Ksenia Zukowsky, PhD, APRN, NNP-BC ○ Section Editor

CE 2.5 HOURS

Necrotizing Enterocolitis

Have We Made Any Progress in Reducing the Risk?

Leslie A. Parker, PhD, NNP-BC

ABSTRACT

Necrotizing enterocolitis (NEC) is a disease affecting premature infants with potentially devastating complications in the neonatal intensive care unit (NICU). Inadequate knowledge regarding the underlying pathophysiology of this disease has contributed to the minimal progress made in decreasing the incidence and severity of NEC. Because of an improved survival rate of the most immature infants, the number of diagnosed cases of NEC is anticipated to increase. Unfortunately, decades of research have failed to reduce the risk or improve the prognosis of NEC, magnifying the need for risk-reduction strategies for decreasing significant NEC-related morbidity and mortality. Advancements in our understanding of this disease process have facilitated the development of several strategies that have been investigated in the risk reduction of NEC. This article discusses the pathophysiology and causes of NEC and presents strategies investigated regarding risk reduction of this devastating disease.

Key Words: NEC, necrotizing enterocolitis, premature infant, prevention

Necrotizing enterocolitis (NEC), a potentially devastating condition occurring predominately in premature infants, affects 7% to 14% of very low-birth-weight (VLBW) infants. The overall mortality rate is 20% to 40%, depending up disease severity and need for surgical intervention, and NEC is associated with complications, including intestinal strictures, short bowel syndrome, and increased risk of neurodevelopmental delay.[1-3] Unfortunately, the number of diagnosed cases of NEC has not decreased with advancements in neonatal care; in fact, it is projected to increase because of improved survival rate of the most premature infants, thereby creating a need for preventive strategies. This article discusses the causes and pathophysiology of NEC and provides information regarding progress in our knowledge of potential strategies to reduce the risk of NEC in premature infants (Table 1).

Author Affiliation: College of Nursing, University of Florida, Gainesville.

The author declares no conflict of interest.

Correspondence: Leslie A. Parker, PhD, NNP-BC, College of Nursing, University of Florida, Box 100187, Gainesville, FL 32610 (parkela@ufl.edu).

DOI: 10.1097/ANC.0b013e31829a872c

PATHOPHYSIOLOGY

Necrotizing enterocolitis predominantly occurs in premature infants especially those weighing less than 1000 g at birth and born prior to 28 weeks' gestation and is characterized by hemorrhagic, ischemic, and often necrotic intestines.[4] Symptoms of NEC include abdominal distension, emesis, lethargy, apnea, and bradycardia and bloody stools. Laboratory assessment may reveal neutrophilia or neutropenia, anemia, electrolyte imbalances including hyponatremia, metabolic acidosis, glucose instability, elevated c-reactive protein level, and thrombocytopenia. If it is severe, NEC can lead to respiratory failure and cardiovascular instability.

A staging system for NEC developed by Bell and colleagues[5] in 1978 and later revised can be used to determine the severity of the disease and guide treatment. Stage I includes nonspecific abdominal symptoms and systemic signs of infection. Stage II pertains to a more advanced disease with continued nonspecific symptoms combined with radiographic findings including pneumatosis intestinalis. Stage III is the most severe stage and is associated with intestinal perforation, peritonitis, and increased mortality.[5]

Radiologic manifestations of NEC include pneumatosis intestinalis, portal venous gas, and pneumoperitoneum. Pneumatosis intestinalis is the presence

TABLE 1. Evidence for Preventive Strategies for Necrotizing Enterocolitis (NEC)

Preventive Strategy	Author and Study Type	Sample Size, Sample Characteristics, Intervention	Salient Findings and Comments
Human milk	Sisk et al[33] Prospective cohort study	202 VLBW infants, grouped according to proportion of human milk consumed during the first 14 d of life.	≥50% breast milk associated with a lower risk of NEC (10.6% vs 3.2%); $P = .01$
Human milk	Furman et al[35] Prospective cohort study	119 VLBW infants grouped according to portion of human milk consumed during first 4 weeks of life.	≥50% breast milk was not associated with a lower risk of NEC. The incidence was decreased but the sample size was not large enough to detect a significant difference.
Human milk	Meinzen-Derr et al[34] Retrospective cohort	1272 infants weighing < 1000 g. Determined whether increased human milk intake during first 14 days of life was associated with decreased NEC.	Every 10% increase in breast milk intake decreased the risk of NEC or death by a factor of 0.83 (95% CI, 0.72-0.96).
Donor human milk	Sullivan et al[39] Prospective controlled trial	207 VLBW infants randomized to receive either breast milk fortified with human milk fortifier with donor milk if no breast milk available or breast milk fortified with bovine fortifier with preterm formula provided if no breast milk available.	Infants fed an exclusively breast milk–based diet had a significantly decrease rate of NEC ($P = .02$).
Donor human milk	McGuire[36] Systematic review	4 randomized controlled trials involving 343 LBW infants. Compared the use of donor milk vs formula.	No individual trial found a difference in the incidence of NEC. The meta-analysis found a borderline statistically significant decrease in NEC in those infants fed donor breast milk. All 4 studies were over 20 years old. Three of the studies were included in all 3 of the systematic reviews.
Donor human milk	Schanler et al[38] Randomized controlled trial	253 VLBW infants. Infants whose mothers intended to breastfeed were randomized to receive either formula or donor milk if mothers' own milk was unavailable.	No difference in NEC between infants who were supplemented with formula or donor breast milk. Both groups received only donor milk or formula when volume of mothers' own milk was insufficient ($P = .27$).
Probiotics	Alfaleh et al[44] Systematic review	16 randomized controlled studies involving 2842 LBW infants. Determined the effect of probiotics on the incidence of NEC.	Probiotics significantly reduced the incidence of severe NEC (RR 0.35, 95% CI: 0.24-0.52). Several different types of probiotics administered and few studies discussed whether human milk or formula was used.

(*continues*)

TABLE 1. Evidence for Preventive Strategies for Necrotizing Enterocolitis (NEC) (*Continued*)

Preventive Strategy	Author and Study Type	Sample Size, Sample Characteristics, Intervention	Salient Findings and Comments
Probiotics	Deshpande et al[45] Systematic review	11 randomized controlled studies involving 2176 LBW infants. Determined the effect of probiotics on the incidence of NEC.	Probiotics significantly reduced the incidence of NEC (RR 0.35, 95% CI: 0.23-0.55). Several different types of probiotics administered and few studies discussed whether human milk or formula was used.
Standardized feeding regimens	Patole and de Klerk[49] Systematic review	6 observational studies that compared rate of NEC before and after implementation of a standardized feeding regimen.	Incidence of NEC was decreased by 87%. When only VLBW infants were included in the analysis, the effect on NEC was reduced (RR 0.57; 95% CI: 0.31-1.06; $P = .08$). Four of the studies were over 10 years old.
Standardized feeding regimens	McCallie et al[50] Retrospective cohort	147 VLBW infants. Compared rate of NEC before and after implementation of a standardized feeding regimen.	Incidence of NEC was decreased from 18% to 3% ($P = .005$).
Oral immuno globulins	Foster and Cole[25] Systematic review	3 randomized controlled trials involving 2095 LBW infants. Determined impact of prophylactic administration of oral immunoglobulins on incidence of NEC.	Oral immunoglobulins did not significantly decrease the incidence of NEC (RR 0.84; 95% CI: 0.57-1.25).
Oral antibiotics	Bury and Tudehope[26] Systematic review	5 randomized or quasi-randomized controlled trials involving 456 LBW or premature infants. Determined effect of administration of enteral antibiotics of incidence of NEC.	Decreased NEC in infants who received enteral antibiotics (RR 0.27, 95% CI: 0.28-0.78) and NEC related deaths (RR 0.32, 95% CI: 0.10-0.96). Four of the 5 studies were over 30 years old.
Polyunsaturated fatty acids	Smithers et al[28] Systematic review	6 randomized controlled trials involving 1496 LBW infants. Determined effect of feeding formula supplemented with polyunsaturated fatty acids on incidence of NEC.	No difference in incidence of NEC in infants who received polyunsaturated fatty acids (RR 1.13, 95% CI: 0.62-2.04). Only 1 study had NEC as a primary outcome. Because the polyunsaturated fatty acids were added to formula, feedings were required for the intervention.
Arginine	Amin et al[31] Randomized controlled trial	152 VLBW infants. Compared infants who received arginine supplementation vs placebo on incidence of NEC.	Infants who received arginine supplementation had a decreased incidence of NEC (6.7% vs 27.3%). A 27.3% incidence of NEC in the control group is unusually high.

Abbreviations: CI, confidence interval; LBW, low birth weight; RR, relative risk; VLBW, very low birth weight.

of gas within the bowel wall and is produced by gas-forming bacteria in the intestinal lumen. Portal venous gas, a poorly understood phenomenon, is thought to occur when intestinal gas dissects into the portal venous system from the intestinal lumen and appears as linear branching vessels in the portal system. A pneumoperitoneum is present when air escapes from perforated intestines and enters the peritoneum.[6]

Treatment of NEC involves withholding feeds, gastric decompression, broad spectrum antibiotic therapy, and monitoring for intestinal perforation through serial abdominal radiographs. Additional treatment depends on disease severity and may include fluid resuscitation, correction of metabolic acidosis, treatment of thrombocytopenia, and cardiovascular/respiratory support. Surgery is required in 20% to 60% of cases and is generally indicated in infants with intestinal perforation and in those with continued clinical deterioration.[7]

RISK FACTORS

Although the causes of NEC is poorly understood, it appears multifactorial and due to gastrointestinal and immune system immaturity, which is strongly associated with prematurity. Gastrointestinal immaturity impairs intestinal motility and decreases nutritional absorption.[8] Impaired intestinal motility prevents movement of substrate through the intestine, resulting in stasis and possible bacterial overgrowth.[9] Impaired nutritional absorption may result in carbohydrate fermentation with subsequent gaseous intestinal distension and damage to the fragile intestinal epithelium as well as bacterial invasion of the intestinal wall.[10]

Deficient antibacterial properties and altered immune cellular production limits the premature infant's defense against infections, including NEC.[11] Moreover, Secretory IgA, which is normally present in the intestinal mucosa and functions to protect the intestines from bacterial invasion, is deficient in premature infants.[12]

Other reported risk factors for NEC include pregnancy-induced hypertension, and intrauterine growth retardation, use of formula feedings, maternal chorioamnionitis, maternal smoking, and the use of systemic antibiotic therapy.[13-15] Although no causal effect has been established, blood transfusions may also be associated with an increased risk of NEC 48 to 72 hours following transfusion.[16]

CAUSES

Events theorized to contribute to the development of NEC include intestinal mucosal injury, intestinal mucosal inflammation, and pathogenic bacterial colonization of the intestinal tract.[17]

Intestinal Injury

Various events, including hypoxia, mild ischemia, intestinal circulatory regulation immaturity, intestinal inflammation, and bacterial infection, can cause initial injury to the intestinal mucosa.[18] Epithelial injury can result in an abnormally increased intestinal inflammatory response in premature infants, further injuring the fragile intestinal mucosa.[19]

Inflammation of the Intestinal Mucosa

Intestinal mucosal inflammation with subsequent activation of the inflammatory cascade can occur because of various factors including intestinal ischemia or injury, enteral feedings, and/or bacterial invasion.[20] Bacterial *translocation*, the passage of pathogenic bacteria across the intestinal barrier into the bowel wall, is common in premature infants; it leads to activation of the inflammatory cascade and the release of chemical mediators, which further damages intestinal epithelial cells and causes necrosis and impairment of intestinal integrity.[11,21]

Intestinal Colonization by Pathologic Bacteria

Normal colonization of the intestinal tract occurs during the first 12 to 24 hours in term healthy infants following exposure to an environment typically containing multiple organisms. When an infant is born prematurely, normal colonization of the intestinal tract is disrupted through administration of antibiotics, delay of enteral feedings, isolation from normal bacteria by housing in incubators, and exposure to abnormal and antibiotic-resistant organisms.[22] Feeding type may also influence colonization of the intestinal tract, and infants receiving breast milk are more likely to be colonized with more beneficial bacteria than formula-fed infants.[23] These beneficial intestinal bacteria help prevent colonization with pathogenic bacteria through competition for nutrition and acidification of the intestinal environment. Pathogenic bacterial colonization of the intestinal tract may result in inflammatory cascade activation with subsequent intestinal damage. Furthermore, colonization with beneficial bacteria helps protect against intestinal injury through prevention of bacterial translocation and promotion of a protective intestinal barrier and maintenance of intestinal integrity.[11]

Unfortunately, despite decades of research, little progress has been made toward prevention of NEC or improvement in prognosis, thereby magnifying the need for risk-reducing strategies.[24]

INVESTIGATED STRATEGIES FOR RISK REDUCTION OF NEC

Several strategies to reduce the risk of NEC have been investigated, which initially appeared promising but, on further examination, have not proved

effective. Prophylactic oral administration of immunoglobulins (IgA and IgG) was reported in individual studies to be associated with a reduced risk of NEC because of their immunoprotective effect on the intestinal mucosa. Unfortunately, these benefits were not supported in a recent systematic review.[25] Although 2 of the studies included in this review used a combination of both IgG and IgA, a preparation of only IgA may be more effective in neonatal gastrointestinal tract protection, but to date, this method has not been investigated.

Another strategy that has been used is oral administration of antibiotics, which has been shown in a systematic review to decrease the risk of NEC through reduction of intestinal pathogenic bacteria, thereby limiting bacterial intestinal wall invasion and decreasing the potential for bacterial translocation.[26] However, 4 of the included studies were conducted in the 1970s prior to the use of antenatal steroids, surfactant, and other modern advances in neonatology, possibly influencing the relevance of these studies to current neonatal care. In addition, only 1 study documented type of feeding, and because feeding type has been shown to influence the incidence of NEC, this lack of clarity may have influenced the reported outcome. Significant concerns regarding administration of oral antibiotics to premature infants, including promotion of resistant organisms and possible systemic antibiotic absorption, have virtually eliminated their use in this population.

Yet another method involves using formula fortified with long-chain polyunsaturated fatty acids (PUFAs), which are inflammatory modulators that may prevent activation of the inflammatory cascade and subsequent intestinal injury.[27] Although individual studies have indicated a decreased incidence of NEC in infants fed formula fortified with PUFA, a systematic review disputed these findings and found no difference in incidence of NEC.[28] In addition, a large, randomized, controlled trial found an increased incidence of NEC in infants who received supplemental PUFA although the difference was not statistically significant.[29]

Finally, because decreased arginine levels have been reported in infants with diagnosed with NEC,[30] it has been surmised that supplementation with L-arginine may have beneficial effects. Arginine is an amino acid necessary for the production of nitric oxide and acts to maintain mucosal integrity, intestinal barrier function, and reduce inflammation. Although a single study found an overall decreased incidence of NEC in infants who received supplementation with L-arginine, no statistically significant difference was found in the incidence of stage II NEC[31]; thus, additional research is necessary before recommending the use of L-arginine supplementation for the prevention of NEC.

PREVENTIVE STRATEGIES SHOWN TO POSSIBLY REDUCE RISK

Human Milk

The protective effects of human milk on the intestinal tract are numerous and include (1) prevention of bacterial attachment to the host mucosa by oligosaccharides, (2) improved intestinal barrier protection, (3) prevention or mediation of inflammatory processes, and (4) provision of beneficial gastrointestinal bacteria, thus preventing pathologic bacterial invasion.[32] Several studies have reported a decreased incidence and severity of NEC in premature infants fed human milk.[32,33] Protection appears dose-dependent with those infants receiving the greatest proportion of breast milk demonstrating the lowest incidence of NEC.[34] In contrast, Furman et al[35] did not find a difference in incidence of NEC when infants were fed 50% or more breast milk although the sample size was small and might not have detected a significant difference between groups.[35]

Donor Human Milk

Donor human milk is commonly provided to premature infants when their mother's own milk is unavailable. Two systematic reviews both reported a decreased incidence of NEC in low-birth-weight infants fed donor milk compared with those fed formula. However, individual studies did not find a difference in the incidence of NEC and the included studies were all over 20 years old, making the relevance of these results questionable in this modern era of neonatology.[36,37]

Schanler et al,[38] in a randomized controlled study of 243 VLBW infants, found no difference in NEC between formula-fed infants and donor human milk–fed infants. However, Sullivan et al,[39] in a randomized trial, found that infants fed an exclusively human milk–based diet of either mothers' own milk or donor milk had a 50% decreased rate of NEC and 90% less surgical NEC.[39] The apparent decreased protective effect of donor milk may be related to donation of milk by mothers of older infants and the need for pasteurization, which may decrease bioactive components including antioxidant properties, T cells, B cells, macrophages, neutrophils, IgA and IgG, lactoferrin.[40]

Probiotics

Probiotics are live nonpathogenic bacteria that colonize the intestinal tract and exert a positive effect on an individual's health and well-being.[41] Probiotics may limit the number of pathogenic bacteria through competition for binding sites and nutrition, production of an acidic environment, and fortification of the intestinal mucosal barrier.[42] Less pathogenic bacteria in the intestinal lumen may reduce the detrimental effects of bacterial translocation,

improve intestinal immune regulation, produce antimicrobial agents and immunoglobulins, and stimulate anti-inflammatory cytokines. Probiotics may also enhance intestinal mobility and maturation, alleviate intestinal inflammation, and produce specific protective agents such as arginine, glutamine, and short-chain fatty acids.[43]

Two meta-analyses report that administration of probiotics to premature infants reduces the incidence and severity of NEC.[44,45] These studies, however, were not homogenous; in fact, nearly every study used a different combination or strain of probiotics. In addition, a recent systematic review suggests fundamental differences in methodology between studies makes combining results for meta-analysis nearly impossible, and indicates there is insufficient evidence to support the routine use of probiotics in this population.[46] Although many believe that sufficient evidence exists to support routine use of probiotics in premature infants, additional research is necessary to determine optimal dose, frequency of dose, most appropriate strain for use, and length of treatment. Additional studies to determine the risk of complications are also advocated. Despite evidence supporting the safety of probiotics in premature infants, the potential for sepsis exists when administering bacteria to immunocompromised patients, and sepsis in compromised infants and children receiving probiotic therapy has been reported.[47,48] The lack of quality-controlled, reliable products is also a concern.

Standardized Feeding Regimens

Standardized feeding regimens consist of a written set of orders with specific instructions regarding type of feeding, volume to be fed, and when fortifiers are to be added. A meta-analysis of 6 observational studies reported a 29% reduction in NEC in those infants fed according to a standardized feeding regimen.[49] These findings were supported by a recent retrospective analysis finding a decrease in NEC from 18% to 3% following initiation of a standardized feeding regimen in 147 VLBW infants.[50] Feeding regimens used by the studies differed, suggesting that the introduction of a feeding regimen may be more important than the specifics of a particular regimen. Introduction of a standardized feeding regimen may impact the risk of NEC through decreased practice variation, increased awareness and possible treatment of early NEC, and the use of evidence-based feeding interventions. Future randomized controlled trials are required to further clarify the effectiveness of this strategy.

NURSING IMPLICATIONS

Because NEC is a major source of mortality and morbidity in the VLBW infant and is a common occurrence in premature infants, it is important for neonatal nurses to understand the pathophysiology, causes, presentation, and treatment of this devastating disease. Neonatal nurses are often closely involved in the care of infants with NEC, so it is critical that they acquire knowledge regarding strategies that may potentially decrease the risk of NEC in these patients (Table 2).

TABLE 2. Suggested Clinical Implications

Strategies that may decrease the incidence of necrotizing enterocolitis
Human milk
Standardized feeding regimens
Fluid restriction
Strategies that appear effective but need additional research
Donor human milk
Probiotics
Arginine
Strategies that do not appear effective
Immunoglobulins
Polyunsaturated fatty acids

Furthermore, because human milk has been shown to decrease both the incidence and severity of NEC in premature infants, it is essential that these vulnerable infants receive as much breast milk as possible. Provision of breast milk, especially colostrum, immediately following delivery may be beneficial for physiologic gastrointestinal development because of its similarity to amniotic fluid.[51] Therefore, nurses need to encourage milk expression as early as possible following delivery to optimize the rate of mothers' own milk being provided as soon after delivery as possible.[52] Nurses play a key role in this capacity: they assist mothers in their decision to provide human milk to their infants, help facilitate an optimal milk supply through assistance with early milk expression, and provide education on milk expression through provision of lactation support. Nursing involvement is also necessary in the formation and implementation of standardized feeding regimens in the NICU. Because nurses are generally the healthcare providers who are charged with provision of enteral nutrition, their input is critical during the initial protocol inception, implementation, and evaluation of standardized feeding regimens in their individual units. Finally, nurses should understand the rationale for use, mechanism of action, and potential

complications of risk-reducing strategies utilized in their NICU.

CONCLUSION

Without question, the most significant risk factor for the development of NEC is prematurity. Because a reduction in premature birth is not expected to occur in the near future, risk-reducing measures are needed to decrease the incidence of this devastating disease. Although identification and minimization of potential side effects of any preventive strategy are imperative, future research is necessary to potentially decrease the risk of NEC in this vulnerable population.

References

1. Lin P, Stoll B. Necrotizing enterocolitis. *Lancet*. 2006;368:1271-1283.
2. Soraisham AS, Amin HJ, Al-Hindi MY, Singhai N, Sauve RS. Does necrotizing enterocolitis impact the neurodevelopmental and growth outcomes in preterm infants with birthweight </+1250 g? *J Paediatr Child H*. 2006;42(9):499.
3. Pike K, Anabrees J, Bassler D, Al-Kharfi T. Outcomes at 7 years for babies who developed neonatal necrotizing enterocolitis: the ORACLE Children Study. *Arch Dis Child Fetal Neonatal Ed*. 2012. http://www.ncbi.nlm.nih.gov/pubmed/22267398. Accessed March, 2012.
4. Holman RC, Stoll BJ, Curns AT, Yorita KL, Steiner CA, Schonberger LB. Necrotizing enterocolitis hospitalizations among neonates in the United States. *Paediatr Perinat Ep*. 2006;20(6):498-506.
5. Bell MJ, Ternber JL, Feigin RD, et al. Neonatal necrotizing enterocolitis: therapeutic decisions based on clinical staging. *Ann Surg*. 1978;187:1-5.
6. Epelman M, Daneman A, Navarro OM, et al. Necrotizing enterocolitis: review of state-of-the-art imaging findings with pathologic correlation. *Radiographics*. 2007;27(2):285-305.
7. Sharma R, Hudak ML, Tepas JJ, et al. Impact of gestational age on the clinical presentation and surgical outcome of necrotizing enterocolitis. *J Perinat*. 2006;26:342-347.
8. Boston VE. Necrotizing enterocolitis and localized intestinal perforation: different diseases or ends of a spectrum of pathology. *Pediatr Surg Int*. 2006;22:477-484.
9. Neu J. Gastrointestinal development and meeting the nutritional needs of premature infants. *Am J Clin Nutr*. 2007;85(suppl):629S-634S.
10. Kliegman R. The relationship of neonatal feeding practices and the pathogenesis and prevention of NEC. *Pediatrics*. 2003;117:S52-S58.
11. Emani CN, Petrosyan M, Giuliani S, et al. Role of the host defense system and intestinal microbial flora in the pathogenesis of necrotizing enterocolitis. *Surg Infect*. 2009;10(5):407-417.
12. Gibbs K, Lin J, Holzman IR. Necrotising enterocolitis: the state of the science. *Indian J Pediatr*. 2007;74(1):67-72.
13. Been JV, Lievense S, Zimmermann LJ, Kramer BW, Wolfs TG. Chorioamniotitis as a risk factor for necrotizing enterocolitis: a systemic review and meta-analysis. *J Pediatr*. 2012. http://www.ncbi.nlm.nih.gov/pubmed/22920508. Accessed October 21, 2012.
14. Cetinkaya M, Ozkan H, Koksal N. Maternal preeclampia is associated with increased risk of necrotizing enterocolitis in preterm infants. *Early Hum Dev*. 2012;88(11):893.
15. Downard CD, Grant SN, Maki AC, et al. Maternal cigarette smoking and the development of necrotizing enterocolitis. *Pediatrics*. 2012;130(1):78-82.
16. Demirel G, Celik IH, Aksoy HT, et al. Transfusion-associated necrotizing enterocolitis in very low birth weight premature infants. *Transfus Med*. 2012;22(5):332-337.
17. Schmolzer G, Urlesberger B, Haim M, et al. Multi-modal approach to prophylaxis of necrotizing enterocolitis: clinical report and review of literature. *Pediatr Surg Int*. 2006;22:573-580.
18. Hsueh W, Caplan M, Qu X, Tan X, De Plaen L, Gonzalez-Crussi F. Neonatal NEC: clinical considerations and pathogenetic concepts. *Pediatr Dev Pathop*. 2002;6:6-23.
19. Lin PW, Nasr TR, Stoll BJ. Necrotizing enterocolitis in premature infants pathophysiology and prevention. *Semin Perinatol*. 2008;32(2):70.
20. Nanthakumar NN, Fusunyan RD, Sanderson I, Walker WA. Inflammation in the developing human intestine: a possible pathophysiologic contribution to necrotizing enterocolitis. *Proc Natl Acad Sc USA*. 2000;97:6043–6048.
21. Annand RJ, Leaphart CL, Mollen KP, Hackam DJ. The role of the intestinal barrier in the pathogenesis of NEC. *Shock*. 2007;27:124-133.
22. Caicedo R, Schanler R, Li N, Neu J. The developing intestinal ecosystem: implications for the neonate. *Pediatr Res*. 2005;58:625-629.
23. Fanaro S, Chierici R, Guerrine P, Virvi V. Intestinal microflora in early infancy: composition and development. *Acta Paediatr Suppl*. 2003;91(441):58-55.
24. Neu J, Walker WA. Necrotizing enterocolitis. *New Engl J Med*. 2011;364:255-264.
25. Foster J, Cole M. Oral immunoglobulin for preventing necrotizing enterocolitis in preterm and low birth-weight neonate. *Cochrane Database Syst Rev*. 2011;(1):CD001816.
26. Bury RG, Tudehope D. Enteral antibiotics for preventing necrotizing enterocolitis in low birthweight or preterm infants. *Cochrane Database Syst Rev*. 2001;(1):CD000405.
27. Uauy R, Mena P, Rojas C. Essential fatty acids in early life: structural and functional role. *P N Soc*. 2000;59(1):3-15.
28. Smithers LG, Gibson RA, McPhee A, Makrides M. Effect of long-chain polyunsaturated fatty acid supplementation of preterm infants on disease risk and neurodevelopment: a systematic review of randomized controlled trials. *Am J Clin Nutr*. 2008;87(4):912.
29. Fewtrell M, Morley R, Abbott RA, et al. Double-blind, randomized trial of long-chain polyunsaturated fatty acid supplementation in formula fed to preterm infants. *Pediatrics*. 2002;110(1 pt 1):73-82.
30. Richter D, Bartmann P, Pohlandt F. Prevention of necrotizing enterocolitis in extremely low birth weight infants by IgG feeding? *Eur J Pediatr*. 1998;157(11):924-925.
31. Amin H, Zamora S, McMillan D, et al. Arginine supplementation prevents necrotizing enterocolitis in the premature infant. *J Pediatr*. 2002;140:425-431.
32. Dvork B, Halpern MD, Holubec H, et al. Maternal milk reduces severity of NEC and increases intestinal IL-10 in a neonatal rat model. *Pediatr Res*. 2003;53(3):426-433.
33. Sisk PM, Lovelady CA, Dillard RG, Gruber KJ, O'Shea TM. Early human milk feeding is associated with a lower risk of necrotizing enterocolitis in very low birth weight infants. *J Perinat*. 2007;27(7):428.
34. Meinzen-Derr J, Poindexter B, Wrage L, Morrow A, Stoll B, Donovan E. Role of human milk in extremely low birth weight infants' risk of NEC or death. *J Perinat*. 2009;29:57-62.
35. Furman L, Taylor G, Minch N, Hack M. The effect of maternal milk on neonatal morbidity of very low birth weight infants. *Arch Pediatr Adolesc Med*. 2003;157(1):55-71.
36. McGuire W, Anthony W. Donor milk versus formula for preventing necrotizing enterocolitis in preterm infants: a systematic review. *Arch Dis Child Fetal Neonatal Ed*. 2003;88(1):F11-F14.
37. Quigley MA, Henderson G, Anthony MY, McGuire W. Formula milk versus donor breast milk for feeding preterm or low birth weight infants. *Cochrane Data Syst Rev*. 2007;17(4):CD002971tm.
38. Schanler RJ, Lau C, Hurst NM, Smith EO. Randomized trial of donor human milk versus preterm formula as substitutes for mothers' own milk in the feeding of extremely premature infants. *Pediatrics*. 2005;116(2):400-406.
39. Sullivan S, Schanler RJ, Kim JH, et al. An exclusively human milk–based diet is associated with a lower rate of necrotizing enterocolitis than a diet of human milk and bovine milk–based products. *J Pediatr*. 2010;156(4):652-657.
40. Silvestre D, Miranda M, Muriach M, Almansa I, Janeno E, Romero FJ. Antioxidant capacity of human milk: effect of thermal conditions for the pasteurization. *Acta Paediatr*. 2008;97(8):1070-1074.
41. Walker WA, Goulet O, Morelli L, Antoine JM. Progress in the science of probiotics: from cellular microbiology and applied immunology to clinical nutrition. *Eur J Nutr*. 2006;45(suppl 1):1-18.
42. Mattar AF, Drongowski RA, Coran AG, Harmon CM. Effect of probiotics on enterocyte bacterial translocation in vitro. *Pediatr Surg Int*. 2001;17:265-268.
43. Schanler RJ. Probiotics and necrotizing enterocolitis in premature infants. *Arch Dis Child*. 2006;91:F395-F397.
44. Alfaleh K, Anabrees J, Bassler D, Al-Kharfi T. Probiotics for prevention of necrotizing enterocolitis in preterm infants. *Cochrane Database Syst Rev*. 2011;(3):CD005496.
45. Deshpande G, Rao S, Patole S, Bulsara M. Updated meta-analysis of probiotics for preventing necrotizing enterocolitis in preterm neonates. *Pediatrics*. 2010;125(5):921-930.
46. Mihatsch WA, Braegger CP, Desci T, et al. Critical systematic review of the level of evidence for routine use of probiotics for reduction of

mortality and prevention of necrotizing enterocolitis and sepsis in preterm infants. *Clin Nutr*. 2012;31:6-15.
47. De Groote MA, Frank DN, Dowell E, Glode MP, Pace NR. *Lactobacillus rhamnosus* GG bacteremia associated with probiotic use in a child with short gut syndrome. *Pediatr Infect Dis J*. 2005;24:278-280.
48. Land MH, Rouster-Stevens K, Woods CR, Cannon ML, Cnota I, Shetty AK. *Lactobacillus* sepsis associated with probiotic therapy. *Pediatrics*. 2005;115:178-181.
49. Patole SK, de Klerk N. Impact of standardized feeding protocols on incidence of necrotizing enterocolitis—a systematic review and meta analysis. *Arch Dis Child Fetal Neonatal Ed*. 2005;90:F147-F151.
50. McCallie KR, Lee HC, Mayer O, Cohen RS, Hintz SR, Rhine WD. Improved outcomes with a standardized feeding protocol. *J Perinatol*. 2011;31:S61-S67.
51. Cilieborg MS, Boye M, Thymann T, Jensen BB, Sangild PT. Diet-dependent effects of minimal enteral nutrition on intestinal function and necrotizing enterocolitis in preterm pigs. *JPEN J Parenter Enteral Nutr*. 2011;35(1):32-42.
52. Parker LA, Sullivan S, Krueger C, Kelechi T, Mueller M. Effect of early breast milk expression on milk volume and timing of lactogenesis stage II among mothers of very low birth weight infants. *J Perinatol*. 2012;32:205-209