in the unadjusted model (odds ratio = 0.99, $p = .031$). This analysis found that the volume per milk per day of PTF was not associated with late-onset sepsis (Table 5.2).

Mortality: Volume of Milk per Day

The volume of MBM per day was significantly associated with a decrease in mortality in both the unadjusted (odds ratio = 0.99, $p < .001$) and adjusted models (odds ratio = 0.99, $p = .013$). DBM and PTF were not significantly associated with mortality (Table 5.2).

Length of Hospital Stay, Weight Gain, and Ventilation

As the volume of MBM per day increased by 1 milliliter (β = *.198; p* < .001), hospital stay also increased by .2 standard deviations (Table 5.3). PTF was also significantly associated with increased hospitalization by .3 days as PTF volume increases by 1 milliliter (β = *.317; p* < .001). Infants are fed daily by calculating ml per kg per day; therefore, as an infant grows, their daily volume of milk will increase. The increased percentage of MBM (β = *.269; p* < .001) and PFT (β = *.367; p* < .001) by 1 milliliter were associated with an increase in weight gain of .27 kg and .37 kg, respectively. An increase in the volume of PTF (β = *.154; p* = .001) by 1 milliliter was associated with an increased total ventilated day of .15 days

Receiver Operating Characteristic Curve

Table 5.4 shows the results for the ROC analysis. An infant diet with at least 138 ml of MBM per day was significantly associated with a decrease in BDP and mortality. A diet of at least 163 ml per day of MBM was associated with a decrease in medical NEC and late-onset sepsis. The ROC analysis revealed that only a very small amount of DBM is needed to be statistically significant for decreasing BPD, medical NEC, and late-on sepsis. On the other hand, only 1.34 ml per day of PTF in an infants' diet was associated with an increase in BPD. None of the variables analyzed had an area under the curve greater than .8 for any milk type. These results, while statistically significant in some cases, do not indicate a strong association, and further investigation is necessary.

CHAPTER 6
Discussion

Dependent Variable: BDP, Medical NEC, Late-onset Sepsis, and Mortality

The results of this study lend some support to the hypothesis that a larger percentage or volume of MBM is associated with a decrease of BPD, medical NEC, late-onset sepsis, and mortality. This study supports the assertion that PTF is associated with an increase of BPD, medical NEC, late-onset sepsis, and mortality. These results are consistent with other studies. The logistic regression results for DBM were statistically significant for decreased BPD, whereas the MBM was not significantly associated in either the adjusted or unadjusted models. In the post hoc analysis, daily exposure to MBM and DBM was associated with a decrease in medical NEC, late-onset sepsis and mortality.

The expected results were that both MBM and DBM would be significantly associated with all four adverse outcomes, with MBM having a stronger association since it is not pasteurized. However, none of the results were significant for reducing NEC or late-onset sepsis. This could be due to the fact that all the infants in this study were fed human milk that was supplemented with a bovine-based milk fortifier. If the current study were replicated with VLBW infants fed an exclusively human milk-based diet, results may show that MBM and DBM would have an association for decreasing BPD, medical NEC, late-onset sepsis, and mortality. More research is necessary to fully understand the effects of bovine-based human milk fortifiers versus human milk-based fortifiers when added to both MBM and DBM. This study does not clearly show that MBM is more beneficial than DBM, although it is clear that an infants' diet is important. Nevertheless, increased gestational age and birthweight are the main factors associated with decreasing BPD, NEC, late-onset sepsis, and mortality.

Dependent Variables: Hospital stays, ventilated days, and weight gain

Consistent with previous research (Carroll & Herrmann, 2013; Chan et al., 2007; Hanson, 2004; Heiman & Schanler, 2006; O'Connor et al., 2015), the current study finds that MBM and DBM were

associated with shorter hospital stays, shorter ventilated days, and slower weight gain. This finding reflects the fact that formula is associated with an increase in BPD, infections, and other morbidities, all leading to an increase in hospital stays and extended mechanical ventilation. Additionally, formula calories are a known constant, whereas MBM and DBM calories vary and are only estimates. Knowing the exact calorie count is important in promoting and predicting an expected growth rate for VLBW infants leading to greater weight gain by discharge.

Covariates

The covariates birthweight, gestational age, and Apgar scores were significantly associated with all primary outcomes in this study. These selected covariates play major roles in preventing adverse outcomes. The results from this study indicate that an infants' diet may only play a supporting role in preventing certain adverse outcomes. Diet is important, and reducing the amount of PTF given to VLBW infants clearly improves outcomes, along with increased gestational age and birthweight.

Gestational age is key for preventing mortality and morbidities in infants. When an infant is born at a young gestational age, they have low birthweight and poor lung development. Proper lung development is important in preventing BPD, and since the final stages of lung development occurs late in the third trimester, premature infants have the highest risk of BPD. The development of antenatal and postnatal steroids has assisted in preventing BPD in recent years, as has the practice of not automatically intubating a premature infant (Stoll et al., 2010). It was common practice to intubate all VLBW infants at birth, but that practice has changed, and now continuous positive airway pressure (CPAP) is attempted first (Stoll et al., 2010). At UWMC, the protocol is to use CPAP or high flow nasal canal before intubation. If the infant fails these, then a noninvasive ventilation (NIV) neutrally adjusted ventilator assist (NAVA) is trialed, which is a ventilation mode controlled by the patient's neural respiratory drive. If all other forms of ventilation fail, then invasive mechanical ventilation is initiated.

Invasive mechanical ventilation is a last resort because increases the potential for infection and mortality.

Gestational age also affects the prematurity of the infants' gut. NEC is a risk because it is a function of an immature gastrointestinal tract with abnormal gut microflora (Neu, 2015). Very young gestational age infants are required to process milk sometimes several months earlier than they are developmentally prepared. A premature infant is exposed not only to early enteral feedings, but also to antibiotics and steroids, all of which may negatively affect a premature gastrointestinal tract (Bode, 2012; C. Hanson et al., 2016; L. Hanson, 2004; Jantscher-Krenn & Bode, 2012; Neu, 2015).

This study's results indicate that having a daily exposure to MBM improves outcomes, including BPD and NEC. This could change clinical practice. It is a frequent practice to feed an infant MBM first, meaning that if a mother only has enough saved breast milk for two full feedings and will not be returning to the hospital for three days, the infant will receive all MBM at the first two feedings. For the remaining time, either DBM or formula will be administered. The preliminary results of this study's post hoc analysis indicate that having a daily minimum of just 138 ml of MBM can reduce the occurrence BPD and mortality; therefore, it may be better to spread the MBM over the three days in order to provide a daily exposure to mothers' milk.

Limitations

Although two of the most theoretically important covariates were included in the current study (gestational age and birthweight), several others were excluded: socio-economic and educational status of the mother, family support, cultural norms about breastfeeding, mothers' breastfeeding experience, mothers' motivation to breastfeed, and mothers' ability to produce adequate breast milk supply. These factors contribute to whether an infant will be fed mostly MBM, DBM, or PTF. Baseline maternal characteristics including maternal age, mode of delivery, multiparity, prenatal care, gestational diabetes, history of smoking, history of alcohol consumption, history of drug and any other maternal health

conditions were not included in this study because this information is located in the mothers' medical chart and not in the infants'.

The current study supports previous work that shows that feeding a premature infant a human milk diet is more beneficial than a bovine-based diet, although none of the infants in this study were fed a 100% exclusive human based milk diet. All the infants in this study were fed a bovine-based human milk fortifier to add calories and protein to both MBM and DBM.

Further research should examine VLBW infants fed an exclusive human based milk diet at multiple sites, and include more infants to adequately detect dosage and threshold effects of DBM and MBM. Another, potential study would be to examine infants fed only MBM versus infants fed only DBM until 1,800 grams. The results in this study showed that DBM was associated with a decrease in BPD and length of stay while MBM was not. These results may be due to the infants' diet of MBM rather than the DBM. On average infants in this study only received 9 % of their diet from DBM (only one infant received 91 %) and on average infants received 65 % of their diet from MBM. This is the same with the ROC results examining volume per day over the length of stay that showed only a very small amount of DBM is needed to be statistically significant for decreasing BPD, medical NEC, and late-on sepsis.

The policies and procedures of the UWMC may have affected the primary outcomes analyzed in this study. The UWMC is a recognized gold level breastfeeding friendly hospital and teaching facility, known for its innovative care and research. UWMC policies and procedures for caring for VLBW infants may differ from other NICU's, thereby affecting the outcomes. For example, at the UWMC will initiate trophic feeding as soon as MBM is available, where other facilities may not introduce any feedings for several days. Since policies may vary between NICUs, it is challenging to compare results to other facilities. For example, not every NICU is recognized as breastfeeding friendly facility, nor does

every NICU provide DBM for VLBW infants. This study at another hospital may yield very different results because it may include infants receiving less than 50% of their diet from human milk.

Conclusions

The main difference between the primary analysis and the post hoc analysis in this study is the examination between total percentage of an infants' diet versus a daily volume of an infants' diet. It appears that a daily exposure to MBM may be more important than having a diet that consist mostly of MBM. The one constant result in both analysis is that PTF is significantly associated with an increase in BPD.

Breastmilk, either MBM or DBM, provides more benefits for the VLBW infant than formula provides. Further research is needed to understand how mixing a bovine-based human milk fortifier with MBM and DBM affects the outcomes of BPD, NEC, late-onset sepsis and mortality.

This study supports the idea that NICUs should encourage mothers to express their own breast milk. Expressing one's own milk can be challenging for some mothers when they are offered the alternative of DBM, but clinicians should be clear that DBM is a volume filler and not a replacement for MBM. Not only does MBM benefit the infants, but there are benefits for the mothers, the most important of which is knowing that they are helping their VLBW infant to grow and heal. So often mothers feel helpless at their child's bedside, but expressing their own breast milk is something only they can do.

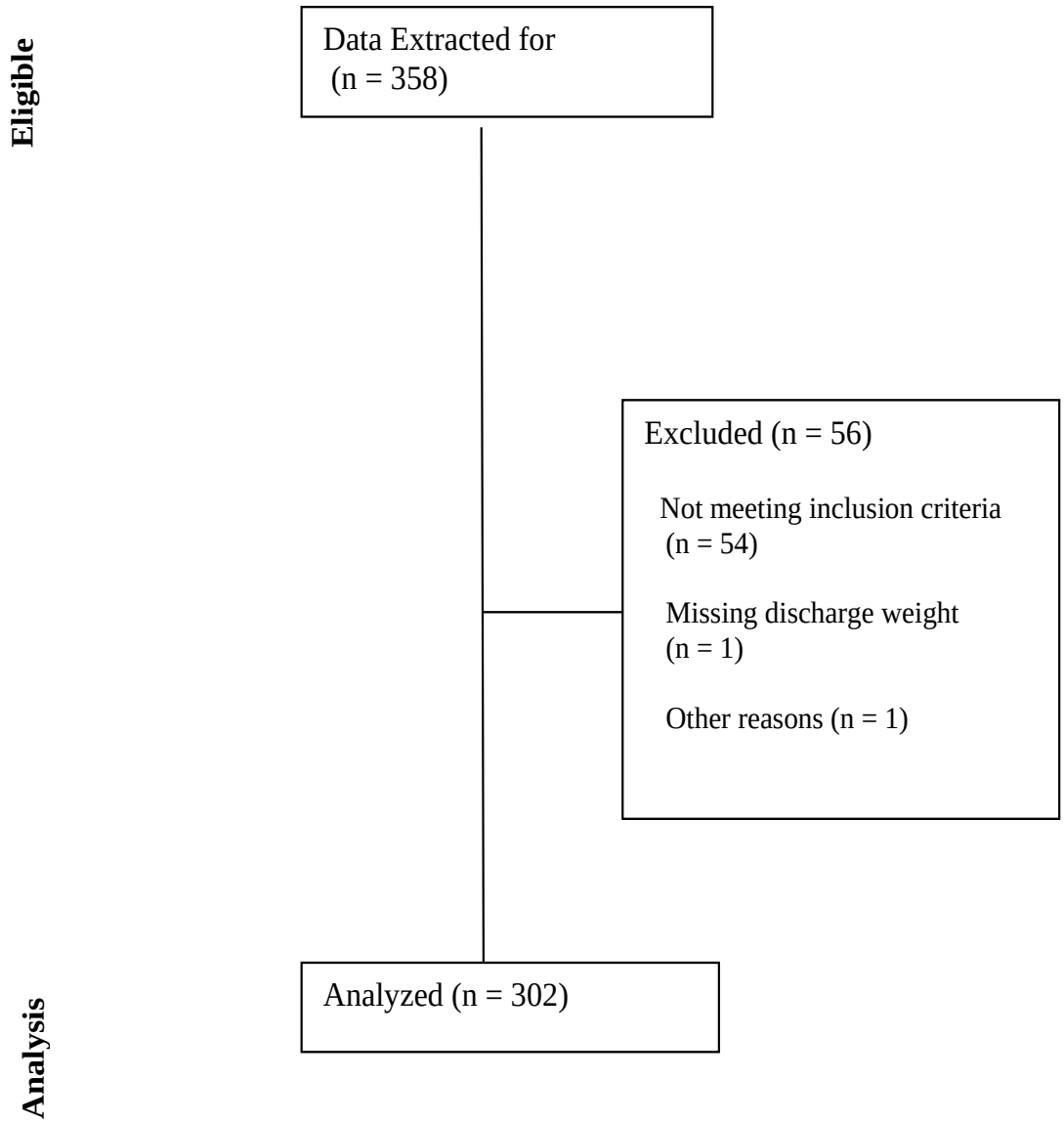

Figure 3.1. Diagram of participates of the study

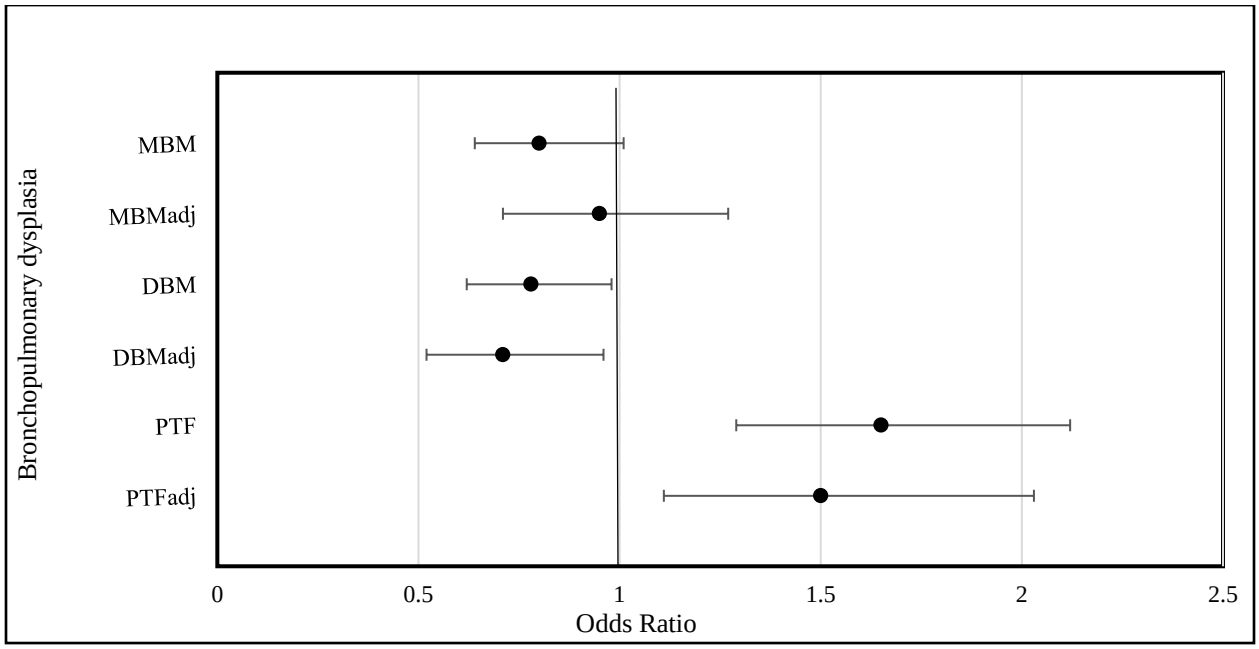

Figure 4.1. Standardized Odds Ratio for Percentage of Milk

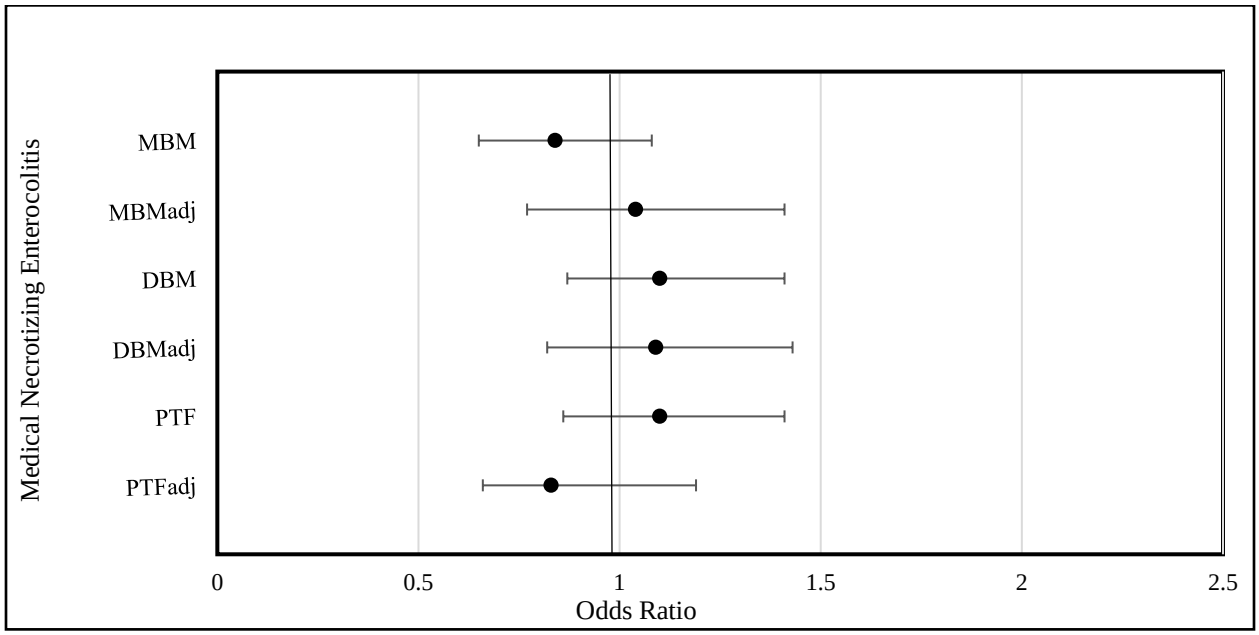

Figure 4.2. Standardized Odds Ratio for Percentage of Milk

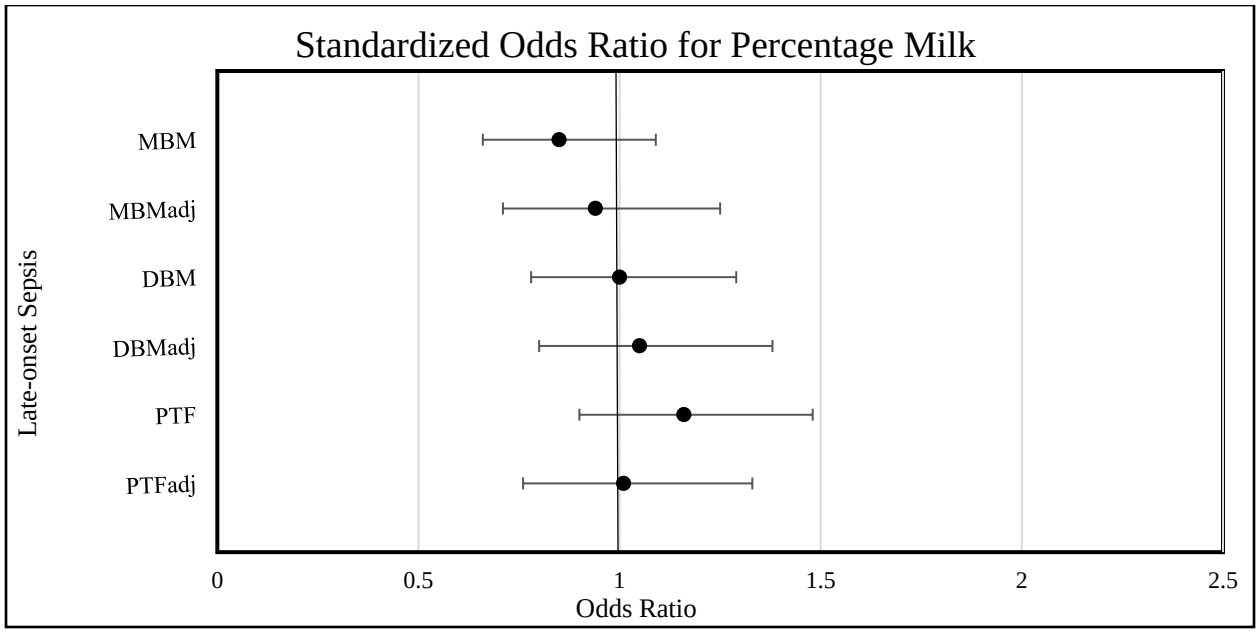

Figure 4.3. Standardized Odds Ratio for Percentage of Milk

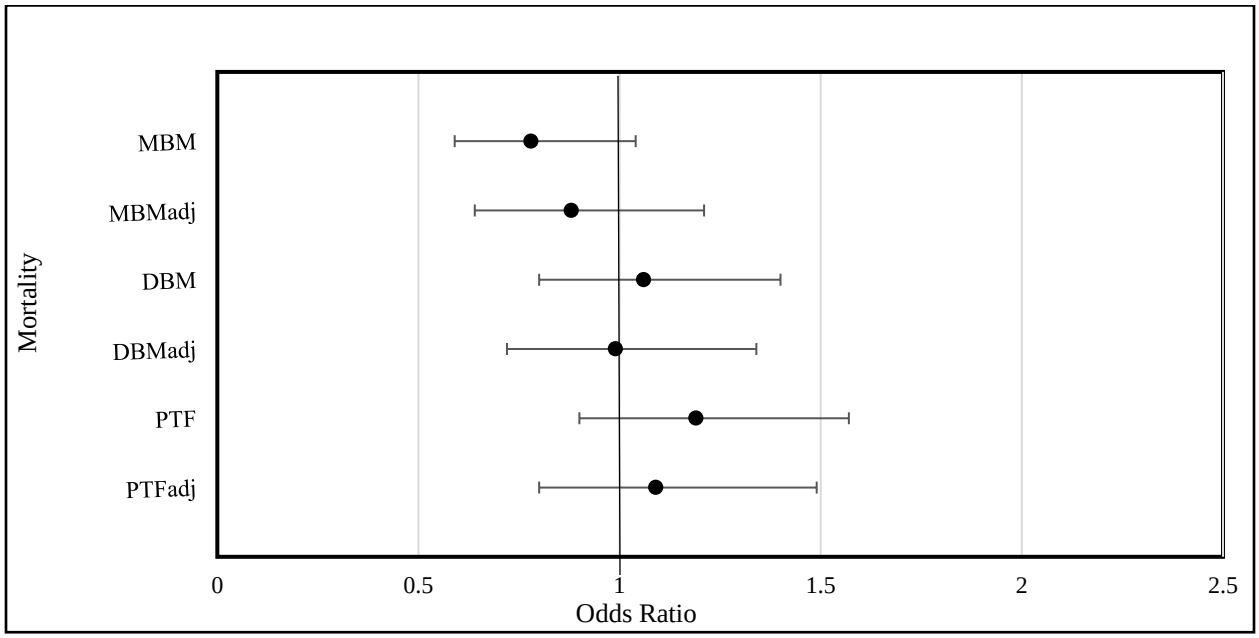

Figure 4.4. Standardized Odds Ratio for Percentage of Milk

Table 2.1.
Nutrient Content of MBM and DBM

	MBM Term Mean ± *SD*	MBM Preterm < 29 weeks Mean ± *SD*	DBM Mean ± *SD*
Protein Source	Human	Human	Human
Calories (kcal/dl)	70 ± 2	78 ± 2	65 ± 2
Protein (g)	1.2 ± 2	2.2 ± 2	1.2 ± 2
Fat (g)	3.6 ± 2	4.4 ± 2	3.2 ± 2
Lactose (g)	7.4 ± 2	7.6 ± 2	7.8 ± 2

Note. MBM = mother's breast milk, DBM = donor breast milk, g = grams, protein measured as total nitrogen, MBM 28 ± 10 days of lactation.
Ballard, O., & Morrow, A. L. (2013). Human milk composition: nutrients and bioactive factors. Pediatr Clin North Am, 60(1), 49-74. doi:10.1016/j.pcl.2012.10.002

Table 2.2.
Nutrient Content of HMF and PTF

	Similac Special care 24	Similac HMF Concentrated Liquid	Enfamil HMF Powder
Protein Source	Bovine	Bovine	Bovine
Calories (kcal/dl)	100	7	14
Protein (g)	3	0.35	1.1
Fat (g)	5.43	0.27	1
Carbohydrate (g)	10.3	0.81	<0.40
Water (g)	109	3.6	
Linoleic Acid (mg)	700	1	140
Potential Rena Solute Load (mOsm)	27.8	3.8	
Vitamin A (IU)	1250	197	950
Vitamin D (IU)	150	35	150
Vitamin E (IU)	4	1	4.6
Vitamin K (mcg)	12	2.4	4.4
Vitamin B1 (mcg)	250	48	150
Vitamin B2 (mcg)	620	123	220
Vitamin B6 (mcg)	250	49	115
Vitamin B12 (mcg)	.55	0.08	0.18
Niacin (mcg)	5000	1040	3000
Folic Acid (mcg)	37	7	25
Pantothenic Acid (mcg)	1900	309	730
Biotin (mcg)	37	7.6	2.7
Vitamin C (mg)	37	7.7	17
Choline (mg)	10	0.9	
Inositol (mg)	40	1.7	
Calcium (mg)	180	35	90
Phosphorous (mg)	100	20	50
Magnesium (mg)	12	2.2	1
Iron (mg)	1.8	0.11	1.44
Zinc (mg)	1.5	0.30	0.72
Copper (mcg)	250	15	44
Manganese (mcg)	12	2.1	10
Sodium (mg)	43	5	16
Potassium (mg)	129	21	29
Chloride (mg)	81	13	13
Iodine (mcg)	6	0.5	
Selenium (mcg)	2	0.2	

Note. Data from Similac and Enfamil website. MBM = mother's breast milk, DBM = donor breast milk, g = grams, mg = milligrams, IU = International Unit, mcg = micrograms, protein measured as total nitrogen, MBM 28 ± 10 days of lactation.

Table 4.1.

Descriptive Statistics for Very Low Birth Weight Infants

Characteristic	*N*	Mean (SD)	[Min., Max.]
Birth weight, kg	302	1.02 (0.30)	[0.44, 1.49]
Gestational age at birth, wk.	302	28.65 (3.08)	[21.71, 40.00]
Discharge weight, kg	302	2.92 (1.09)	[0.46, 6.01]
Apgar 1 minutes	292	4.51 (2.18)	[1.00, 9.00]
Apgar 5 minutes	292	6.76 (1.61)	[0.00, 9.00]
Maternal antenatal steroids, *n* (%)	302	0.57 (0.50)	[0.00, 1.00]
Postnatal steroids, *n* (%)	302	0.11 (0.32)	[0.00, 1.00]
Gender	302		
Male	161 (53.3%)		
Female	141 (46.7%)		
Race			
Black	33 (10.9%)		
Caucasian	172 (57.0%)		
Native American or Alaska Native	7 (2.3%)		
Asian	26 (8.6%)		

Mexican or Hispanic	17 (5.6%)
Native Hawaiian or Pacific Islander	5 (1.7%)
Not Reported	42 (13.9%)

Table 4.2.

Descriptive Statistics for Outcomes	Yes	No
Bronchopulmonary Dysplasia	168 (55.6%)	134 (44.4%)
Medical Necrotizing Enterocolitis	82 (27.2%)	220 (72.8%)
Late-onset Sepsis	82 (27.2%)	220 (72.8%)
Mortality	56 (18.5%)	246 (81.5%)
	Mean (SD)	[Min., Max.]
Length of hospital stay, days	67.37 (37.01)	[1.00, 175.00]
Weight gain, kg	1.90 (1.12)	[-0.04, 5.21]
Number of days on ventilator, days	20.65 (26.09)	[0.00, 128.00]

Note. $N = 302$.

Table 4.3.

Descriptive Statistics for Total Percentage of Milk Type

	N	Mean	Standard Deviation	Median	[Min., Max.]
Mother's breast milk	302	65.41	36.87	81.59	[0.0, 100]
Donor breast milk	302	9.40	17.44	.46	[0.0, 91]
Preterm formula	302	24.19	28.46	9.79	[0.0, 88]

Table 4.4.

Univariate Standardized Odds Ratios for Covariates upon Four Outcomes

	Bronchopulmonary Dysplasia OR [95% CI]; *p*	Medical Necrotizing Enterocolitis OR [95% CI]; *p*
Birth Weight	0.33 [0.25, 0.44]; ***	0.30 [0.22, 0.42]; ***
Gestational Age	0.23 [0.16, 0.34]; ***	0.40 [0.29, 0.56]; ***
Apgar 1 minute	0.57 [0.44, 0.73]; ***	0.55 [0.42, 0.73]; ***
Apgar 5 minutes	0.59 [0.46, 0.77]; ***	0.69 [0.53, 0.89]; **
Prenatal Steroids	1.14 [0.91, 1.43]; ns	0.83 [0.65, 1.07]; ns
Postnatal Steroids	1.86 [1.32, 2.61]; ***	1.04 [0.81, 1.34]; ns
Gender	1.04 [0.83, 1.31]; ns	1.06 [0.82, 1.37]; ns

	Late-onset Sepsis OR [95% CI]; *p*	Mortality OR [95% CI]; *p*
Birth Weight	0.43 [0.32, 0.57]; ***	0.48 [0.35, 0.67]; ***
Gestational Age	0.41 [0.30, 0.58]; ***	0.57 [0.40, 0.79]; ***
Apgar 1 minute	0.54 [0.40, 0.71]; ***	0.60 [0.44, 0.83]; **
Apgar 5 minutes	0.50 [0.38, 0.66]; ***	0.69 [0.52, 0.91]; **
Prenatal Steroids	0.75 [0.58, 0.97]; *	1.40 [1.03, 1.91]; *
Postnatal Steroids	0.99 [0.77, 1.28]; ns	1.60 [1.26, 2.03]; ***
Gender	1.20 [0.85, 1.41]; ns	1.09 [0.81, 1.45]; ns

Note. CI = confidence interval; OR = odds ratio.

* $p < .05$, ** $p < .01$, *** $p < .00$, ns = not significant.

Table 4.5.
Results for Logistic Regressions with Standardized Odds Ratios (OR) for Percentage of Milk

	Not adjusted for covariates		Adjusted for covariates	
	OR [95% CI]	*p* Value	OR [95% CI]	*p* Value
Bronchopulmonary Dysplasia				
MBM	0.80 [0.64, 1.01]	ns	0.95 [0.71, 1.27]	ns
DBM	0.78 [0.62, 0.98]	*	0.71 [0.52, 0.96]	*
PTF	1.65 [1.29, 2.12]	***	1.50 [1.11, 2.03]	**
Medical Necrotizing Enterocolitis				
MBM	0.84 [0.65, 1.08]	ns	1.04 [0.77, 1.41]	ns
DBM	1.10 [0.87, 1.41]	ns	1.09 [0.82, 1.43]	ns
PTF	1.10 [0.86, 1.41]	ns	0.83 [0.66, 1.19]	ns

Late-onset Sepsis				
MBM	0.85 [0.66, 1.09]	ns	0.94 [0.71, 1.25]	ns
DBM	1.00 [0.78, 1.29]	ns	1.05 [0.80, 1.38]	ns
PTF	1.16 [0.90, 1.48]	ns	1.01 [0.76, 1.33]	ns
Mortality				
MBM	0.78 [0.59, 1.04]	ns	0.88 [0.64, 1.21]	ns
DBM	1.06 [0.80, 1.40]	ns	0.99 [0.72, 1.34]	ns
PTF	1.19 [0.90, 1.57]	ns	1.09 [0.80, 1.49]	ns

Note. Covariates were birthweight, gestational age, Apgar scores at both 1 and 5 minutes, prenatal and postnatal steroids, and gender. CI = confidence interval; OR = odds ratio
* $p < .05$, ** $p < .01$, *** $p < .001$, ns = not significant.

Table 4.6.
Results for Linear Regressions with Coefficients for Percentage of Milk

	Unstandardized Coefficients		Standardized Coefficients		
Variables	*B*	*(SE)*	β	*t*	*p*
Length of hospital stay(days)					
MBM	-0.186	0.057	-0.186	3.274	**
Adjusted	-0.096	0.053	-0.096	1.812	ns
DBM	-0.389	0.120	-0.183	3.230	**
Adjusted	-0.393	0.106	-0.188	3.714	***
PTF	0.532	0.068	0.409	7.773	***
Adjusted	0.420	0.065	0.323	6.471	***
Weight gain (kg)					
MBM	-0.005	0.002	-0.163	2.869	**
Adjusted	-0.003	0.002	-0.095	1.708	ns
DBM	-0.015	0.004	-0.229	4.069	***

Adjusted	-0.014	0.003	-0.223	4.238	***
PTF	0.016	0.002	0.411	7.804	***
Adjusted	0.013	0.002	0.342	6.554	***
Total days on ventilation					
MBM	-0.166	0.040	-0.234	4.171	***
Adjusted	-0.085	0.034	-0.119	2.533	*
DBM	-0.121	0.086	-0.081	1.402	ns
Adjusted	-0.124	0.069	-0.083	1.801	ns
PTF	0.339	0.049	0.370	6.901	***
Adjusted	0.246	0.042	0.264	5.845	***

Note. *N*= 302. Covariates were birthweight, gestational age, Apgar scores at both 1 and 5 minutes, prenatal and postnatal steroids, and gender. MBM = mother's breast milk; DBM=donor breast milk; PTF = preterm formula
* $p < .05$, ** $p < .01$, *** $p < .001$, ns = not significant.

Table 4.7.
Percentage of Milk Type Thresholds Derived from Receiver Operating Characteristic Curve

	Threshold	OR [95% CI]	*P* value
Bronchopulmonary Dysplasia			
MBM	91.80 %	0.472 [0.296, 0.754]	**
DBM	.11 %	0.474 [0.294, 0.762]	**
PTF	5.48 %	2.963 [1.850, 4.747]	***
Medical Necrotizing Enterocolitis			
MBM	90.64 %	0.489 [0.285, 0.839]	**
DBM	.05 %	0.380 [0.226, 0.641]	***
PTF	7.26 %	1.591 [0.949, 2.667]	ns
Late-onset Sepsis			
MBM	88.80 %	0.558 [0.329, 0.945]	*
DBM	.07 %	0.386 [0.230, 0.650]	***
PTF	7.26 %	1.706 [1.016, 2.866]	ns
Mortality			
MBM	65.50 %	0.556 [0.310, 0.998]	*
DBM	.11 %	0.676 [0.377, 1.211]	ns
PTF	31.15 %	1.939 [1.074,3.500]	ns

Note. OR = odds ratio; CI = confidence interval; MBM = mother's breast milk; DBM = donor breast milk; PTF = preterm formula.
* $p < .05$, ** $p < .01$, *** $p < .001$, ns = not significant.

Table 5.1.
Descriptive Statistics for Volume of Milk per Day in milliliters

	N	Mean	Standard Deviation	Median	[Min., Max.]
Mother's breast milk	302	140.24	115.87	140.57	[0.0, 1013]
Donor breast milk	302	16.55	29.70	.98	[0.0, 179]
Preterm formula	302	54.61	71.77	21.31	[0.0, 350]

Table 5.2.
Results for Logistic Regressions with Standardized Odds Ratios (OR) for Volume of Milk per Day in milliliters

	Not adjusted for covariates		Adjusted for covariates	
	OR [95% CI]	*p* Value	OR [95% CI]	*p* Value
Bronchopulmonary Dysplasia				
MBM	0.99 [0.99, 1.00]	ns	1.00 [1.00, 1.01]	ns
DBM	0.99 [0.98, 0.99]	**	0.99 [0.98, 1.00]	*
PTF	1.01 [1.00, 1.01]	**	1.01 [1.00, 1.01]	*
Medical Necrotizing Enterocolitis				
MBM	0.99 [0.99, 1.00]	***	1.00 [0.99, 1.00]	ns
DBM	0.99 [0.98, 1.00]	*	0.99 [0.98, 1.00]	ns
PTF	0.99 [0.99, 1.00]	ns	1.00 [0.99, 1.00]	*

Late-onset Sepsis				
MBM	0.99 [0.99, 1.00]	***	0.99 [0.99, 1.00]	ns
DBM	0.99 [0.98, 1.00]	*	0.99 [0.98, 1.01]	ns
PTF	1.00 [0.99, 1.00]	ns	1.00 [0.99, 1.00]	ns
Mortality				
MBM	0.99 [0.99, 1.00]	***	0.99 [0.99, 1.00]	*
DBM	1.00 [0.99, 1.01]	ns	1.00 [0.99, 1.02]	ns
PTF	1.00 [0.99, 1.00]	ns	1.00 [0.99, 1.00]	ns

Note. Covariates were birthweight, gestational age, Apgar scores at both 1 and 5 minutes, prenatal and postnatal steroids, and gender. MBM = mother's breast milk; DBM = donor breast milk; PTF = preterm formula.
* $p < .05$, ** $p < .01$, *** $p < .001$.

Table 5.3.

Results for Linear Regressions with Coefficients for Volume of Milk per Day in milliliters

	Unstandardized Coefficients		Standardized Coefficients		
Variables	*B*	*(SE)*	β	*t*	*p*
Length of hospital stay(days)					
MBM	0.019	0.018	.061	1.050	ns
Adjusted	0.063	0.018	.198	3.57	***
DBM	-0.125	0.072	-0.100	-1.741	ns
Adjusted	-0.052	0.064	-0.042	-0.811	ns
PTF	0.179	0.028	0.348	6.429	***
Adjusted	0.166	0.026	0.317	6.474	***
Weight gain (kg)					
MBM	-0.002	0.001	0.167	2.935	**
Adjusted	-0.003	0.001	0.269	4.702	***
DBM	-0.004	0.002	-0.116	-2.024	*
Adjusted	-0.003	0.002	-0.069	-1.274	ns
PTF	0.006	0.001	0.409	7.769	***
Adjusted	0.006	0.001	0.367	7.296	***

Total ventilated days (days)					
MBM	-0.055	0.013	-0.245	-4.371	***
Adjusted	-0.010	0.012	-0.042	-0.833	ns
DBM	-0.123	0.050	-0.141	-2.460	*
Adjusted	-0.036	0.041	-0.041	-0.873	ns
PTF	0.072	0.021	0.197	3.484	**
Adjusted	0.058	0.017	0.154	3.366	**

Note. *N*= 302. MBM = mother's breast milk; DBM=donor breast milk; PTF = preterm formula
* $p < .05$, ** $p < .01$, *** $p < .001$.

Table 5.4.

Volume per Day of Milk Types Thresholds Derived from Receiver Operating Characteristic Curve

	Threshold	OR [95% CI]	*P* value
Bronchopulmonary Dysplasia			
MBM	138 ml	0.565 [0.357, 0.894]	*
DBM	.307 ml	0.451 [0.281, 0.727]	**
PTF	1.34 ml	2.764 [1.687, 4.529]	***
Medical Necrotizing Enterocolitis			
MBM	163 ml	0.177 [0.095, 0.328]	***
DBM	.058 ml	0.385 [0.227, 0.651]	**
PTF	70 ml	.691 [0.388, 1.233]	ns
Late-onset Sepsis			
MBM	163 ml	0.234 [0.130, 0.421]	***
DBM	.117 ml	0.352 [0.209, 0.594]	***
PTF	30 ml	1.268 [0.763, 2.108]	ns
Mortality			
MBM	138 ml	0.233 [0.119, 0.455]	***
DBM	.167 ml	0.654 [0.364, 1.175]	ns
PTF	49 ml	1.56 [0.863,2.803]	ns

Note. OR = odds ratio; CI = confidence interval; MBM = mother's breast milk; DBM = donor breast milk; PTF = preterm formula.

* $p < .05$, ** $p < .01$, *** $p < .001$.

REFERENCES

Aaltonen, J., Ojala, T., Laitinen, K., Poussa, T., Ozanne, S., & Isolauri, E. (2011). Impact of maternal diet during pregnancy and breastfeeding on infant metabolic programming: a prospective randomized controlled study. *Eur J Clin Nutr, 65*(1), 10-19. doi:10.1038/ejcn.2010.225

Afjeh, S. A., Sabzehei, M. K., Khoshnood Shariati, M., Shamshiri, A. R., & Esmaili, F. (2017). Evaluation of Initial Respiratory Support Strategies in VLBW Neonates with RDS. *Arch Iran Med, 20*(3), 158-164. doi:0172003/aim.008

Assad, M., Elliott, M. J., & Abraham, J. H. (2016). Decreased cost and improved feeding tolerance in VLBW infants fed an exclusive human milk diet. *J Perinatol, 36*(3), 216-220. doi:10.1038/jp.2015.168

August, D., & Kandasamy, Y. (2017). The effects of antenatal glucocorticoid exposure on fetal and neonatal skin maturation. *J Perinat Med*. doi:10.1515/jpm-2016-0338

Ballard, O., & Morrow, A. L. (2013). Human milk composition: nutrients and bioactive factors. Pediatr Clin North Am, 60(1), 49-74. doi:10.1016/j.pcl.2012.10.002

Bertino, E., Arslanoglu, S., Martano, C., Di Nicola, P., Giuliani, F., Peila, C., . . . Moro, G. (2012). Biological, nutritional and clinical aspects of feeding preterm infants with human milk. *J Biol Regul Homeost Agents, 26*(3 Suppl), 9-13.

Bertino, E., Giuliani, F., Occhi, L., Coscia, A., Tonetto, P., Marchino, F., & Fabris, C. (2009). Benefits of donor human milk for preterm infants: current evidence. *Early Hum Dev, 85*(10 Suppl), S9-s10. doi:10.1016/j.earlhumdev.2009.08.010

Bloom, B. T. (2016). Safety of donor milk: a brief report. *J Perinatol*. doi:10.1038/jp.2015.207

Bode, L. (2012). Human milk oligosaccharides: every baby needs a sugar mama. *Glycobiology, 22*(9), 1147-1162. doi:10.1093/glycob/cws074

Cabrera-Rubio, R., Collado, M. C., Laitinen, K., Salminen, S., Isolauri, E., & Mira, A. (2012). The human milk microbiome changes over lactation and is shaped by maternal weight and mode of delivery. *Am J Clin Nutr, 96*(3), 544-551. doi:10.3945/ajcn.112.037382

Carlo, W. A., McDonald, S. A., Fanaroff, A. A., Vohr, B. R., Stoll, B. J., Ehrenkranz, R. A., . . . Higgins, R. D. (2011). Association of antenatal corticosteroids with mortality and neurodevelopmental outcomes among infants born at 22 to 25 weeks' gestation. *Jama, 306*(21), 2348-2358. doi:10.1001/jama.2011.1752

Carroll, K., & Herrmann, K. R. (2013). The cost of using donor human milk in the NICU to achieve exclusively human milk feeding through 32 weeks postmenstrual age. *Breastfeed Med, 8*(3), 286-290. doi:10.1089/bfm.2012.0068

Chan, G. M. (2003). Effects of powdered human milk fortifiers on the antibacterial actions of human milk. *J Perinatol, 23*(8), 620-623. doi:10.1038/sj.jp.7211003

Chan, G. M., Lee, M. L., & Rechtman, D. J. (2007). Effects of a human milk-derived human milk fortifier on the antibacterial actions of human milk. *Breastfeed Med, 2*(4), 205-208. doi:10.1089/bfm.2007.0015

Cohen, J. (1977). *Statistical Power Analysis for Behavior Science*. San Diego: Academic Press, Inc.

Colaizy, T. T. (2015). Donor human milk for very low birth weights: patterns of usage, outcomes, and unanswered questions. *Curr Opin Pediatr, 27*(2), 172-176. doi:10.1097/mop.0000000000000201

Collaco, J. M., Aherrera, A. D., & McGrath-Morrow, S. A. (2017). The influence of gender on respiratory outcomes in children with bronchopulmonary dysplasia during the first 3 years of life. *Pediatr Pulmonol, 52*(2), 217-224. doi:10.1002/ppul.23520

Cristofalo, E. A., Schanler, R. J., Blanco, C. L., Sullivan, S., Trawoeger, R., Kiechl-Kohlendorfer, U., . . . Abrams, S. (2013). Randomized trial of exclusive human milk versus preterm formula diets in extremely premature infants. *J Pediatr, 163*(6), 1592-1595.e1591. doi:10.1016/j.jpeds.2013.07.011

De Halleux, V., Pieltain, C., Senterre, T., & Rigo, J. (2017). Use of donor milk in the neonatal intensive care unit. Semin Fetal Neonatal Med, 22(1), 23-29. doi:10.1016/j.siny.2016.08.003

Delfosse, N. M., Ward, L., Lagomarcino, A. J., Auer, C., Smith, C., Meinzen-Derr, J., . . . Morrow, A. L. (2013). Donor human milk largely replaces formula-feeding of preterm infants in two urban hospitals. *J Perinatol, 33*(6), 446-451. doi:10.1038/jp.2012.153

Edwards, T. M., & Spatz, D. L. (2012). Making the case for using donor human milk in vulnerable infants. *Adv Neonatal Care, 12*(5), 273-278; quiz 279-280. doi:10.1097/ANC.0b013e31825eb094

Eidelman, A. I. (2012). Breastfeeding and the use of human milk: an analysis of the American Academy of Pediatrics 2012 Breastfeeding Policy Statement. *Breastfeed Med, 7*(5), 323-324. doi:10.1089/bfm.2012.0067

Ganapathy, V., Hay, J. W., & Kim, J. H. (2012). Costs of necrotizing enterocolitis and cost-effectiveness of exclusively human milk-based products in feeding extremely premature infants. *Breastfeed Med, 7*(1), 29-37. doi:10.1089/bfm.2011.0002

Gartner, L. M., Morton, J., Lawrence, R. A., Naylor, A. J., O'Hare, D., Schanler, R. J., & Eidelman, A. I. (2005). Breastfeeding and the use of human milk. *Pediatrics, 115*(2), 496-506. doi:10.1542/peds.2004-2491

Hair, A. B., Blanco, C. L., Moreira, A. G., Hawthorne, K. M., Lee, M. L., Rechtman, D. J., & Abrams, S. A. (2014). Randomized trial of human milk cream as a supplement to standard fortification of an exclusive human milk-based diet in infants 750-1250 g birth weight. *J Pediatr, 165*(5), 915-920. doi:10.1016/j.jpeds.2014.07.005

Hair, A. B., Hawthorne, K. M., Chetta, K. E., & Abrams, S. A. (2013). Human milk feeding supports adequate growth in infants </= 1250 grams birth weight. *BMC Res Notes, 6*, 459. doi:10.1186/1756-0500-6-459

Hair, A. B., Peluso, A. M., Hawthorne, K. M., Perez, J., Smith, D. P., Khan, J. Y., . . . Abrams, S. A. (2016). Beyond Necrotizing Enterocolitis Prevention: Improving Outcomes with an Exclusive Human Milk-Based Diet. *Breastfeed Med, 11*, 70-74. doi:10.1089/bfm.2015.0134

Hanson, C., Lyden, E., Furtado, J., Van Ormer, M., & Anderson-Berry, A. (2016). A Comparison of Nutritional Antioxidant Content in Breast Milk, Donor Milk, and Infant Formulas. *Nutrients, 8*(11). doi:10.3390/nu8110681

Hanson, L. (2004). *Immunobiology of Human Milk: How Breastfeeding Protects Babies*: Pharmasoft Publishing.

Heiman, H., & Schanler, R. J. (2006). Benefits of maternal and donor human milk for premature infants. *Early Hum Dev, 82*(12), 781-787. doi:10.1016/j.earlhumdev.2006.09.009

Herrmann, K., & Carroll, K. (2014). An exclusively human milk diet reduces necrotizing enterocolitis. *Breastfeed Med, 9*(4), 184-190. doi:10.1089/bfm.2013.0121

Huston, R. K., Markell, A. M., McCulley, E. A., Pathak, M., Rogers, S. P., Sweeney, S. L., . . . Gardiner, S. K. (2014). Decreasing Necrotizing Enterocolitis and Gastrointestinal Bleeding in the Neonatal Intensive Care Unit The Role of Donor Human Milk and Exclusive Human Milk Diets in Infants ≤1500 g Birth Weight *ICAN: Infant, Child, & Adolescent Nutrition*. doi:10.1177/1941406413519267

Ikonen, R., Paavilainen, E., & Kaunonen, M. (2015). Preterm Infants' Mothers' Experiences With Milk Expression and Breastfeeding: An Integrative Review. Adv Neonatal Care, 15(6), 394-406. doi:10.1097/anc.0000000000000232

Jantscher-Krenn, E., & Bode, L. (2012). Human milk oligosaccharides and their potential benefits for the breast-fed neonate. *Minerva Pediatr, 64*(1), 83-99.

Johnson, T. J., Patel, A. L., Jegier, B. J., Engstrom, J. L., & Meier, P. P. (2013). Cost of morbidities in very low birth weight infants. *J Pediatr, 162*(2), 243-249.e241. doi:10.1016/j.jpeds.2012.07.013

Lomax, R. G., & Vaughn, D. L. (2012). *Statistical Concepts A second Course* (4 ed.). New York, NY: Taylor and Francis Group.

Lonnerdal, B. (2014). Infant formula and infant nutrition: bioactive proteins of human milk and implications for composition of infant formulas. *Am J Clin Nutr, 99*(3), 712s-717s. doi:10.3945/ajcn.113.071993

Meier, P., Patel, A., & Esquerra-Zwiers, A. (2016). Donor Human Milk Update: Evidence, Mechanisms, and Priorities for Research and Practice. *J Pediatr*. doi:10.1016/j.jpeds.2016.09.027

Neu, J. (2015). Preterm infant nutrition, gut bacteria, and necrotizing enterocolitis. *Curr Opin Clin Nutr Metab Care, 18*(3), 285-288. doi:10.1097/mco.0000000000000169

Neu, J., & Bernstein, H. (2002). Update on host defense and immunonutrients. *Clin Perinatol, 29*(1), 41-64.

O'Connor, D. L., Ewaschuk, J. B., & Unger, S. (2015). Human milk pasteurization: benefits and risks. *Curr Opin Clin Nutr Metab Care, 18*(3), 269-275. doi:10.1097/mco.0000000000000160

Organization, W. H. (2011). WHO Guidelines Approved by the Guidelines Review Committee. In *Guidelines on Optimal Feeding of Low Birth-Weight Infants in Low- and Middle-Income Countries*. Geneva: World Health Organization.

Patel, A. L., Johnson, T. J., Engstrom, J. L., Fogg, L. F., Jegier, B. J., Bigger, H. R., & Meier, P. P. (2013). Impact of early human milk on sepsis and health-care costs in very low birth weight infants. *J Perinatol, 33*(7), 514-519. doi:10.1038/jp.2013.2

Pepe, M. (2003). The Statistical Evaluation of Medical Tests for Classification and Prediction. New York: Oxford University Press.

Ramani, M., & Ambalavanan, N. (2013). Feeding practices and necrotizing enterocolitis. *Clin Perinatol, 40*(1), 1-10. doi:10.1016/j.clp.2012.12.001

Ressel, G. (2002). AAP updates statement for transfer of drugs and other chemicals into breast milk. American Academy of Pediatrics. Am Fam Physician, 65(5), 979-980.

Roberts, D., Brown, J., Medley, N., & Dalziel, S. R. (2017). Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. *Cochrane Database Syst Rev, 3*, Cd004454. doi:10.1002/14651858.CD004454.pub3

Schanler, R. J. (2015). In time: human milk is the feeding strategy to prevent necrotizing enterocolitis. *Rev Paul Pediatr, 33*(2), 131-133. doi:10.1016/j.rpped.2015.01.001

Schanler, R. J., Lau, C., Hurst, N. M., & Smith, E. O. (2005). Randomized trial of donor human milk versus preterm formula as substitutes for mothers' own milk in the feeding of extremely premature infants. *Pediatrics, 116*(2), 400-406. doi:10.1542/peds.2004-1974

Schanler, R. J., Shulman, R. J., & Lau, C. (1999). Feeding strategies for premature infants: beneficial outcomes of feeding fortified human milk versus preterm formula. *Pediatrics, 103*(6 Pt 1), 1150-1157.

Sherman, P. M., Cabana, M., Gibson, G. R., Koletzko, B. V., Neu, J., Veereman-Wauters, G., . . . Walker, W. A. (2009). Potential roles and clinical utility of prebiotics in newborns, infants, and children: proceedings from a global prebiotic summit meeting, New York City, June 27-28, 2008. In *J Pediatr* (Vol. 155, pp. S61-70). United States.

Spiegler, J., Preuss, M., Gebauer, C., Bendiks, M., Herting, E., & Gopel, W. (2016). Does Breastmilk Influence the Development of Bronchopulmonary Dysplasia? J Pediatr, 169, 76-80.e74. doi:10.1016/j.jpeds.2015.10.080

Stoll, B. J., Hansen, N. I., Bell, E. F., Shankaran, S., Laptook, A. R., Walsh, M. C., . . . Higgins, R. D. (2010). Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. *Pediatrics, 126*(3), 443-456. doi:10.1542/peds.2009-2959

Sullivan, S., Schanler, R. J., Kim, J. H., Patel, A. L., Trawoger, R., Kiechl-Kohlendorfer, U., . . . Lucas, A. (2010). An exclusively human milk-based diet is associated with a lower rate of necrotizing enterocolitis than a diet of human milk and bovine milk-based products. *J Pediatr, 156*(4), 562-567.e561. doi:10.1016/j.jpeds.2009.10.040

Tabachnick, B. G., & Fidell, L. S. (2013). *Using Multivariate Statistics* (6 ed.). New York, NY: Pearson.

Underwood, M. A. (2013). Human milk for the premature infant. *Pediatr Clin North Am, 60*(1), 189-207. doi:10.1016/j.pcl.2012.09.008

Varga, P., Berecz, B., Gasparics, A., Dombi, Z., Varga, Z., Jeager, J., . . . Kornya, L. (2017). Morbidity and mortality trends in very-very low birth weight premature infants in light of recent changes in obstetric care. *Eur J Obstet Gynecol Reprod Biol, 211*, 134-139. doi:10.1016/j.ejogrb.2017.01.051

Wojcik, K. Y., Rechtman, D. J., Lee, M. L., Montoya, A., & Medo, E. T. (2009). Macronutrient analysis of a nationwide sample of donor breast milk. *J Am Diet Assoc, 109*(1), 137-140. doi:10.1016/j.jada.2008.10.008

VITA

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Appendix A

Contrasting the Upper Quartile with the Lower Quartile of MBM

Table A.1 show the results for the logistic regressions with standardized odds ratios for contrasting the upper quartile with the lower quartile of percentage of MBM, controlling for birthweight, gestational age, gender, Apgar scores at both 1 and 5 minutes, prenatal and postnatal steroids. This analysis found that when comparing the upper and the lower quartile of MBM there was not an association for BPD, NEC, late-onset sepsis, and mortality.

Length of Hospital Stay, Weight Gain, and Ventilation

Table A.2 shows the results for the linear regression contrasting the upper quartile with the lower quartile percentage of MBM, controlling for birthweight, gestational age, gender, Apgar scores at both 1 and 5 minutes, prenatal and postnatal steroids. MBM was not associated with length of stay or weight gain. On the other hand, MBM was significantly associated with decreased total ventilated days (β= -.*207; p* = .006).

Table A.1.

Results for Logistic Regressions with Standardized Odds Ratios (OR) for Contrasting the Upper Quartile with the Lower Quartile of Percentage Mother's Breast Milk

	Not adjusted for covariates		Adjusted for covariates	
	OR [95% CI]	*p* Value	OR [95% CI]	*p* Value
Bronchopulmonary Dysplasia				
MBM	0.78 [0.57, 1.08]	ns	0.92 [0.60, 1.42]	ns
Medical Necrotizing Enterocolitis				
MBM	0.84 [0.58, 1.21]	ns	1.24 [0.75, 2.03]	ns
Late-onset Sepsis				
MBM	0.93 [0.65, 1.34]	ns	0.91 [0.58, 1.43]	ns
Mortality				
MBM	0.81[0.55, 1.22]	ns	0.94 [0.57, 1.57]	ns

Note. *N*= 150. Covariates were birthweight, gestational age, Apgar scores at both 1 and 5 minutes, prenatal and postnatal steroids, and gender. MBM = mother's breast milk.
* $p < .05$, ** $p < .01$, *** $p < .001$, ns = not significant.

Table A.2.
Results for Linear Regressions with Coefficients for Contrasting the Upper Quartile with the Lower Quartile Percentage of Mother's Breast Milk

	Unstandardized Coefficients		Standardized Coefficients		
Variables	*B*	*(SE)*	β	*t*	*p*
Length of hospital stay(days)					
MBM	-0.197	0.062	-0.253	-3.177	**
Adjusted	-0.113	0.062	-0.149	-1.808	ns
Weight gain (kg)					
MBM	-0.005	0.002	-0.206	-2.561	**
Adjusted	-0.003	0.002	-0.120	-1.433	ns

Total days on ventilation					
MBM	-0.160	0.038	-0.326	-4.190	***
Adjusted	-0.102	0.037	-0.207	-2.774	**

Note. *N*= 302. Covariates were birthweight, gestational age, Apgar scores at both 1 and 5 minutes, prenatal and postnatal steroids, and gender. MBM = mother's breast milk.
* $p < .05$, ** $p < .01$, *** $p < .001$, ns = not significant.