Proposal: A Retrospective Cohort Study to Analyze if Type of Feeding Decreases the Incidence of Necrotizing Enterocolitis in Very Low Birthweight Infants

By

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Abstract

Necrotizing enterocolitis (NEC) is a devastating disorder that primarily impacts pre-term infant's intestines, which are believed to be invaded by bacteria causing local infection and inflammation. It has been consistently observed that pre-term infants who are primarily fed human breast milk have reduced incidence of NEC, which is believed to be due to a component of human breast milk known as human milk oligosaccharides (HMOs). Therefore, the purpose of this retrospective cohort multi-center study is to further analyze if the type of feeding decreases the incidence of NEC in very low birthweight (VLBW) infants. Data, including amount and type of enteral feedings and NEC diagnosis, will be collected through the electronic medical record (EMR) of the participant. The anticipated results of the study are expected to demonstrate human breast milk as a preventative measure against the incidence of NEC. This study will likely demonstrate support for optimization of enteral feedings with a strong recommendation for human breast milk in the neonatal intensive care unit (NICU) setting, specifically for the first 28 days of life.

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CHAPTER 1: Introduction

Necrotizing enterocolitis (NEC) is a devastating disorder that develops when a pre-term infant's intestines are invaded by bacteria causing local infection and inflammation (Children's Hospital of Los Angeles [CHLA], n.d.). While this disease can occur in any infant admitted to the neonatal intensive care unit (NICU), over 90% of all NEC cases are pre-term infants (CHLA, n.d.). NEC cases are inversely associated with pre-term infants' gestational age and weight (National Organization for Rare Disorders [NORD], 2015). The mortality rate also differs depending on the birthweight of the infant (National Center for Advancing Translational Sciences [NCATS], 2013). Pre-term infants are specifically at higher risk for numerous reasons including increased permeability of their intestines, potential for enteral feeding intolerances, and underdevelopment of the infant gut flora (CHLA, n.d.).

It has been consistently observed that infants who predominately consumed human breast milk have a decreased risk of NEC (Gephart et al., 2012). A large prospective study indicated that infants fed human breast milk were six to ten times less likely to develop NEC compared to infants who were fed infant formula (Lucas & Cole, 1990). The authors also found that infants who were fed a mixture of both human breast milk and infant formula were three times less likely to develop NEC compared to those fed only infant formula (Lucas & Cole, 1990).

Human milk oligosaccharides (HMOs) are a type of complex carbohydrates found abundantly in human breast milk, but not infant formula, that are believed to be the component responsible for reducing the risk of NEC (Underwood et al., 2015). HMOs are not digested by the infant for energy but are thought to move into the large intestine intact where they serve as a prebiotic (Underwood et al., 2015).

Background

Gestational age and low birthweight are the greatest risk factors for developing NEC (NORD, 2015). The inverse association of gestational age and low birthweight is likely due to the underdeveloped GI tract of the pre-term infant (Mugambi et al., 2012; NORD, 2015). An immature GI tract leads to increased permeability, allowing pathogenic bacteria to translocate from the intestines to other areas of the body causing infection (Mugambi et al., 2012). There is also evidence demonstrating that an immature intestinal immune response may be the primary factor causing NEC (Feinberg et al., 2017).

A potential mechanism as to why a pre-term infant may have an insufficient intestinal immune response is due to a failure to establish a population of gut flora (Feinberg et al., 2017). In conjunction with increased permeability of the intestines and underdeveloped immune system, this then can lead to pathogens translocating across the mucosa allowing for potential infection, such as NEC (Feinberg et al., 2017).

Emerging research has shown that infants who are fed human breast milk are less likely to develop NEC than infants who are fed infant formula (Gephart et al., 2012). While more research is needed, it is theorized that HMOs are beneficial to a pre-term infant by playing a role in the development of the microbiome (Gritz & Bhadari, 2015). HMOs, which are found in abundance in human breast milk, are not common in other natural sources (Bode, 2012). While specific HMOs are added to some infant formulas, they are structurally different from the oligosaccharides found in human breast milk (Gritz & Bhandari, 2015).

It has been observed that once HMOs reach the large intestine, beneficial bacteria are attracted to the HMOs allowing them to grow and proliferate (Jantscher-Krenn & Bode, 2012). Multiple strains of bacteria feed on HMOs with the *Bifidobacteria* species being best known (Jantscher-Krenn & Bode, 2012). However, HMOs do not enable harmful or pathogenic bacteria to grow (Jantscher-Krenn & Bode, 2012). In addition, HMOs further prevent the growth of pathogenic bacteria by mimicking structures of viral receptors and preventing attachment to the intestinal cells (Morozov et al., 2018).

There have been studies with findings that human breast milk reduces the risk of NEC in pre-term infants; however, gestational age and birthweight of subjects have greatly ranged in the studies previously conducted. This has led to insufficient knowledge regarding human breast milk and the association with NEC in VLBW infants.

Problem Statement

While very low birthweight (VLBW) infants are at high risk for developing NEC, it is unclear if human breast milk serves as a preventative measure for this specific population, as research subjects vary in gestational age and weight between studies. This study proposal will further analyze if human breast milk decreases the incidence of NEC in VLBW infants. This study will also analyze if over 50% of calories from human breast milk will decrease incidence of NEC in VLBW infants.

Purpose of the Study

The purpose of this retrospective cohort study is to further analyze if human breast milk intake decreases the incidence of NEC in VLBW infants compared to VLBW infants fed infant formula. The primary objective of this study is to determine if a VLBW

infant's risk of NEC is reduced by being fed human breast milk in the first 28 days of life compared to those fed infant formula for the first 28 days of life. The secondary objective is to determine if over 50% calories from human breast milk will significantly decrease the incidence of NEC in VLBW infants compared to VLBW infants receiving 50% or less calories from human breast milk. Infants will be divided into groups depending on what type of feeding they have, which will be monitored through the electronic medical record (EMR) for enteral intake and diagnosis of NEC.

Potential Significance

Results supporting the research hypothesis that human breast milk reduces the incidence of NEC will demonstrate the need for an optimized practice resulting in more infants receiving human breast milk within the first 28 days of life. The potential significance of this study could support the need of optimizing practice to increase enteral feedings of human breast milk (through mother's own milk or donor breast milk) for VLBW infants admitted into the NICU. This could result in decreased incidence of NEC, decreased mortality in the NICU, and prevention of medical complications or additional surgeries.

Furthermore, this study will provide insight into the type and amount of feeding to decrease a VLBW infant's risk of NEC. Due to the lack of sufficient data regarding whether human breast milk does indeed reduce risk of NEC in VLBW infants, this study will investigate the benefits of human breast milk intake and determine if over 50% calories from human breast milk reduces the risk of NEC in this population.

Research Question

***RQ1*.** Does type of feeding decrease the incidence of necrotizing enterocolitis in VLBW infants?

Sub-Problems

1. Will over 50% of calories from human breast milk reduce the incidence of NEC in VLBW infants?

Hypotheses

H1o: There will be no significant difference between the incidence of NEC in VLBW infant's fed human breast milk compared to VLBW infants fed infant formula.

H1a: There will be a significantly lower incidence of NEC in VLBW infants fed human breast milk compared to VLBW infants fed infant formula.

H2o: The incidence of NEC will not be significantly decreased in VLBW infants who receive over 50% of calories from human breast milk compared to VLBW infants fed 50% or less calories from human breast milk.

H2a: The incidence of NEC will be significantly decreased in VLBW infants who receive over 50% of calories from human breast milk compared to VLBW infants fed 50% or less calories from human breast milk.

Dependent and Independent Variables

NEC diagnosis will serve as the independent variable whereas type of formula, amount of feeding, and calories received from each feeding will serve as dependent variables.

Nature of the Study

Subjects will include VLBW infants admitted to one of the six hospitals in the Los Angeles area participating in the study. Participants will be categorized based on the two objectives of the study (first objective groups: NEC cases compared to No-NEC cases; second objective: human breast milk group compared to infant formula group). Data will be collected retrospectively through the hospital's EMR. Information to be gathered includes the type of feeding, amount of feeding, and whether the infant was diagnosed with NEC by day of life 28. A retrospective design is appropriate for this study due to VLBW infants diagnosed with NEC being a specialized and rare population.

To meet the first objective, NEC cases (infants diagnosed with NEC in the first 28 days of life) will be compared to No-NEC cases (infants without a diagnosis of NEC in the first 28 days of life) to determine whether infants who received any amount of human breast milk or "HBM-Yes" differed significantly between groups. Infants who did not receive any amount of human breast milk will be categorized as "HBM-No". To meet the second objective, participants will be categorized under one of the two groups: human breast milk group or infant formula group. The human breast milk group will include VLBW infants who were predominately fed human breast milk or received over 50% of calories from human breast milk throughout the first 28 days of life through mother's own milk or donor breast milk. The infant formula group will include VLBW infants who were predominately fed infant formula or received over 50% of calories from infant formula throughout the first 28 days of life. Data will be non-parametric and categorical, thus making the chi-square test appropriate for statistical analysis.

Definition of Terms

***Abdominal X-ray*:** A type of radiation called electromagnetic waves that creates a picture of the inside of the abdomen (National Institute of Health: U.S. National Library of Medicine [NIH], n.d.)

***Birthweight*:** The first weight of a baby taken just after they are born (NIH, n.d.)

***Breastfeeding*:** Infant fed human milk from their mother (World Health Organization [WHO], n.d.)

***Day of Life*:** The number of days the infant has been alive for (WHO, n.d.)

Extremely Low Birthweight (ELBW): Infant with a birthweight of under 1000 grams (2 pounds, 3 oz) (Wejyrd et al., 2018)

***Full-term Infant*:** An infant born at or after 37 weeks gestation (NIH, n.d.)

Gestational Age: Measurement in weeks of how far along a pregnancy is, usually starting from the woman's last menstrual cycle. A normal pregnancy is 37-40 weeks gestation. (NIH, n.d.)

***Gut Dysbiosis*:** Condition in which there is an imbalance of microorganisms within the intestines (Rao & Patole, 2019).

***Human Breast Milk*:** Milk produced by mammary glands in the breast of a female to feed a child (NIH, n.d.)

***Human Milk Oligosaccharides (HMOs)*:** A type of complex carbohydrates found abundantly in human breast milk that serves as a prebiotic to an infant (Wejyrd et al., 2018)

Infant Formula: An artificial substitute for human breast milk (NIH, n.d.)

Lactation: The secretion of milk by the mammary glands (NIH, n.d.)

Low Birthweight (LBW): Infants with a birthweight of under 2500 grams (5 pounds, 8 ounces) (NIH, n.d.)

***Mother's Own Milk*:** Human breast milk expressed from the infant's biological mother (Corpeleijn et al., 2016)

Necrotizing Enterocolitis (NEC): A disease that mostly affects the intestines of a pre-term infant. The infection is often due to invasion of pathogenic bacteria (Wejyrd et al., 2018)

***Neonate*:** An infant in the first 4 weeks of life (NIH, n.d.)

Pre-term Infant or Prematurity: An infant born before 37 weeks gestation (NIH, n.d.)

***Short-gut Syndrome*:** A malabsorption disorder caused by lack of functional small intestines (NCATS, 2013)

Very Low Birthweight: Infants with a birthweight of under 1500 grams (3 pounds, 4 ounces) (NIH, n.d.)

Assumptions

1. Human breast milk is beneficial to a pre-term infant.
2. Clinical providers and the participants' parents want the best outcome for the VLBW infant.
3. The methodology of this study is appropriate to determine the association between NEC and human breast milk.

Limitations

1. This study may have a limited number of participants due to the small population of VLBW infants.
2. This study is only able to show an association between human breast milk intake and NEC incidence, not causality.
3. This study has no control over enteral feedings the infant receives, such as amount given in a day.
4. This study has potential for bias due to its study design.

5. Results have the potential to be confounded by the following variables: clinical condition of the infant, treatment interference (i.e. surgery or antibiotic treatment), or an NPO diet order.

Delimitations

1. This study will only include VLBW infants admitted to one of the six NICU sites in the Los Angeles area participating in the study.
2. This study will the rely on the EMR for information, such as volume of feeding the infant consumed, which is subject to human error.
3. This study will only include infants born with a birthweight <1500 grams.
4. Parents of participants must provide written informed consent prior to the data collection.
5. Any infant transferred to another facility before the 28th day of life will be excluded from analysis.
6. Any infant born with a congenital malformation will not be included in the study.
7. Donor breast milk will not be differentiated from human breast milk for this study.
8. All types of infant formula will be grouped together under one category for this study.

Summary

In summary of this chapter, this research proposal is set up to determine if type of feeding is associated with reduced incidence of NEC in VLBW infants, as there is currently insufficient knowledge on this topic. Chapter 2 (The Literature Review) will further investigate the information known and what research is currently available on this subject. Chapter 3 (Methodology) will include the methodology specific to this

research proposal. Chapter 4 (Anticipated Results) will include the expected results of the study. Lastly, Chapter 5 (Discussion and Potential Significance) will discuss the anticipated results and determine the potential significance for clinical practice and what future studies should focus on.

CHAPTER 2: The Literature Review

Introduction

Necrotizing enterocolitis (NEC) is a devastating gastrointestinal (GI) disease commonly diagnosed in pre-term infants (Gephart et al., 2012). NEC develops when the pre-term infant's intestines are invaded by bacteria causing local infection and inflammation (CHLA, n.d.). If untreated, NEC can cause a destruction of the intestinal wall, which allows stool to leak through, and lead to an overwhelming infection and potentially even death (CHLA, n.d.). While modern medicine and technology have improved to support growth and development in pre-term infants, unfortunately, about 10% of this population still develop this disease (CHLA, n.d.). While full-term infants can also be diagnosed, pre-term infants make up 90% of all NEC cases (Gephart et al., 2012). The mortality rate of this disease varies depending on the birthweight of the infant and severity of the disease (NCATS, 2013). Infants who weigh over 2500 grams (about 5.5 pounds) at birth have a mortality rate of 0 to 20% (NCATS, 2013). For infants born at less than 1500 grams (about 3.3 pounds), the mortality rate is 10 to 50% (NCATS, 2013). For infants weighing less than 1000 grams (about 2.2 pounds), the mortality rate is 40 to 100% (NCATS, 2013). Of the infants that survive, up to 80% of infants diagnosed with NEC Bell stage II or greater will require surgical intervention (Zamrik et al., 2018). Furthermore, of the 50% who survive are likely to develop a long-term complication such as an intestinal stricture or short-gut syndrome (NCATS, 2013).

In addition to the complications directly due to this disease, pre-term infants face another challenge: meeting their nutritional needs with increased risk of developing a feeding intolerance. Pre-term infants 1) are at risk for nutritional deficiencies due to decreased nutrient stores of fat, protein, glycogen, vitamins, and minerals, 2) have

increased nutritional needs due to rapid growth and 3) may have mothers with limited or insufficient milk production, especially during the first week postnatal (Feinberg et al. 2017; Mugambi et al., 2012). When the mother is unable to produce enough milk, infant formula or donor human milk is used (Feinberg et al., 2017). Initiation of enteral feedings is preferred over parenteral nutrition for the pre-term infant, as it promotes growth and development of the GI tract; thus, is imperative in the neonatal intensive care unit (NICU) (Feinberg et al., 2017). Parenteral nutrition is indicated when an infant has a feeding intolerance; however, this method is undesirable as it has morbidities associated with it (Feinberg et al., 2017). A feeding intolerance is likely to be one of the first signs shown of NEC and often results in discontinuation of enteral nutrition (CHLA, n.d.).

The type of enteral nutrition, such as human breast milk or infant formula, may also put an infant at risk for developing NEC (CHLA, n.d.). A risk factor that has been consistently observed for this disease is feeding infant formula to pre-term infants (CHLA, n.d.). It has been reported that a large prospective study indicated that infants fed human breast milk were six to ten times less likely to develop NEC compared to infants who were fed infant formula (Lucas & Cole, 1990). This study also found that infants who were fed a mixture of both human breast milk and infant formula were three times less likely to develop NEC compared to those fed only infant formula (Gephart et al., 2012). However, it is currently unknown whether something harmful in infant formula increases risk or if there is a beneficial property of human breast milk decreasing the risk, such as immune-modulating components (Underwood et al., 2015).

Emerging research is showing the impact human breast milk may have in preventing NEC due to the promotion of adequate bacterial colonization.

Bacterial colonization in the infant's gut occurs rapidly after birth and is heavily influenced by the infant's diet (Gritz & Bhandari, 2015). Human milk oligosaccharides (HMOs) are a type of complex carbohydrates found abundantly in human breast milk, but not infant formula (Underwood et al., 2015). While the number of HMOs differ amongst women, it is the third largest component of human breast milk (Underwood et al., 2015). However, HMOs are not digested by the infant for energy (Underwood et al., 2015). Instead, HMOs have been shown to pass through the stomach and small intestine, arriving in the large intestine intact where they serve as a prebiotic (Bering, 2018). As a prebiotic, the HMOs help to shape the developing microbiome and prevent against pathogenic bacterial overgrowth (Bering, 2018). HMOs are one of the components of human breast milk that are believed to be preventative against NEC (Underwood et al., 2015).

While there is a fair case supporting HMOs having an influence on an infant's gut flora, there is not much research to support this claim. With a disease as devasting as NEC, it is imperative that clinicians and other healthcare providers understand the factors associated with this disease and work aggressively to prevent it. Therefore, the purpose of this literature review is to analyze the current research available on human breast milk and HMOs and their association with NEC and determine what further research is needed to be conducted in the future.

To gather literature, the following search terms were used: human milk oligosaccharides and necrotizing enterocolitis; Human milk and necrotizing enterocolitis;

Human milk oligosaccharides and neonates; Human milk oligosaccharides and pre-term infant; Human milk oligosaccharides and neonates and gut flora; and, human breast milk and necrotizing enterocolitis.

Background of Necrotizing Enterocolitis & Emerging Research

As common as NEC is in the NICU, there is still much unknown about this disease and what prevents it from occurring; thus, with mortality rates so high for certain pre-term infants, and the risk of delayed enteral nutrition for proper growth and development, it is crucial that healthcare providers understand and work to prevent the development of NEC.

Risk Factors

Prematurity and low birthweight are the greatest risk factors to the infant, as prematurity and birthweight are inversely related to the risk of NEC (NORD, 2015). Full-term infants who develop this disease usually have a specific risk factor such as congenital heart disease, sepsis, or low blood pressure (NORD, 2015). Other known risk factors for NEC include formula feeding, low oxygen at birth, and infections of the intestine (Stanford Children's Health, n.d.).

Prematurity. Pre-term infants are at high risk for the development of NEC due to immaturity of their organs, specifically the GI tract (Mugambi et al., 2012). Overall, five to ten percent of pre-term infants weighing less than 1500 grams are affected by NEC (NORD, 2015). An immature GI tract leads to increased permeability, allowing pathogenic bacteria to translocate from the intestine to other areas of the body causing infection (Mugambi et al., 2012). In addition, pre-term infants often have challenges fighting off infections due to poor blood circulation (Stanford Children's Health, n.d.).

Formula Feeding. As stated above, emerging research has shown that infants who are fed human breast milk are less likely to develop NEC than those who are fed infant formula (Gephart et al., 2012). While more research is needed, it is believed that human breast milk is beneficial to the infant because it 1) contains HMOs whereas infant formula does not and 2) is easier to digest and contains immunological components that help to develop the gut (Stanford Children's Health, n.d.). Benefits and certain properties of human breast milk are discussed in further detail in this literature review.

Low Oxygen Levels. Infants who have low blood oxygen levels at birth are at risk for developing NEC (Stanford Children's Health, n.d.). When the infant has too little oxygen, their body prioritizes circulation to the heart and brain reducing the amount of oxygen sent to the GI tract and increasing the risk for infection (Stanford Children's Health, n.d.). When the cells of the intestines receive insufficient blood supply it can lead to cellular injury and necrosis, which could then lead to the development of NEC (NORD, 2015).

Signs and Symptoms

Symptoms of NEC often appear within the first two weeks of life (CHLA, n.d.). One of the first symptoms of NEC is often a feeding intolerance (CHLA, n.d.). Feeding intolerances are often associated with abdominal distension and vomiting bile (CHLA, n.d.). Also, feedings may not move through the intestines in the presence of NEC, which may be indicated by symptoms of gastric aspiration, abdominal distention and tenderness, and vomiting (NORD, 2015). Other symptoms include bloody stools, diarrhea, lethargy, apnea, and decreased blood pressure (CHLA, n.d.).

Diagnosis

The diagnosis of NEC is most often confirmed by the presence of gas or air bubbles in the wall of the intestine, abdominal cavity outside of the intestines, or in the portal vein, via abdominal X-ray (CHLA, n.d.). Intestinal fluid in the abdominal cavity indicating a hole in the intestine can also diagnose NEC (Stanford Children's Health, n.d.). Blood tests may also indicate risk for infection such as decreased number of platelets and white blood cells (CHLA, n.d.). The severity of NEC diagnosis is classified into stages.

Stages of NEC. The three stages of NEC: suspected, definite, and advanced are summarized under **Table 1** utilizing the Bell Staging Criteria (Gregory et al., 2011). Most research in pre-term infants with NEC involves those infants who were diagnosed with NEC Bell stage II or greater.

Table 1

Bell criteria staging for necrotizing enterocolitis

Stage	Intestinal Signs	Systemic Signs	Radiologic Signs
Stage I, suspected			
IA	• Increased gastric residuals • Mild abdominal distention • Emesis	• Apnea • Bradycardia • Temperature instability • Lethargy	• Normal or dilated intestines • Mild ileus
IB	• *Same as above, plus* • Bloody stool	• *Same as above*	• *Same as above*
Stage II, definite			
IIA	• *Same as above, plus* • Absent bowel sounds • Tender abdomen	• *Same as above*	• Ileus • Pneumatosis intestinalis
IIB	• Definite abdominal tenderness • Absent bowel sounds • Abdominal cellulitis • Right lower quadrant mass	• *Same as above, plus* • Mild metabolic acidosis • Mild thrombocytopenia	• Portal venous gas with or without ascites
Stage III, advanced			
IIIA	• Marked tenderness • Abdominal exam shows marked distention • Peritonitis	• *Same as above* • Hypotension • Respiratory failure • Severe metabolic acidosis • Coagulopathy • Neutropenia	• Ascites
IIIB	• *Same as above, plus* • Perforated bowel	• *Same as above*	• *Same as above, plus* • Pneumoperitoneum

Note. Adapted from "Necrotizing Enterocolitis Risk: State of the Science" by Gephart, S. M., McGrath, J. M., Effken, J. A., & Halpern, M. D, 2012, *Adv Neonatal Care, 12*(2): 77-89. https//doi.org/10.1097/ANC.0b013e31824cee94

Treatment

The current treatment for NEC depends on the disease stage, symptoms, and the infant's age (Stanford Children's Health, n.d.). Treatment may vary but typically involves the following: discontinuation of enteral nutrition, insertion of a nasogastric tube to remove air in stomach and intestines, intravenous fluids and nutrition, antibiotics, frequent abdominal X-rays, and isolation of the infant to prevent further spread of infection (Stanford Children's Health, n.d.). Infants who respond to treatment can resume enteral feedings once the signs of infection have disappeared, often taking a minimum of five to seven days (CHLA, n.d.). However, each case is individual and overall medical treatment depends on the infant.

In cases of NEC Bell stage I, suspected NEC, treatment typically consists of bowel rest, discontinuation of enteral nutrition, nasogastric decompression, blood cultures, and antibiotic treatment (NORD, 2015). While enteral nutrition is discontinued an infant is likely to receive parenteral nutrition (NORD, 2015). The infant is then monitored closely via serial examinations and radiographs for further signs and symptoms of NEC (NORD, 2015).

In cases of NEC Bell stage II or III, treatment often requires surgical intervention (NORD, 2015). Surgical intervention is indicated when an infant has a perforated or necrotic intestine (NORD, 2015). Other indications include clinical deterioration or severe abdominal distension causing abdominal compartment syndrome (NORD, 2015). Two surgical approaches may be used to treat NEC: laparotomy with resection (removal of the infected intestines) or primary peritoneal drainage, which involves inserting a drain into the space of the abdomen that holds the intestines (NORD, 2015).

If the infant requires an ostomy, they will be in recovery for six to eight weeks before they then go to surgery again to reverse the procedure (CHLA, n.d.).

Prognosis

The survival rates of those diagnosed with NEC has improved over the years due to advancement in medical technology (NCATS, 2013). Currently, about 3% of infants with birthweight between 1251 to 1500 grams are diagnosed with NEC (NORD, 2015). Infants who are born weighing less than 750 grams have a higher rate of NEC, which is 11% (NORD, 2015). Overall, while it varies depending on severity of disease and other factors, the survival rate of infants affected by NEC is currently 60 to 70% (NCATS, 2013). However, up to 80% of infants diagnosed with NEC stage II or greater will require surgical intervention (Zamrik et al., 2018). Of the infants who survive, 50% are likely to develop a long-term complication, such as intestinal stricture or short-gut syndrome (NCATS, 2013). Short-gut syndrome is the most common post-surgical complication affecting up to 23% infants, which is a post-operative malabsorption syndrome due to critical components of the small bowel being removed (NCATS, 2013). The infant's gut tends to develop and adapt over time; however, those who are unable to tolerate enteral feedings or develop severe liver disease may require a bowel or liver transplant (NCATS, 2013). Recurrent NEC is uncommon (occurs in four to six percent of cases), but can occur (NCATS, 2013). Of the infants who graduate from the NICU, most are likely to grow and develop by childhood (NCATS, 2013).

A study was conducted in 2002 of children ages five to ten who were diagnosed with NEC Bell Stage II or greater as a pre-term infant (Stanford et al., 2002). Results of the study showed that all participants were able to eat by mouth but were under the 50th

percentile for height and weight (Stanford et al., 2002). The study showed that 83% of children were enrolled in school full-time, indicating that those who survive can expect a favorable long-term outcome (Stanford et al., 2002).

Pre-term Infant's Gut Flora

The gut flora of infants has been investigated for over 150 years and is still not fully understood (Perez-Muñoz et al., 2017). It has been previously thought that an infant was born with a sterile gut and exposed to microorganisms during delivery and quickly after birth; however, emerging research is indicating the microbiome develops even earlier than previously believed (Gritz & Bhandari, 2015). Due to modern sequencing technology, new research proposes that neither the fetus, placenta, nor amniotic fluid (which is swallowed by the fetus in the womb) is sterile, indicating that the infant's gut flora begins to develop in utero (Perez-Muñoz et al., 2017).

The idea that an infant was born with a sterile gut was determined via traditional microscopy, which is highly limited and deficient compared to the modern technology used to study the infant's gut flora (Perez-Muñoz et al., 2017). Recently, four studies were included in a review that stated over 90% of infant meconium tested positive for bacteria (as cited in Perez-Muñoz et al., 2017). This supports the theory that the fetal environment in the womb and the placenta are not sterile (as cited in Perez-Muñoz et al., 2017). These findings were found via bacterial culture techniques and high-throughput sequencing (Perez-Muñoz et al., 2017). Previous research had shown 62% of infant meconium negative for bacteria; however, traditional microscopy was used to analyze the data of this research (as cited in Perez-Muñoz et al., 2017). As stated above, research has also indicated that not only bacteria are present in the fetal

environment, but also that the placenta may also have a microbiome to further colonize the infant's gut (as cited in Perez-Muñoz et al., 2017). While this research is relatively new, it proposes that the microbiota exists prenatally and helps with intestinal development in utero, which in turn helps prepare the infant for digesting and absorbing enteral nutrition (as cited in Perez-Muñoz et al., 2017).

A pre-term infant has an increased risk of having microbiome dysbiosis due to their conditions after birth (Gritz & Bhandari, 2015). Pre-term infants who spend time in the NICU develop a different gut flora than a healthy, breastfed infant (Mugambi et al., 2012). This is mostly due to decreased exposure to the maternal gut flora, increased exposure to organisms that are found abundantly in the NICU, antibiotic treatment, and delays in enteral feedings (Mugambi et al., 2012). Nutrition has been demonstrated to play a major role in the infant's gut flora, as it can vary the composition of the gut flora based on the type of nutrition (Gritz & Bhandari, 2015).

It has been observed that the gut microbiota of full-term infants has a high abundance of *Bifidobacteria* and *Bacteroidetes* compared to a pre-term infant's gut microbiota (Chernikova et al., 2018). Interestingly, the *Bifidobacteria* species are also the main consumers of HMOs (as cited in Bering, 2018). It has also been noted that pre-term infants have a higher colonization of *Enterobacteriaceae* and other *Proteobacteria,* of which certain types are associated with higher incidence of NEC (as cited in Bering, 2018).

There was a study conducted by Chernikova et al. (2018) that evaluated the impact of gestational age on infant gut microbiota in pre-term infants in the first six weeks of life compared to full-term infants. Results of the study showed that pre-term

infants had a lower abundance of the *Bifidobacteria* than full-term infants (Chernikova et al., 2018). Results also indicated that the lower the gestational age of the pre-term infant (infants born before 32 weeks gestation compared to infants born after 32 weeks gestation), the lower the diversity of the infant's gut flora (Chernikova et al., 2018). In addition, results showed that the greater the gestational age of a pre-term infant, the more similar their gut flora was to full-term infants (Chernikova et al., 2018). Antibiotic use was also a factor for low diversity of the infant's gut flora (Chernikvoa et al., 2018).

The structure of the gut is heavily influenced by both diet and antibiotic use (Cresci & Bawden, 2015). Cresci and Bawden (2015) explain that while emerging research is suggesting that the womb is not sterile as it was once believed to be, most of the microbes will be acquired post-partum. The normal development of the infant gut flora is believed to be chaotic and goes through stages in the first year of life (Cresci & Bawden, 2015). Early on, the infant's gut is colonized by aerobic bacteria that have the potential to be pathogenic such as *Enterobacteria*, *Staphylococci*, and *Streptococci* (Cresci & Bawden, 2015). This early colonization begins to change the environment of the gut, which allows anaerobic bacteria to colonize (Cresci & Bawden, 2015). After these rapid changes in the first year of life, the gut then continues to change based on external factors, such as diet (Cresci & Bawden, 2015).

Microbiota and Human Breast Milk. It has been shown that bacteria in an infant's gut is like those in their mother's milk (Chong et al., 2018). It has been observed that there is shared bacterial DNA in mother's milk samples and the infant's fecal samples (Chong et al., 2018). This contrasts with a comparison of a pre-term

infant's feces and an unrelated mother's breast milk where this was not seen (Chong et al., 2018).

It has been observed that certain carbohydrates in human breast milk pass through the stomach and small intestines undigested, which help to promote growth of the gut flora (Gritz & Bhandari, 2015). Full-term breastfed infants have been observed to have a gut flora with a high abundance of predominately *Bifidobacterium* with other strains such as *Staphylococcus, Streptococcus, Lactobacillus* and decreased amounts of *Enterobacteria,* which are bacteria known to cause GI symptoms (Gritz & Bhandari, 2015).

Bifidobacteria are one of the major components of the large intestine's microbiome and are shown to have beneficial effects to the host (Biradar et al., 2004). *Bifidobacteria* have shown to inhibit pathogenic bacteria from growing by creating an acidic environment by its ability to lower the pH level of the large intestine (Biradar et al., 2004). This probiotic does so by producing acetic and lactic acid, increasing intestinal acidity, and thus making it less desirable for the pathogenic bacteria to colonize (Biradar et al., 2004).

Patole et al. (2016) researched whether a probiotic supplement of a *Bifidobacterium* species in pre-term neonates would reduce the incidence of NEC. Findings of the study showed that there was a significant reduction in NEC in neonates given the *Bifidobacterium* species probiotic (Patole et al., 2016). The authors concluded that probiotic supplementation was associated with reduced risk of NEC Bell Stage II or III in neonates born less than 34 weeks gestation (Patole et al., 2016). The authors also

concluded that probiotic supplementation was reduced in neonates less than 28 weeks gestation; however, this result was not statistically significant (Patole et al, 2016).

Microbiota and Infant Formula. Formula fed infants are exposed to different types of carbohydrates, bacteria, and other nutrients than infants fed human milk, which have a different impact on the development of the infant's microbiome (Gritz & Bhandari, 2015). It has been demonstrated that HMOs play a role in the development of the microbiome (Griz & Bhandari, 2015). While oligosaccharides are added to some infant formulas, they are structurally different from the oligosaccharides found in human breast milk (Gritz & Bhandari, 2015). HMOs, which are found in abundance in human milk, are not common in other natural sources (Bode, 2012). Because of this, HMOs are limited in supply (Bode, 2012). Currently, galacto-oligosaccharides and fructo-oligosaccharides, which are not HMOs and not naturally found in human milk, are the replacement for HMOs in infant formula (Bode, 2012). Infants fed with formula have been observed to have a microbiome primarily consisting of *Escherichia coli, Clostridium difficile, Bacteroides, Prevotella,* and *Lactobacillus* (Gritz & Bhandari, 2015).

It has been observed that an infant who is predominately fed with infant formula has a more diverse microbiome compared to an infant who was fed human breast milk (Benno et al., 1984). The infants who were fed predominately formula had higher levels of facultative anaerobes and strict anaerobes, compared to those fed human breast milk who had higher amounts of aerobes, which demonstrates the impact nutrition has on the development of the infant gut flora (Benno et al., 1984). It is currently believed that once solid foods are introduced, the differences in bacterial composition between formula fed and breastfed infants are lost (as cited in Chong et al., 2018).

Influence of Human Milk Oligosaccharides

The discovery of HMOs has a long history starting at the end of the 19th century, during a time when the first-year mortality rate was as high as 30% (Bode, 2012). It was first observed that infants who were breastfed were more likely to survive the first year of life than formula fed infants (as cited in Bode, 2012). In 1886, it was discovered that human breast milk was linked to intestinal mucosa, and then was later found that fecal bacteria differed between breast fed and formula fed infants in 1900 (as cited in Bode, 2012). It has also been observed that human breast milk contained the same type of lactose as bovine milk except with a different carbohydrate portion, which was called gynolactase (as cited in Bode, 2012). A breakthrough in research of HMOs started when the "bifidus factor", or a factor that enhances the growth of *Bifidobacteria*, was theorized to be the gynolactase, and then later confirmed that this "bifidus factor" was indeed the oligosaccharides found in human breast milk (as cited in Bode, 2012). This breakthrough then led scientists to discover different types of these HMOs, of which over 100 different HMOs have now been identified in human breast milk (as cited in Bode, 2012).

HMOs are comprised mostly of glucose, galactose, *N*-acetyl glucosamine, fucose, and sialic acid or fucose (Bering, 2018). HMOs are complex carbohydrates that are highly abundant in human breast milk that serve as a prebiotic for bacteria in the gut to help shape the microbiome (Bering, 2018). A prebiotic is a compound that is fermented in the lower GI tract resulting in changes in the GI microflora that is beneficial to the host (Bode, 2012). For something to serve as a prebiotic, it must be able to withstand the pH of the stomach and avoid digestion through enzymes in the small

intestine (as cited in Bode, 2012). HMOs meet these criteria, as only about 1% are absorbed before making it to the large intestine (Bode, 2012).

When HMOs reach the large intestine, bacteria are attracted to the HMOs and grow on them (Jantscher-Krenn & Bode, 2012). Multiple strains of bacteria feed on HMOs with the *Bifidobacteria* being best known; however, HMOs do not allow for harmful or pathogenic bacteria to grow (Jantscher-Krenn & Bode, 2012). In addition, HMOs further prevent the growth of pathogenic bacteria by mimicking structures of viral receptors and preventing attachment to intestinal cells (Morozov et al., 2018).

While HMOs have been demonstrated to benefit an infant, the composition of HMOs in human breast milk varies depending on genetic factors of the mother, including the secretor status (whether a person secretes or does not secrete antigens into bodily fluids) and the Lewis blood group (blood group classification based on glycoprotein antigens present or not present) characteristics (Bering, 2018). Secretor status varies because of a mutation to the gene for the enzyme fucosyltransferase-2 (FUT2) gene (Underwood et al., 2015). If a mother is homozygous for this mutation, she is unable to produce alpha-1,2 fucosylated glycans (HMOs are a type of glycan) in her secretions, including human breast milk (Underwood et al., 2015). Mothers with non-secretor status are observed to have infants with lower levels of *Bifidobacteria* in their GI tract and are at higher risk for developing certain infections, such as NEC (Underwood et al., 2015). The blood group determines the expression of certain enzymes, which gives rise to one of the four different milk groups: Group 1 (Secretor-Lewis positive [Se+, Le+]) has the highest abundance of HMOs and the most common, Group 2 (Nonsecretor-Lewis positive [Se-, Le+]), Group 3 (Secretor-Lewis negative

[Se+, Le-]), and Group 4 (Nonsecretor-Lewis negative [Se-, Le-]) being lowest in HMOs (Bering, 2018). Lacto-*N*-tetraose (LNT) and Lacto-*N*-neo-tetraose (LNnT) are two specific HMOs that account for over 90% HMOs across all four different groups, with LNT in the highest concentration in milk for pre-term infants (Gabrielli et al., 2011. Further details of the most common HMOs are summarized under **Table 2** (Bering, 2018).

Table 2

HMOs secreted in milk depends on mother's genetic factors

Group	Human Milk Oligosacchardies
1 – Se+,Le+	• Lacto-N-tetraose (LNT) • Lacto-N-neo-tetraose (LNnT) • 2'-Fucosyllactose (2'-FL) • Lacto-N-fucopentaose I (LNFP I) • Lacto-N-difucohexaose (LNDFH)
2 – Se-, Le+	• Lacto-N-tetraose (LNT) • Lacto-N-neo-tetraose (LNnT) • Lacto-N-difucohexaose (LNDFH) • Lacto-N-fucopentaose II (LNFP II) • 3'-Sialyllactose (3'SL)
3 – Se+, Le-	•Lacto-N-tetraose (LNT) • Lacto-N-neo-tetraose (LNnT) • 2'-Fucosyllactose (2'-FL) • Lacto-N-fucopentaose I (LNFP I)
4 – Se-, Le-	•Lacto-N-tetraose (LNT) • Lacto-N-neo-tetraose (LNnT) • Monofucosyllacto-N-hexaose II (MFLNH II) • Lacto-N-fucopentaose III (LNFP III)

Note. Adapted from "Human Milk Oligosaccharides to Prevent Gut Dysfunction and Necrotizing Enterocolitis in Preterm Neonates" by Bering, S. B., 2018, *Nutrients*, *10*(10): 1461. http://doi.org/10.3390/nu10101461

It is important to note that it has been observed that Group 1's pre-term breast milk has an even higher concentration of HMOs than a full-term infant's breast milk,

specifically of LNT, which then decreases in the first month of life (Gabrielli et al., 2011). However, this observation varies among research studies due to lack of standardized methods of measuring HMO concentration from human breast milk (Gabrielli et al., 2011). The authors concluded that HMO variation in pre-term infant breast milk may influence certain biological functions (Gabrielli et al., 2011).

In vitro studies have been conducted on HMOs and their impact on the intestinal microbiota (Bode, 2012). Ward et al. (2007) conducted a study to investigate the fermentation and catabolism of HMOs by five different strains of *Bifidobacterium*. This study suggested that HMOs may serve as a prebiotic for certain strains of bacteria (Ward et al., 2007). Other studies have also observed that certain strains of *Bifidobacterium*, which are beneficial bacteria to the host, have been observed to grow well when HMOs are the only source of carbohydrate to the bacteria (as cited in Bode, 2012). It has also been observed that HMOs act to prevent pathogenic bacteria from adhering to the intestinal lining, preventing colonization of pathogenic bacteria to cause infection (as cited in Bode, 2012). It is believed that HMOs adhere to these pathogens by what is called a lectin-glycan interaction, in which the HMOs and a protein on the pathogen bind together (as cited in Bode, 2012).

Another study compared different variations of HMOs (4-25 mixtures) that were supplemented to pre-term piglets (Rudloff et al., 2019). Pre-term piglets were supplemented 5-10 g/L of the HMOs mixture through formula feedings for five or eleven days (Rudloff et al., 2019). After five days, there were no significant differences between the intervention and control group (Rudloff et al., 2019). After eleven days, the 4-HMO mixture was showing a minor trend in the reduction of NEC in the intervention

group compared to the control indicating early supplementation of HMOs did not affect NEC incidence (Rudloff et al., 2019). The authors believe that a potential reason for these results may be due to the highly variable excretion of HMOs in each pre-term piglet (Rudloff et al., 2019).

Disiallacto-N-tetraose (DSLNT) is an HMO that has been demonstrated in neonatal rat studies to be the most effective HMO in preventing NEC (Jantscher-Krenn et al., 2012). In this study, the neonatal rats who were supplemented with 10 mg/mL of HMOs were shown to have a 95% survival rate of NEC as compared to a 73% survival rate in the neonatal rats who were not supplemented (Jantscher-Krenn et al., 2012). The mechanism as to how these specific HMOs reduce the risk of NEC remains unclear, as rats have a different physiology and mechanisms for gut development than human subjects (Autran et al., 2017). It is also important to note that humans and other mammals have been observed to have higher diversity and complexity of HMOs in their milks when compared to other animals, therefore leading to animal studies to be even more inconclusive.

There is evidence demonstrating that an immature intestinal immune response may be the primary factor causing NEC (Feinberg et al., 2017). A potential mechanism as to why a pre-term infant may have an insufficient intestinal immune response is due to a failure to establish a population of gut flora (Feinberg et al., 2017). In conjunction with increased permeability of the intestines and underdeveloped immune system, this then can lead to pathogens translocating across the mucosa allowing for potential infection, such as NEC (Feinberg et al., 2017). Human breast milk, as discussed, is believed to have beneficial components to help prevent these complications, specifically

HMOs (Wejyrd et al., 2018). HMOs have been observed to prevent pathogenic bacteria from adhering to the intestinal lining by binding and inhibiting the microorganism (Wejyrd et al., 2018). Certain HMOs have also shown to stimulate anti-inflammatory responses in the intestines (Wejyrd et al., 2018). However, research is still needed to further identify HMOs role in preventing against NEC in pre-term infants.

There are currently some randomized controlled trials on human breast milk and NEC; however, the research on HMOs and NEC specifically is limited to cohort studies at this time. Further review of the literature on whether human breast milk reduces the incidence of NEC is discussed in more detail in the following section.

Breast Milk's Association With NEC: Current Research

The following section summarizes the current research on human breast milk and the prevention of and NEC.

Autran et al. (2017) conducted a multi-center clinical prospective cohort study to evaluate if human breast milk fed to infants who develop NEC contains lower amounts of DSLNT than human breast milk fed to infants who do not develop NEC. This study recruited 200 mothers and their very low birth weight infants (VLBW) who met the following criteria: birth weight under 1500 grams (VLBW) and predominately received human breast milk for at least the first 28 days of life (Autran et al., 2017). Exclusion criteria included the following: infant who received infant formula or had a known congenital bowel abnormality (Autran et al., 2017). Each case of NEC was then matched with five controls (those without a NEC diagnosis) based on factors such as location, gestational age, birth weight, mode of delivery, race, ethnicity, and availability of milk samples (Autran et al., 2017).

Results showed that DSLNT concentrations were significantly lower in NEC cases when compared to controls (OR 0.86; $p<0.001$); however, total HMO concentration did not differ significantly between case and control groups (Autran et al., 2017). Limitations include that the quantity of milk each participant consumed was not recorded and selection bias due to this being a case control study (Autran et al., 2017). Strengths of the study included selection bias was minimized by conducting a prospective study, matching five controls to each case of NEC, and confounding factors were minimized (Autran et al., 2017).

Wejyrd et al. (2018) conducted a prospective multi-center randomized-controlled trial to investigate the composition of the 15 dominant HMOs in human breast milk during the neonatal period and examine how this is correlated to NEC in extremely low birth weight (ELBW) infants who were exclusively fed human breast milk. Inclusion criteria for these infants were a birthweight under 1000 grams, gestational age between 23 and 27 weeks, and admitted to one of the two NICUs included in the study (Wejyrd et al., 2018). Exclusion criteria included major congenital or chromosomal abnormalities, low likelihood of survival, the infant could not be fed within three days, or an infant was included in another intervention trial (Wejyrd et al., 2018).

Results showed that HMO composition had greater variability between mothers (R-value 0.7; $p=0.001$) than within the same mother over time (R-value 0.9; $p=0.001$) (Wejyrd et al., 2018). Unlike the results from Autran et al., this study showed that NEC development was associated with low levels of the HMO Lacto-N-difucohexaose (LNDH I) and lower concentrations of all 15 HMOs was not associated with incidence of NEC; however, diversity of all 15 HMOs was lower in samples with NEC cases (Wejyrd et al.,

2018). Limitations of this study included exclusion of infants if they were exclusively fed with infant formula or donor milk, small sample size, and little diversity in the research population (Wejyrd et al., 2018). Strengths of the study include inclusion of ELBW infants (highest risk for NEC) only, prospective design, collection of samples throughout the entire neonatal period, human breast milk sampling standardization, and measurement of the 15 most abundant HMOs (Wejyrd et al., 2018).

Cristofalo et al. (2013) conducted a blind, randomized controlled trial study that analyzed whether infants fed an exclusively human breast milk-based diet have better short-term health outcomes. Inclusion criteria included infants with birthweights 500-1250 grams, whose mother did not intend to provide milk, who received parenteral nutrition within 48 hours of birth, who received enteral feedings before day 21 of life, and who were admitted to one of the seven NICUs included in the study (Cristofalo et al., 2013). Exclusion criteria include infants who had major congenital abnormalities, were transferred to another facility outside of the study, or were participating in another study affecting nutrition management (Cristofalo et al., 2013).

The human milk group was shown to have lower incidence of NEC and NEC-requiring surgery when compared to the infant formula group (Cristofalo et al., 2013). The human milk group had an overall NEC incidence of 11.3%, whereas the infant formula group was 21% (Cristofalo et al., 2013). Limitations of this study include the small sample size and possible confounding variables unaccounted for during the study (Cristofalo et al., 2013). Strengths of this study does include that it is a randomized control trial, investigators were blinded to limit bias, and the study was conducted on ELBW infants who are most at risk for NEC (Cristofalo et al., 2013).

Corpeleijn et al. (2016) conducted a double-blind, parallel randomized clinical trial at six NICU locations. Inclusion criteria for this study included infants who were born less than 1500 grams and whose parents gave written informed consent (Corpeleijn et al., 2016). Exclusion criteria included maternal drug or alcohol abuse during pregnancy, major congenital abnormalities or defects, congenital infection, perinatal asphyxia with umbilical or first neonatal pH less than 7.0, and any cow's milk-based product intake before randomization (Corpeleijn et al., 2016).

Results indicated that when over 50% of total enteral intake is from mother's own milk the VLBW infants have reduced risk of serious infections such as NEC (Corpeleijn et al., 2016). This observation is also supported by Cristofalo et al.'s research in 2013, another randomized control trial, regarding the findings of the mother's own milk. A limitation of this study includes that donor milk was used instead of mother's own milk (which may not have resulted in the same findings) (Corpeleijn et al., 2016). Strengths of this study include that it was a double-blind randomized control trial and subjects were VLBW infants who were at high risk for developing NEC (Corpeleijn et al., 2016).

Feinberg et al. (2017) conducted a single center quality initiative study to analyze whether the early introduction of human breast milk feedings would reduce the rate of NEC by fostering development of a beneficial population of commensal bacteria. Participants included all pre-term infants admitted to their NICU during the length of the study (Feinberg et al., 2017).

Results showed a decreased incidence of NEC from 4.1% at baseline to 0.4% post-intervention (Feinberg et al., 2017). Furthermore, incidence of NEC decreased

from 8.3% in VLBW infants to 1.0% and from 2.4% to 0.2% in non-VLBW infants (Feinberg et al., 2017). These findings are in further support of Cristofalo et al. and Corpeleijn et al. stating that the beneficial properties found in human breast milk help to prevent the incidence of NEC (Feinberg et al., 2017). Limitations of the study included a retrospective design which increases the risk for bias, this research study was conducted at a single site preventing results to be applicable to the entire population, the decline of NEC did not completely align with the timeframe of the post-intervention, and other unmeasured factors may have played a part (Feinberg et al., 2017). The strengths of the study include a dramatic change in the incidence of NEC comparisons were able to be made between the two groups to show an association between human breast milk and prevention of NEC, and a large sample size (Feinberg et al., 2017).

Conclusion

NEC, as explained above, is a devastating disease of the intestines in the pre-term infant. It is crucial that healthcare providers understand NEC and the role human breast milk has in decreasing the risk because this can help shape the clinical practice in the NICU. Even with optimizing care to prevent NEC in the NICU, there are still issues that will arise, such as a mother who cannot or chooses not to provide her own milk. This makes determining the component of human breast milk that reduces NEC risk even more imperative. If future research determines that HMOs are the component of human breast milk preventative against NEC, this could lead to potential supplementation or fortification of infant formulas with HMOs so that even infants who are on pre-term formula can receive adequate amounts of HMOs to reduce their risk.

In summary, current research supports that human breast milk is a preventative measure against NEC. Cristofalo et al.'s (2013) study found that there was a difference in the incidence of NEC and NEC-requiring surgery in pre-term infants receiving human milk group (NEC incidence 11.3%) compared to infants receiving formula (NEC incidence 21%) ($p=0.08$). Similarly, Corpeleijn et al.'s (2016) showed that VLBW infants who were fed a diet over 50% of total enteral intake for the first 10 days of life had a decreased risk of serious infection such as NEC. Feinberg et al., published in 2017, had a significant reduction in their NEC Bell stage II or greater: 4.1% at baseline to 0.4% post-intervention overall ($p<0.001$), 8.3% in at baseline to 1.0% post-intervention in VLBW infants ($p<0.001$), and 2.4% at baseline to 0.2% in non-VLBW infants ($p<0.001$). While these studies are important in supporting human breast milk a preventative measure, they are unable to determine what the component of human breast milk is responsible for the reduced incidence of NEC in pre-term infants.

The studies conducted by Autran et al. in 2017 and Wejyrd et al in 2018 showed evidence that HMOs are one of the components of human breast milk responsible for reduced incidence of NEC; however, these studies differed in what HMOs had the strongest association with the reduction in NEC. The results by Autran et al. (2017) found that total HMO concentration did not differ significantly between the case and control groups, meaning that the differences in total of HMO concentration was not shown to reduce the risk of NEC in pre-term infants. The results of this study did, however, show that lower concentrations of the specific HMO, DSLNT, were associated lower incidence of NEC (Autran et al., 2017). The study conducted by Wejyrd et al. (2018) showed that increased incidence of NEC development was

associated with low concentrations of the HMO LNDH I and did not support DSLNT being associated with the development of NEC, as previously suggested. Wejyrd et al. (2018) also had findings that low diversity of all 15 HMOs were associated with increased risk for NEC, which indicates that the diversity of HMOs in the mother's milk may be more important than one single HMO.

While the research supports that feeding pre-term infants human breast milk is associated with decreased incidence of NEC, it remains unclear if HMOs are the component of human breast milk responsible for preventing NEC. While studies in vitro and on animal subjects are supportive of HMOs preventing NEC, data on humans remains inconclusive. Furthermore, the amount of HMOs or human breast milk needed to prevent NEC from occurring in the pre-term infant is unknown. Further research is needed in the subject to clearly show an association between HMOs and the incidence of NEC, how much human breast milk is needed to decrease the incidence of NEC, and the impact of breastfeeding in certain groups of pre-term infants.

CHAPTER 3: Methodology

There have been several in vitro and animal studies conducted that have shown components of human breast milk have improved survival and reduced the incidence of NEC; however, there is limited data on human subjects (Autran et al, 2017). Of the research available, weight and gestational age of the participants are variable and limit comparability between the studies. Every category of birthweight or gestational age is physiologically different from the other, leaving research on pre-term infants as a general category inappropriate to compare to a specific group such as VLBW infants. There is also insufficient knowledge on the amount of human breast milk required to be beneficial to a VLBW infant. Therefore, the purpose of this retrospective cohort study is to further analyze if the type of feeding decreases the incidence of NEC in VLBW infants.

Research Question & Sub-Problems

This study will aim to answer the following questions: 1) Does type of feeding decrease the incidence of necrotizing enterocolitis in VLBW infants? 2) Will over 50% of calories from human breast milk reduce the incidence of NEC in VLBW infants?

Hypotheses

This study will aim to determine whether there is significant evidence to support the following hypotheses:

H1o: There will be no significant difference between the incidence of NEC in VLBW infant's fed human breast milk compared to VLBW infants fed infant formula.

H1a: There will be a significantly lower incidence of NEC in VLBW infants fed human breast milk compared to VLBW infants fed infant formula.

H2o: The incidence of NEC will not be significantly decreased in VLBW infants who receive over 50% of calories from human breast milk compared to VLBW infants fed 50% or less calories from human breast milk.

H2a: The incidence of NEC will be significantly decreased in VLBW infants who receive over 50% of calories from human breast milk compared to VLBW infants fed 50% or less calories from human breast milk.

Study Design & Objectives

This study will be a retrospective cohort study to investigate whether human breast milk is beneficial to a VLBW infant by decreasing their risk of NEC. The primary objective of this study is to determine if a VLBW infant's risk of NEC is reduced by being fed human breast milk in the first 28 days of life compared to VLBW infants fed infant formula for the first 28 days of life. The secondary objective is to determine if over 50% calories from human breast milk will significantly decrease the incidence of NEC in VLBW infants compared to VLBW infants receiving 50% or less calories from human breast milk. This percentage was determined based on a previous research finding in Corpeleijn et al. (2016); however, there is limited research on this percentage, regardless it was chosen to be comparable to the results of the previous research.

Subjects

Subjects will be VLBW infants admitted to one of the six hospitals in the Los Angeles area participating in the study. Enrollment will occur between October 1, 2020 and September 30, 2021. Inclusion criteria for this study will be any infant with a birthweight under or equal to 1500 grams whose parents give written informed consent (**Appendix B**). Infants must receive enteral nutrition consisting of human breast milk or

infant formula within the first 72 hours of life. Exclusion criteria for this study include any infant with a birthweight over 1500 grams, born with a congenital malformation, transferred to another facility during the period of the study, received probiotic supplementation, or for whom enteral nutrition could not be initiated within the first 72 hours of life. Participants who receive supplemental parenteral nutrition will be included in the study if their enteral intake is able to meet the intake requirements for one of the groups. The goal is to include a minimum of 400 subjects total in the study. A total of 381 subjects was determined to detect significant differences in the primary outcome (Qualtrics, 2019). A 95% confidence interval and 5% margin error were used to determine significant sample size.

Data Collection

Data will be collected through the hospital's electronic medical record (EMR). Information to be gathered includes the type of feeding (i.e. mother's own milk, donor breast milk, or the type of infant formula), amount of feeding, and whether the infant was diagnosed with NEC by day of life 28. Data will be collected retrospectively when the infant has reached day of life 28. Donor milk will not be differentiated from human breast milk nor will type of infant formula be differentiated from each other for this study. Energy each infant receives from human breast milk or infant formula will be calculated for each participant.

The amount of each feeding will be retrieved through the EMR and documented each day for the first 28 days of life. Whether a patient will be receiving human breast milk or infant formula will be retrieved from the EMR. Any changes in enteral feedings

such as increasing volumes of feedings, holding or discontinuing enteral nutrition, or fortification will be determined by the clinical team.

A NEC diagnosis will be determined by the healthcare providers using the Bell Staging Criteria. The EMR will be monitored for a diagnosis of NEC, which an infant is considered to have if they were diagnosed with a Bell Stage II or greater.

Study Protocol

Once data is collected, infants will be categorized into groups to meet the objectives of the study. The study protocol to meet the two objectives are discussed in further detail below.

Study Protocol: First Objective

VLBW infants will first be divided into one of the following groups: NEC cases (infants who were diagnosed with NEC in the first 28 days of life) or No-NEC cases (infants who were not diagnosed with NEC in the first 28 days of life). Each participant will then be labeled as “HBM-Yes” (received any amount of human breast milk in the first 28 days of life) or “HBM-No” (did not receive any amount of human breast milk in the first 28 days of life). The following data will be collected from the EMR for this part of the study: NEC diagnosis and type of nutrition (yes or no response to receiving any amount of human breast milk). The number of infants who were “HBM-Yes” will be compared between NEC cases and No-NEC cases for statistical significance. This part of the analysis will be used to determine if any amount of human breast milk is associated with decreased incidence of NEC.

Study Protocol: Second Objective

For the second part of the study, VLBW infants will be divided into groups depending on the type of feeding received: human breast milk or infant formula. To meet the second objective, the human breast milk group will include VLBW infants who were predominately fed human breast milk, or received over 50% of calories from human breast milk, throughout the first 28 days of life through mother's own milk or donor breast milk. The infant formula group will include those who were fed predominately infant formula, or received over 50% of calories from infant formula, throughout their first 28 days of life. The following data will be collected from the EMR: type of feeding, amount of feeding, calories from each feeding, and NEC diagnosis.

Statistical Analysis

The chi-square test will be used for data analysis. Potential confounding variables include clinical condition of the infant, treatment interference (i.e. surgery or antibiotic treatment), or an NPO diet order for the infant. Amount of formula will be measured in mL in a 24-hour timespan. Calories consumed from each type of feeding will be based off a 28-day average. Energy intake will be measured as kcalories. Descriptive and inferential statistics for this specific study are described in further detail below.

Descriptive Statistics

For descriptive statistics, measures of central tendency will be used. Mode will be used to measure the first objective (number of infants who are categorized as "HBM-Yes" compared to "HBM-No" between NEC cases and No-NEC cases). Mean will be

used to measure the second objective (28-day average of calories consumed from each type of feeding).

Inferential Statistics

For inferential statistics, all data will be extracted from the EMR during timeline of this study, which is provided in further detail in **Table 3**.

Table 3
Data Collection Summary

Variable Name	Variable Name	Variable Source	Breast Milk or Formula	NEC Diagnosis	Level of Measurement
Dependent	Amount of feeding	EMR	Yes	No	Ordinal
Dependent	Calories from each feeding	EMR	Yes	No	Nominal
Independent	NEC diagnosis	EMR	No	Yes	Nominal
Dependent	Type of feeding	EMR	Yes	No	Nominal

Threats to Validity

Threats to validity include variation in infants' clinical condition, an NPO diet order, and treatment interference such as antibiotic treatment or surgical intervention.

Infant's Clinical Condition

An infant can be admitted to the NICU for a variety of reasons; therefore, the infant's clinical condition can be a threat to the validity of this study. While infants will be excluded if they are diagnosed with a congenital malformation, the complexity of each infant's condition is unique and may respond to treatment or enteral nutrition differently.

NPO Diet Order

Oftentimes, patients in a hospitalized setting are made NPO for procedure, bowel rest, etc. As discussed in chapter 2, nutrition plays a major role in the development of an infant's gut flora, and delays in enteral feedings can change the composition (Mugambi et al., 2012). Therefore, an NPO diet order for a participant is a threat to the validity of this study.

Treatment Interference

An infant receiving antibiotic treatment could be a threat to validity due to antibiotics having the potential to alter the composition of the infant's gut flora (Mugambi et al., 2012).

An infant requiring surgery, especially a GI surgery, may also be a threat to validity for a variety of reasons. First, Rao & Patole (2019) discuss that neonates who have GI surgery are at risk for gut dysbiosis due to receiving parenteral nutrition, exposure to antibiotics, and undergoing invasive procedures. Second, an infant may develop a feeding intolerance from the surgery which could potentially alter the infant's gut flora development as well (Mugambi et al., 2012).

Ethical Procedures

This study will be approved by the Institutional Review Board prior to the start of the study (**Appendix A**). This retrospective cohort study will be conducted be retrieving information from the EMR regarding the VLBW infant's feeding and diagnosis of NEC. All information gathered from the EMR are protected under the HIPAA law. In addition, all participants will be given a unique number so no data protected under the HIPAA law needs to be recorded outside of the EMR. Treatments will be determined by the

healthcare providers. Written informed consent will be received from the parents of the VLBW infant prior to the start of the study (**Appendix B**). Participants' parents or guardians have the right to withdraw their infant at any point of the study.

Summary

The methodology to meet the first and second objectives of study are summarized under **Table 4** and **Table 5**.

Table 4
First objective methodology summary

First objective	
Groups	Participants of the study will first be divided into the following groups: ▪ NEC cases ▪ No-NEC cases Each participant will then be categorized as one of the following (if they received any breast milk in the first 28 days of life): ▪ HBM-Yes ▪ HBM-No
Research Question/Sub-Problems	The first objective will aim to meet the following research question: Does type of feeding decrease the incidence of necrotizing enterocolitis in VLBW infants?
Hypothesis	The first objective will aim to meet one of the following hypotheses: ***H1o:*** There will be no significant difference between the incidence of NEC in VLBW infant's fed human breast milk compared to VLBW infants fed infant formula. ***H1a:*** There will be a significantly lower incidence of NEC in VLBW infants fed human breast milk compared to VLBW infants fed infant formula.
Data Collected	To meet the first objective, the following information will be obtained from the EMR: ▪ NEC diagnosis ▪ Received any amount of human breast milk (HBM) – Yes or No
Statistical Analysis	To meet the first objective, chi-square will be used to determine statistical significance.
Comparison	NEC cases will be compared to No-NEC cases based on infants who received any amount of human breast milk (HBM-Yes).

Table 5
Second objective methodology summary

Second Objective	
Groups	Participants of the study will be divided into the following groups: ▪ Human Breast Milk Group ▪ Infant Formula Group
Research Question/Sub-Problems	The second objective will aim to meet the following sub-problem: Will over 50% of calories from human breast milk reduce the incidence of NEC in VLBW infants?
Hypothesis	The second objective will aim to meet the following hypotheses: ***H2o:*** The incidence of NEC will not be significantly decreased in VLBW infants who receive over 50% of calories from human breast milk compared to VLBW infants fed 50% or less calories from human breast milk. ***H2a:*** The incidence of NEC will be significantly decreased in VLBW infants who receive over 50% of calories from human breast milk compared to VLBW infants fed 50% or less calories from human breast milk.
Data Collected (EMR)	To meet the second objective, the following data will be obtained through the EMR: ▪ NEC Diagnosis ▪ Amount of feeding (mL) ▪ Calories from each feeding (kcal) ▪ Type of feeding
Statistical Analysis	To meet the second objective, chi-square will be used to determine statistical significance.
Comparison	NEC cases in breast milk group will be compared to NEC cases in infant formula group.

CHAPTER 4: Anticipated Results

The anticipated results of the study are expected to demonstrate human breast milk as a preventative measure against the incidence of NEC. Specifically, this study will likely observe that VLBW infants who consumed any amount of human breast milk in the first 28 days of life will be significantly higher in those who were not diagnosed with NEC compared to those who were diagnosed with NEC. Furthermore, this study will also likely observe that the higher intake of human breast milk (over 50% of calories from human breast milk), the lower the risk of NEC. Thus, the two objectives of the study will be met.

For the primary objective of the study, VLBW infants who consumed any amount of human breast milk who developed NEC (NEC cases) were compared to VLBW infants who did not develop NEC (No-NEC cases). If an infant received any amount of human breast milk in the first 28 days of life they were categorized as “HBM-Yes” whereas if an infant did not receive any amount of human breast milk in the first 28 days of life they were categorized as “HBM-No”. The results will likely show that VLBW infants who developed NEC will have significantly lower intake of human breast milk when compared to VLBW infants who did not develop NEC after the first 28 days of life ($p<0.05$). Anticipated results of the first objective are further summarized under **Table 6**.

Table 6
Anticipated results of NEC cases compared to no-NEC cases

Participants (n=400)*	HBM-Yes	HBM-No	% HBM-Yes	P-value
NEC Cases (n=30)**	24^	6^	80%^	p<0.05^^
No-NEC Cases (n=370)	329^	41^	89%^	p<0.05^^

**Note*: Anticipated number of participants based on goal of minimum subjects discussed in chapter 3.

***Note*: Anticipated NEC cases based on 7.5% of VLBW infants developing NEC (five to ten percentage of VLBW infants reported to develop NEC) (NORD, 2015).

^*Note*: Predicted results based off findings in study conducted by Feinberg et al. (2017).

^^*Note*: P-value anticipated to be statistically significant.

For the secondary objective, this study will likely observe that VLBW infants who received over 50% of calories from human breast milk will have a significantly lower incidence of NEC when compared to VLBW infants who received 50% or less calories from human breast milk ($p<0.05$). Anticipated results of the second objective are further summarized under **Table 7**.

Table 7

NEC incidence in breast milk group compared to infant formula group

Groups (n=400)*	Average % Breast Milk	Average % Formula	NEC Cases	P-value
Breast milk group (n=207)	68%**	32%**	7^	p<0.05^^
Infant formula group (n=193)	27%**	73%**	23^	P<0.05^^

**Note*: Anticipated number of participants based on goal of minimum subjects discussed in chapter 3.

***Note*: Predicted results based off study conducted by Corpeleijn et al. (2016).

^*Note*: Predicted results based off study conducted by Feinberg et. al (2017).

^^*Note*: P-value anticipated to be statistically significant.

CHAPTER 5: Discussion

While current research indicates that human breast milk is associated with decreased incidence of NEC in pre-term infants, there is still much unknown. Therefore, more research is needed in this field to strengthen the findings of the current research and to identify the unknown, such as how human breast milk impacts specific groups of pre-term infants and how much human breast milk is needed to be consumed to reduce risk of NEC in these specific groups. In addition, future studies need to be conducted specifically on HMOs and the role they potentially play in the prevention of NEC.

Potential Problems

There are a few potential problems regarding this study. First, this population is very high-risk and complex, leading to many VLBW infants being excluded from the study. In addition, there is potential for a high dropout rate. This study could potentially address these problems by being a multi-centered study, which gives more access to potential candidates and allowing for more participants to be included in the study.

Another potential problem regarding this study is that healthcare providers and the participants' family make the decision as to how the patient is fed, how much they are fed, and whether they are to receive infant formula or human breast milk. While it is important that the participant receives adequate and appropriate nutrition, this leads to a high variation of human breast milk and infant formula consumption amongst the participants for both first and second objective groups. For the second objective, while participants may be in the same group (receiving predominately human breast milk or predominately infant formula), an infant could be receiving 100% of their nutrition

through human breast milk whereas another infant could only be receiving 60% of their nutrition from human breast milk, leaving consumption to be highly variable. For the first objective, this also holds true due to groups being measured as “any human breast milk intake” compared to “no human breast milk intake”. An infant who receives human breast milk for one out of the first 28 days of life will be categorized under “HBM-Yes”, which an infant receiving human breast milk every day for the first 28 days of life would also be under the same category. In the future, it may be advisable to further break down groups based on percentage of the participant’s intake.

Another potential problem of the study is that data would be collected based on information collected through the EMR; therefore, there is potential for documentation to be inaccurate due to human error. It may be advisable that prior to starting this study healthcare workers are educated on proper documentation and reinforce the importance of accuracy for this study.

Another potential problem of this study would be that both donor breast milk and mother’s own breast milk are included under the same group. Donor breast milk has many of the same beneficial properties as mother’s own milk; however, donor breast milk goes through sanitation and storage that mother’s own milk does not, leading samples to have potential differences in protection against NEC. While donor breast milk is recognized as the next best alternative to mother’s own milk, the pasteurization process does impact the beneficial properties of the milk (Martin et al., 2016). Martin et al. (2016) explain that the pasteurization process reduces the number of beneficial microorganisms, live immune cells, bioactive proteins, and enzymes, that are known to benefit an infant. Regarding HMOs, a study conducted by Marx et al. in 2013 showed

that total concentrations of HMOs in donor breast milk were significantly lower than the mother's own milk concentrations; however, HMOs were still present in the milk. In addition, all types of infant formula were grouped together into one category. However, no known infant formula contain HMOs found in human breast milk and no harmful component of infant formula has been identified to alter the results of this study.

Lastly, results could be confounded by the following variables: clinical condition of the infant, treatment interference (i.e. antibiotic treatment or surgical intervention), or an NPO diet order for the infant. As discussed in chapter 2, Mugambi et al. (2012) discussed the importance of nutrition for the pre-term infant, as it plays a major role in the development of an infant's gut flora, and delays in enteral nutrition can change the microbiome composition. Thus, an infant made NPO or enteral nutrition is delayed, this could be a confounding variable to the study. This confounding variable can be slightly minimized from excluding any infants who are unable to be fed enterally within the first 72 hours of life. Regarding an infant's clinical condition, this has potential to be a confounding variable due to each infant responding differently to treatment in the NICU. Furlong-Dillard et al. (2018) explained that infants who require surgical intervention, diagnosed with heart failure, diagnosed with a genetic disorder, have complex neuroendocrine responses, or born with a gastrointestinal disorder are at increased risk for developing a feeding intolerance which may lead to delay or discontinuation of enteral feedings. Regarding surgical intervention, it has been reported that 50% of infants who require surgical intervention due to NEC and survive are likely to develop an intestinal stricture or short-gut syndrome, which would put these infants at a disadvantage for receiving enteral nutrition compared to infants who did not develop

one of these conditions (NCATS, 2013). In terms of antibiotic treatment, Mugambi et al. (2012) explains how pre-term infants who spend time in the NICU develop a different gut flora partly due to many infants receiving antibiotic treatment (NORD, 2015).

Clinical Application

The anticipated results of this study will likely demonstrate the need for optimization of enteral nutrition with a strong recommendation of human breast milk in the NICU setting. From the research conducted and this current study, human breast milk will likely provide as a preventative measure against NEC in VLBW infants. Therefore, it is strongly recommended that VLBW infants receive human breast milk for the first 28 days of life at least. Education should also be provided to the family of the pre-term infant so they can make decisions regarding the type of feeding the infant should receive.

Education

Educating the family of the pre-term infant on the benefits of human breast milk can result in significant outcomes. The quality initiative study findings conducted by Feinberg et al. (2017) indicated that process changes, one of which including the improvement mechanisms to encourage and support breastfeeding in the NICU, to have significant reductions in NEC incidence post-intervention.

Research findings have also shown that healthcare professionals have limited knowledge and skills regarding breastfeeding and providing human breast milk in a NICU setting; therefore, education should be provided for both healthcare professionals and the pre-term infant's family (Bernaix et al., 2008).

Educating Healthcare Staff. There has been research supporting that a NICU-specific lactation education program is effective in improving the knowledge and attitudes of NICU nurses regarding breastfeeding (Bernaix et al., 2008). Outcomes of this study found that after nurses attended a 4-hour educational program on lactation, human breast milk feeding rates in the NICU improved and maintained over time (Bernaix et al., 2008). Therefore, lactation education programs should be provided to nurses and other healthcare members working with women and infants to obtain adequate knowledge to educate the families in the NICU setting.

The United States Breastfeeding Committee (USBC) also developed breastfeeding and lactation competencies that are applicable for all healthcare providers caring for women and infants (2010). These core competencies were developed to provide healthcare professionals with evidence-based guidelines that can be integrated into standardized practices (USBC, 2010). Integrating these core competencies with current practices in the hospital setting could help improve healthcare providers' knowledge on human breast milk in the NICU setting for the families. These competencies are listed and discussed further under **Appendix C** (USBC, 2010).

Educating the Family. It has been observed that mothers may be discouraged from providing human breast milk to their infant due to conflicting information they receive from healthcare providers (Meier et al., 2010). The first way to perform appropriate education for the family is to standardize the advice given from the staff (Meier et al., 2010). Through the development of policies and procedures to guide education, it addresses human breast milk feedings as a NICU therapy and limits healthcare professionals providing information based off personal advice (Meier et al.,

2010). As a NICU therapy with a developed policy and procedure, this would provide the knowledge needed for healthcare workers to give appropriate education to the infant's family and decrease contradicting information (Meier et al., 2010).

It is also imperative that a healthcare professional who is competent on the subject educates the family (Meier et al., 2010). While educational programs as discussed above may help, employing lactation specialists in the NICU to educate mothers may also benefit families by helping them to become more comfortable with providing human breast milk to their infant (Meier et al., 2010). Lactation specialists can assume the position of teaching a mother how to use a breast pump, how to appropriately clean equipment, how to appropriately store and transport human breast milk, and more (Meier et al, 2010).

Future Studies

Future studies should focus on whether HMOs are the component of human breast milk that prevents against NEC in pre-term infants. While in vitro and animal studies are supportive of HMOs and their beneficial properties in preventing NEC, studies on human subjects are currently lacking. Research conducted by Autran et al. (2017) and Wejyrd et al. (2018) have contradicting results and raise further questions on the benefits of HMOs in terms of NEC. Studies should focus on dose-response of total HMO concentrations to determine the strength of the association with NEC in VLBW infants. In addition, specific HMOs need to be identified to determine which have the greatest impact on preventing NEC in pre-term infants.

As previously mentioned, infant formula does not contain the naturally occurring HMOs found in human breast milk, but some may contain man-made HMOs similar in

structure. While human breast milk is the ideal form of nutrition, it may not be readily available to all pre-term infants in the NICU regardless of incentive to increase infants receiving human breast milk. Therefore, companies manufacturing pre-term infant formula should work towards a formula that mimics the HMO composition of human breast milk.

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APPENDIX A: Institutional Review Board Application

Office use only: IRB Approval #: _____

Mount Mary University

Institutional Review Board (IRB)

for the Protection of Human Subjects

Application for IRB Review

DATA COLLECTION CANNOT BEGIN

UNTIL THE IRB HAS APPROVED THIS PROJECT

Directions:

- Faculty and student researchers, as well as student research advisors, should **read all relevant information on the University IRB page in My Mount Mary before initiating an application**. This includes full knowledge of the US Department of Health and Human Services Code of Federal Regulations Title 45 (Public Welfare), Part 46 (Protection of Human Subjects). http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.html
- All applicants must verify completion of Human Subjects Training. See http://www.citiprogram.org
- The IRB application must be filed and approved by the IRB **prior** to any Mount Mary University faculty, staff, or student (undergraduate or graduate), initiating a research project/study.
- If there is a cooperating institution, attach a copy of their IRB approval.
- In the case of a student research project, the student may complete the IRB application but the student's research advisor must sign and submit the application to the IRB for approval. It is the responsibility of the faculty research advisor to ensure that student applications and all attachments (e.g., informed consent forms and survey instruments) are in their final edited form. Even though a student research project may qualify as **exempt** from full IRB review, the research advisor may request the student to complete and submit a full IRB application.
- Complete this application using your word processing program (ex. Word), then print it out and obtain signatures from all investigators and advisors. (**Handwritten applications will not be accepted**.) For your benefit, save the completed application on your computer in case it needs to be revised and resubmitted.

- This is a professional document; please check spelling, grammar and punctuation.
- Submit an electronic copy, via email, of the completed application with required signatures and attachments, **in a single pdf**, to Tammy Scheidegger, IRB Chair, scheidet@mtmary.edu . You will receive an email verifying receipt of the application from the IRB Board Chair.
- Allow a **minimum of 30 working days** to process your application. Make sure this timeframe is accounted for when considering initiation of data collection and due dates for student projects. Please be aware that if, upon completion of the application, you find that ***no exemptions apply to your research, your application will need to go through a full IRB Committee review which can take as many as 60 days to be completed.***
- For class projects you must submit IRB applications to the IRB Chair by October 31st of the fall semester and March 31st for the spring semester. For summer classes, please consult with the IRB Chair.
- Upon receipt of the IRB letter of approval, data collection may begin.

I. Required Documentation - No action will be taken without these attachments.

Are the following attached to the IRB application?

Informed Consent Document	☒ Yes	Informed Consent Documents should include an explanation of procedures, risk, safeguards, freedom to withdraw, confidentiality, offer to answer inquiries, third party referral for concerns, signature and date. See Appendix.A and use the **MMU Informed Consent Template** to avoid delays in the process.
Questionnaire/Survey Instrument(s)	☐ Yes	If a survey is being administered in any written format (e.g., survey monkey, qualtrics), a copy of that survey must accompany this application. If a survey is being conducted verbally, a copy of the introductory comments and survey questions being asked must be attached to this application. If survey includes focus group questions, a complete list of the question must be attached. For research using a published/purchased instrument, a photocopy of the instrument will suffice.

Verification of Human Subjects Training	☒ Yes	Copy of transcript, certificate or other evidence that ALL members of the research team have completed the required training. .
Copy of cooperating institution's IRB approval.	☒ Yes	Not required if there is no cooperating institution. .

II. Investigator(s):

Name: Anne Schmidt Phone: 724-549-5341

Affiliation with Mount Mary University (e.g. faculty, student, etc.): Graduate Student

Email: schmidta@mtmary.edu

Signature: _____ Date: 03/10/2020

Name: Phone:

Affiliation with Mount Mary University:

Email:

Signature: _____ Date:

If student, list Research Advisor and complete the application. Research Advisor must provide requested information and verify.

	Department: Dietetics
Research Advisor's Name: Dr. Dana Scheunemann	
Email: scheuned@mtmary.edu	Phone: 414-930-3658
Research Advisor: Have you completed Human Subject's Training?	☒ Yes ☐ No
Research advisor's signature indicates responsibility for student compliance with all IRB requirements.	
Signature: _____ Research Advisor	Date:

III. Project Description – Required by all applicants

Instructions: Briefly describe the proposed project including the sample and methodology (e.g. human subjects, data collection, data analysis and instruments).

1) Objectives (purpose of project):

This study will be a retrospective cohort study to investigate whether human breast milk is beneficial to a very low birthweight infant by decreasing their risk of Necrotizing Enterocolitis (NEC). The primary objective of this study is to determine if a VLBW infant's risk of NEC is reduced by being fed predominately human breast milk in the first 28 days of life. The secondary objective of this study is to determine if over 50% intake of human milk is associated with risk of NEC.

2) Relevance to practice/body of knowledge:

The potential significance of this study could support the need of optimizing practice regarding enteral feedings of human breast milk for VLBW infants admitted into the NICU via mother's own milk or donor breast milk. With a optimizing practice resulting in more infants receiving breast milk within the first few days of life, this could result in decreased incidence of NEC, decrease mortality in the NICU, and prevent medical complications and unneeded surgeries.

3) Describe the research design (e.g. subject/participant selection and assignment, design, intervention, data analysis):

This study will be a retrospective cohort study to investigate whether human breast milk is beneficial to a VLBW infant by decreasing their risk of NEC. Participants of the study will be recruited through VLBW infants admitted to one of six hospitals in the Los Angeles area participating in the study. VLBW infants included in the study will be divided into the following groups to meet objectives of the study. Data will be collected through the hospital's Electronic Medical Record (EMR) during a 1-year period. Information to be gathered include the VLBW infant's type of feeding, amount of feeding, calories received from the feeding, and whether the infant was diagnosed with NEC. The EMR will also be monitored for a diagnosis of NEC, which an infant was considered to have if they were diagnosed with a Bell Stage II or greater. The chi-square test will be used for statistical analysis.

4) What measurement/data collection tools are being used?

Data will be collected through the hospital's Electronic Medical Record (EMR) during a 1-year period. Information to be gathered include the VLBW infant's type of feeding, amount of feeding, calories received from each feeding, and whether the infant was diagnosed with NEC. The EMR will also be monitored for a diagnosis of NEC, which an infant was considered to have if they were diagnosed with a Bell Stage II or greater.

IV. Additional Project Information – Required by all applicants

1) What human subjects training has the researcher completed (e.g. course work, online certification)?

CITI program – Research Ethics and Compliance Training

2) What process is used for obtaining informed consent (attach the informed consent application)? See Appendix for consent application.

Participants' parent of the study will be given an informed consent for signature prior to the patient's participation of the study.

3) Does the research include special populations?

Minors under 18 years of age?	☒ Yes	☐ No
Persons legally incompetent?	☐ Yes	☒ No

Prisoners?	☐ Yes	☒ No
Pregnant women, if affected by research?	☐ Yes	☒ No
Persons institutionalized?	☐ Yes	☒ No
Persons mentally incapacitated?	☐ Yes	☒ No

4) If **YES,** describe additional precautions included in the research procedures.

This study includes VLBW infants. All information extracted from the EMR will be protected under the HIPAA law. No part of this study will directly influence the care of the VLBW infants included.

5) Does the research involve any of the following procedures?

False or misleading information to subjects?	☐ Yes	☒ No
Withholds information such that their informed consent might be questioned?	☐ Yes	☒ No
Uses procedures designed to modify the thinking, attitudes, feelings, or other aspects of the behavior of the subjects?	☐ Yes	☒ No

6) If **YES**, describe the rationale for using procedures, how the human subjects will be protected and what debriefing procedures are used.

7) Does the research involve measurement in any of the following areas?

Sexual behaviors?	☐ Yes	☒ No
Drug use?	☐ Yes	☒ No
Illegal conduct?	☐ Yes	☒ No
Use of alcohol?	☐ Yes	☒ No

8) If **YES**, describe additional precautions included in the research procedures.

9) Are any portions of the research being conducted online?

Survey posted on a website?	☐ Yes	☒ No	If yes, assure anonymity
URL for survey includes information that could identify participants?	☐ Yes	☒ No	If yes, assure anonymity
Invitation to participate sent by email?	☐ Yes	☒ No	If yes, assure anonymity
Items use drop-down box?	☐ Yes	☒ No	If yes, assure that items allow choice of "no response"

10) If **YES**, describe additional procedures.

11) Describe the methods used to ensure confidentiality of data obtained.

Each participant of the study will be given an identification number. Data obtained from the EMR will be documented with this identification number. Patient's information protected under the HIPAA law will be honored.

Risks and Benefits

1) Describe risks to the subjects and the precautions that will be taken to minimize them. (Risk includes any potential or actual physical risk of discomfort, harassment, invasion of privacy, risk of physical activity, risk to dignity and self-respect, and psychological, emotional or behavioral risk.)

No risks to the participants have been identified in this study.

2) Describe the benefits to subjects and/or society. (These will be balanced against risk.)

This study could potentially benefit the subjects by reduction of NEC in the NICU.

V. Is the proposed project "research" as defined by Institutional Review Board requirements? - Required by all applicants

- Research is defined as a systematic investigation, including research development, testing and evaluation, designed to develop or contribute to generalizable knowledge.
- A human subject is defined as a living individual about whom an investigator obtains either 1) data through intervention or interaction with the individual; or 2) identifiable private information.

Does the research involve human subjects or official records about human subjects?

☒ Yes

☐ No

***If NO STOP here, and SUBMIT application*.**

If the results will be available in the library, presented at a professional conference (includes any presentation to group(s) outside of the classroom), or published, please check the Yes box:

☒ Yes

☐ No

If the YES box is CHECKED, proceed to SECTION VI.

***If the NO box is CHECKED, STOP here, and SUBMIT application*.**

VI. Exemptions - Required by all applicants

Are you requesting exemption from IRB review in one of the federally approved categories?

If yes, please reference OHRP website http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.html and continue with application.

1) Does the research meet the criteria for exempt category 1 (education)? [45 CFR 46.101 (b) (1)]

Is the research conducted in established or commonly accepted educational settings (e.g. schools, Universities or other sites where educational activities regularly occur)?

☐ Yes

☒ No

☐ Yes

Does the research study involve only normal education practices (e.g. instructional strategies, techniques, curricula, or classroom management techniques)?	☒ No

*If **both** questions are answered **"yes"**, stop here, and **submit** application.*

2) Does the research meet the criteria for exempt category 2 (specific procedures)? [45 CFR 46.101 (b) (2)]

Does the research involve only the use of educational tests, survey procedures, interview procedures or observation of public behavior?	☐ Yes ☒ No
Is the information obtained recorded in such a manner that human subjects cannot be identified directly or through identifiers linked to the subjects? (See Appendix B)	☒ Yes ☐ No

*If **both** questions are answered **"yes"**, stop here, and **submit** application.*

3) Does the research meet the criteria for exempt category 3 (public officials)? [45 CFR 46.101 (b) (3)]

Does the research involve only the use of educational tests, survey procedures, interview procedures or observation of public behavior?	☐ Yes ☒ No
Are the human subjects elected or appointed public officials or candidates for public office? **If no, proceed to Category 4.**	☐ Yes ☒ No
Does any federal statute require without exception that the confidentiality of the personally identifiable information will be maintained throughout the research and thereafter? (See Appendix B)	☐ Yes ☐ No

*If **all** questions are answered **"yes"**, stop here, and **submit** application.*

4) Does the research meet the criteria for exempt category 4 (existing data/specimens)? [45 CFR 46.101 (b) (4)]

Does the research involve only the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens?

☒ Yes
☐ No

☒ Yes

Will the information be recorded by the investigator in such a manner that the subjects cannot be identified directly or through identifiers linked to the subjects? (See Appendix B)

☐ No

*If **both** questions are answered **"yes",** stop here, and **submit** application.*

5) Does the research meet the criteria for exempt category 5 (federal program research)? [45 CFR 46.101 (b) (5)]

Does the research involve studying, evaluating or examining federal public benefit or service programs?

☐ Yes
☐ No

Is the research conducted through a federal agency?

☐ Yes
☐ No

*If **both** questions are answered **"yes"**, stop here, and **submit** application.*

6) Does the research meet the criteria for exempt category 6 (taste and food quality)?

[45 CFR 46.101 (b) (6)]

Does the research involve a taste and food quality evaluation or consumer acceptance study?

☐ Yes
☐ No

Does the food consumed contain no additives, or a limited amount of food additives at or below a level approved by the FDA or EPA or the Food Safety and Inspection Service of the U.S. Department of Agriculture?

☐ Yes
☐ No

*If **both** questions are answered **"yes"**, stop here, **submit** application.*

If no exemptions apply, your application will need to go through a full IRB Committee review. Be advised that this process can take as many as 60 days to be completed.

APPENDIX B: Information and Consent Form

Research Participant Information and Consent Form

Mount Mary University

Title of Study: A Retrospective Cohort Study to Analyze if Type of Feeding Decreases the Incidence Necrotizing Enterocolitis in Very Low Birthweight Infants

Invitation to Participate and Purpose of the Research

You are invited to participate in a research study that seeks to investigate whether human breast milk is beneficial to a very low birthweight infant (birthweight <1500 grams) by decreasing the occurrence of a disease called Necrotizing Enterocolitis (NEC). Participants must be infants admitted to the NICU born with a birthweight <1500 grams.

Information will be obtained through the participant's electronic medical chart for the first 28 days of life. Information that will be obtained include type of feeding, amount of feeding, and whether the infant was diagnosed with NEC.

Benefits and Risks

There are no known risks to this study. All data will be collected through the electronic medical record. All interventions will be determined on the clinical team.

This study is designed to potentially benefit infants who are admitted to a NICU. Findings of this study will help to determine how human breast milk could potentially prevent Necrotizing Enterocolitis from occurring.

Confidentiality

All information obtained will be kept confidential by the researchers who will be the only people with access to the data. Information obtained will be stored electronically and will be password protected. Paper files will be shredded, and electronic files will be deleted. Individual participants will not be identified in any report or publication about this study.

Contact Information

If you have any questions or concerns about this study, please contact Anne Schmidt of Mount Mary University at 724.549.5341 or email schmidta@mtmary.edu. You may also contact the Institutional Review Board of Mount Mary University at 414.930.3434 or email schediet@mtmary.edu.

Consent

By signing below, you are indicating that you have read this consent form, have been given the opportunity to ask questions, and have agreed to voluntarily participate. You may withdraw from participation at any time, or refuse to answer any question herein, without penalty or loss of benefits to which other participants are entitled.

You may request a copy of this page for your records. Thank you for your participation.

Signature of participant_____ Date _____

APPENDIX C: USBC Core Competencies

Core Competencies in Breastfeeding Care and Services for All Health Professionals

Revised Edition

About USBC

The United States Breastfeeding Committee (USBC) is an independent nonprofit coalition of more than 40 nationally influential professional, educational, and governmental organizations. Representing over half a million concerned professionals and the families they serve, USBC and its member organizations share a common mission to improve the Nation's health by working collaboratively to protect, promote, and support breastfeeding. For more information on USBC, visit ***www.usbreastfeeding.org***.

Background

Breastfeeding is a basic and cost-effective measure that has a significant positive impact on short- and long-term health outcomes for individuals and populations.[1] The greatest health impact is found with early initiation, exclusive breastfeeding for the first six months of life, and continued breastfeeding with appropriate complementary foods for the first year of life and beyond.[2] Lack of breastfeeding is a significant risk to the public health of our nation and increases health care spending.[3]

In order to establish and maintain breastfeeding, women need education and support from a knowledgeable health care community.[4] Evidence-based knowledge, skills, and attitudes are lacking among health professionals in many disciplines.[5] The volume of new information, advances in treatments and technologies, and health care system challenges, combined with the relative paucity of professional training in human lactation and breastfeeding, leave many providers without satisfactory answers for their patients.[6,7]

Purpose

These core competencies in breastfeeding care and services were developed to provide health professionals with a guideline and framework to integrate evidence-based breastfeeding knowledge, skills, and attitudes into their standard health care delivery practices.

The United States Breastfeeding Committee recommends that *all* health professionals possess the core competencies identified in this document in order to integrate breastfeeding care effectively and responsibly into current practice and thus provide effective and comprehensive services to mothers, children, and families.

Effecting Change

Educators are in a unique position to lead the way by incorporating these core competencies into the undergraduate, graduate, and post-graduate curricula of health professionals.[8 9 10 11] These core competencies provide a structure for educators to respond to the emerging necessity of educating all health care providers regarding breastfeeding and human lactation in the context of findings from the World Health Organization (WHO)[12] and the Agency for Healthcare Research and Quality (AHRQ).[13]

Maternal and child health (MCH) education and practice is based upon a life cycle framework that recognizes that there are pivotal periods in human development that present both risks and opportunities for improving health outcomes for individuals and populations.[14] In particular, USBC calls upon MCH leaders to emphasize the synergistic value of these breastfeeding core competences to the health of women, children, and families.

Breastfeeding Core Competencies

Competence in the following areas represents the ***minimal*** knowledge, skills, and attitudes necessary for health professionals from ***all*** disciplines to provide patient care that protects, promotes, and supports breastfeeding.

At a minimum, every health professional should understand the role of lactation, human milk, and breastfeeding in:

- The optimal feeding of infants and young children[2 15]
- Enhancing health and reducing:
  - long-term morbidities in infants and young children[2 15]
  - morbidities in women[15 16]

All health professionals should be able to facilitate the breastfeeding care process by:

- Preparing families for realistic expectations
- Communicating pertinent information to the lactation care team[17]
- Following up with the family, when appropriate, in a culturally competent manner after breastfeeding care and services have been provided[18]

USBC proposes to accomplish this by recommending that health professional organizations:

- Understand and act upon the importance of protecting, promoting, and supporting breastfeeding as a public health priority[2 3 16 19 20]
- Educate their practitioners to:
  - appreciate the limitations of their breastfeeding care expertise[17 21]
  - know when and how to make a referral to a lactation care professional[17 21]
- Regularly examine the care practices of their practitioners and establish core competencies related to breastfeeding care and services[20 22]

Knowledge

All health professionals should understand the:

1.1 basic anatomy and physiology of the breast[8, 23]
1.2 role of breastfeeding and human milk in maintaining health and preventing disease[2, 15]
1.3 importance of exclusive breastfeeding, and its correlation with optimal health outcomes[15, 24]
1.4 impact of pregnancy, birth, and other health care practices on breastfeeding outcomes[19, 25]
1.5 role of behavioral, cultural, social, and environmental factors in infant feeding decisions and practices[26, 27]
1.6 potentially adverse outcomes for infants and mothers who do not breastfeed[28]
1.7 potential problems associated with the use of human milk substitutes[29]
1.8 few evidence-based contraindications to breastfeeding[30, 31]
1.9 indications for referral to lactation services[17]
1.10 resources available to assist mothers seeking breastfeeding and lactation information or services[30, 32]
1.11 effects of marketing of human milk substitutes on the decision to breastfeed and the duration of breastfeeding[1, 33, 34]

Skills

All health professionals should be able to:

2.1 practice in a manner that protects, promotes, and supports breastfeeding[2, 3, 16, 22]
2.2 gather breastfeeding history information sufficient to identify mothers and families who would benefit from specific breastfeeding support services[35]
2.3 seek assistance from and refer to appropriate lactation specialists[22, 24]
2.4 safeguard privacy and confidentiality[36]
2.5 effectively use new information technologies to obtain current evidence-based information about breastfeeding and human lactation[22, 37]

Attitudes

All health professionals should:

3.1 value breastfeeding as an important health promotion and disease prevention strategy[20, 28]
3.2 recognize and respect philosophical, cultural, and ethical perspectives influencing the use and delivery of breastfeeding care and services[18, 22]
3.3 respect the confidential nature of the provision of breastfeeding care and services[36]
3.4 recognize the importance of delivering breastfeeding care and services that are free of commercial conflict of interest or personal bias[22, 23, 34]
3.5 understand the importance of tailoring information and services to the family's culture, knowledge, and language level[18, 39]
3.6 seek coordination and collaboration with interdisciplinary teams of health professionals[17]
3.7 recognize the limitations of their own lactation knowledge and breastfeeding expertise[17]
3.8 recognize when personal values and biases may affect or interfere with breastfeeding care and services provided to families[8]
3.9 encourage workplace support for breastfeeding[40]
3.10 support breastfeeding colleagues[41, 42, 43]
3.11 support family-centered policies at federal, state, and local levels[9]

All health professionals do not need to have the level of competence expected of those practitioners who care for childbearing women, infants, and young children. Health professionals who care for childbearing women, infants, and young children can be further divided into two groups:

1. Those that provide **primary care** are front-line practitioners who care for women of childbearing age and/or infants and young children.
2. Those that provide **secondary care** may be front-line practitioners or practitioners with enhanced knowledge and skills specifically referable to the use of human milk and breastfeeding.

Those health professionals who provide primary and secondary care for childbearing women, infants, and young children should be able to:

4.1 understand the evidence-based *Ten Steps to Successful Breastfeeding*[25, 44]
4.2 obtain an appropriate breastfeeding history[45]
4.3 provide mothers with evidence-based breastfeeding information[24]
4.4 use effective counseling skills[18]
4.5 offer strategies to address problems and concerns in order to maintain breastfeeding[24, 46]
4.6 know how and when to integrate technology and equipment to support breastfeeding[26]
4.7 collaborate and/or refer for complex breastfeeding situations[47]
4.8 provide and encourage use of culturally appropriate education materials[33]
4.9 share evidence-based knowledge and clinical skills with other health professionals[25, 48]
4.10 preserve breastfeeding under adverse conditions[24, 49]

In addition, those health professionals who provide secondary or more direct "hands-on" care for childbearing women, infants, and young children should also be able to:

5.1 assist in early initiation of breastfeeding[50]
5.2 assess the lactating breast[51]
5.3 perform an infant feeding observation[37, 51]
5.4 recognize normal and abnormal infant feeding patterns[51, 52]
5.5 develop and appropriately communicate a breastfeeding care plan[51, 52]

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