

December 18, 2007

Thank you for the thoughtful review of the manuscript entitled *Clinical Predictors of Necrotizing Enterocolitis in Premature Infants*. I appreciate the reviewer's comments, particularly their enthusiasm for the study and clinical significance of the findings. Below is a point-by-point response to the comments of the reviewers, as required for resubmission to *Nursing Research*. All revisions have been noted in bold font in the text of the revised manuscript. All revisions required by the Checklist for Style have been made accordingly.

REVIEWER #1

Reviewer Comments:
Literature Review: References are appropriate, most recent cited is 2003. Consider searching for more recent literature

Literature Review:

- Literature review has been updated with more recent references, including:
 - Bakewell-Sachs, S. & Blackburn, S. (2003). State of the Science: Achievements and Challenges across the spectrum of care for preterm infants. *JOGNN*, 32, 683-695.
 - Hallstrom, M. et al. (2006). Laboratory parameters predictive of developing necrotizing enterocolitis in infants born before 33 weeks of gestation. *Journal of Pediatric Surgery*, 41, 792-798.
 - Lin, H.C., Su, B.H., Chen, A.C., Lin, T.W., Tsai, C.H., Yeh, T.F. & Oh, W. (2005). Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. *Pediatrics*, 115 (1), 1-4.
 - Hintz, S.R, et al. (2005). Neurodevelopmental and growth outcomes of extremely low birth weight infants after necrotizing enterocolitis. *Pediatrics*, 115 (3), 696-703.
 - Moss, R.L., et al. (2006). Laparotomy versus peritoneal drainage for necrotizing enterocolitis and perforation. *NEJM*, 354, 21 2225-2234.
 - Neu, J. (2007). Gastrointestinal development and meeting the nutritional needs of premature infants. *American Journal of Clinical Nutrition*, 85(supp), 629S-634S.
 - Pietz, J., Achanti, B., Lilien, L., Stepka, E., & Mehta, S. (2007). Prevention of Necrotizing Enterocolitis in Preterm Infants: A 20-year Experience. *Pediatrics*, 119, e164-e170.
- Outdated literature has been omitted from the manuscript so that no more than 40 references are included as required for publication in *Nursing Research*.

Reviewer Comments:
Data Analysis:
Pg 12, line 15 - clarify the 28 variables used for the Bonferroni correction; from Tables 1 and 2 it is difficult to pick out 28, depending on how you classified them there are either more or less but it is not clear what 28 were used (e.g. in Table 2 p values were reported for 27 variables)

Pg 12, lines 22-23 and Pg 13, lines 2-4 - although all preterm infants were the sample, there does not seem to be a logical reason why some info was reported and some not (e.g. 92.7% of the total sample was born before 32 weeks - but info on age of those who developed NEC was not reported in text. Or, one would not expect that subjects who did not have NEC would have surgery for the condition, why report surgical management of NEC by percentage of the total sample?); while it is best not to repeat data reported in the tables it would be more pertinent in text to report percentages of those who developed NEC from the total sample since this is the concept of interest.

Data Analysis:

- The 28 variables used for the Bonferroni correction have been clarified. These variables are numbered 1-28 in Table 2. The text refers the reader to this table for clarification of this point.
- Reported information on the sample has been revised as follows:
 - Average age, birth weight, gender, race and ethnicity of infants in the NEC sample has been reported
 - Percentage of subjects who required surgery for NEC has been reported from the NEC sample only.
 - See page 13, lines 6-10.

Specific comments:

Pg 8, lines 14-19 - this paragraph does not fit under the review of the literature; move discussion to the Methods section where you then are presenting your current study, Table 1 presents the points you discuss in this paragraph so can condense the discussion, eliminating much of the elaboration.

In your discussion under Methods please speak to the classification of race - why were there only 2 groups - White, and all other?

Provide legend for abbreviations in Tables.

Make it clear that it is lack of change to fortification of feeding that is the risk factor.

Specific Comments:

- Page 8, lines 14-19 have been condensed and moved to page 15, lines 22-23 – page 16, lines 1-2.
- Race and ethnicity was classified in two groups, white non-Hispanic and all others, to provide an adequate sample size in each group for analysis purposes. This data was initially collected in 7 groups, in accordance with the NIH classification of race and ethnicity for research subjects. This data was collapsed after initial analysis indicated that the sample was overwhelming white, non-Hispanic in race, and the number of subjects in the 6 other groups were less than 30% of the total sample. This issue has been clarified in the text on page 12, lines 10-13.
- A legend for abbreviations has been provided for Tables 1 and 2.
- Lack of change to fortification of feeding has been made clear as the risk factor. See Page 17, lines 5-7 and Page 18, lines 10-12.

REVIEWER #2

Reviewer Comments:

1). The purpose of the paper is stated inconsistently. On page 5, line3-4, and page 6, line 17, the purpose of the study is stated as being examination of the relationship between variables related to intestinal inflammation on the incidence of NEC, when in fact the study examines the relationship between specific clinical events that may result in decreased mesenteric blood flow and intestinal inflammation and the development of NEC. You do state this clearly on pg, 8, line9-11, however this is too late. On page 13, line18-20, you suggest that increase in respiratory support and increase in perfusion support 'represent' inflammation & ischemia-however, these changes in physiological stability represent a wide spectrum of disease processes. Along this line, on page 5, line 6, it is stated that "The study proposes." while in fact I think you mean the study "assumes".

Comment #1

- The purpose of the study has been clarified as the examination of the relationship between specific clinical events that may result in decreased mesenteric blood flow and intestinal

inflammation leading to the development of NEC. Please see the following text: page 5, lines 6-7, and page 6, line 22- page 7, line 1.

- Page 14, lines 5-7, has been revised to read: “Increase in respiratory support and increase in perfusion support required to maintain hemodynamic perfusion of the neonate, both physiologic stability factors **that may play a role in** inflammation and ischemia, were entered in the first block.”
- Page 5, line 9 has been revised to read: “The study **assumes** that hypoxia, hypotension and hypothermia, as well as decreased superior mesenteric artery flow during the early neonatal period decrease blood flow to the premature intestine causing the bowel to be at especially high risk for ischemic injury.”

Reviewer Comments:

2). There are some sections (page 5, lines 6-16; 8, lines 14-19) that need citations.

Comment #2

Citations included as follows:

- Page 5, lines 6-16 (revised lines 9-13): Crissinger, 1999, Neu, 2007
- Page 8, lines 14-19: the majority of text condensed and moved to Methods section, per Reviewer #1.

Reviewer Comments:

3). The references are very old; the latest is 2003 and some are from the 1970's. While much of the beginning work on NEC started in the 70's, there continues to be a great deal of research being done currently on both the risk factors and management of NEC, for instance the use of probiotics. There is also new literature concerning the timing of enteral feeding (see Pietz et al, pediatrics, 2007, e164-70). Newer literature needs to be incorporated into the introduction and discussion.

Comment #3

Referenced literature updated throughout manuscript, as listed above in response to Reviewer #1 comments.

Reviewer Comments:

4). Page 12: you set the alpha for the selection of variables for inclusion in the logistic regression at .002. This is a very restrictive and there needs to be an explanation given. Additionally, this restriction resulted in key variables (i.e. breast milk) being left out of the regression even though their p values were significant by standard criteria.

Comment #4

The alpha of significance was restrictive as required by the analysis plan for this study and as a result, some key variables such as breast milk were excluded from the logistic regression model. This is a limitation of the study that has been discussed in the text on page 19, lines 10-14. However, even if the p value had been made less restrictive at $p=.01$, the same set of variables would have been included in the logistic regression model.

Reviewer Comments:

5). On my initial reading of the paper I found that the finding that the lack of fortified breast milk resulted in a 4.4 times increase in NEC to be confusing, as there is strong literature support for the reduction of the incidence of NEC

when breast milk is given. Realizing that breast milk alone was left out of the regression analysis made this clearer, and your explanation in the discussion was adequate. My initial confusion was related to the fact that supplementation of breast milk or formula can increase the osmolality of the feeding which is known to increase the risk of NEC. I was also surprised that you were referring to supplementation that included MCT oil and polyose which are not common, as well as HMF which is almost routine. This needs to be included in the methods section of the paper.

Comment #5

The methods section refers the reader to Table 1 for a description of the study variables. The study variable description of *type of enteral feeding* has been revised to include a note indicating that nutritional fortification of enteral feedings includes HMF, MCT oil, and Polyose. These compounds were commonly used in this practice setting at the time of this study. Supplementation of breast milk does increase the osmolality of feedings and has been thought to play a role in NEC. However, there are other enteral substrates with higher osmolalities, namely medications, that pose a far greater risk to the neonatal gut (Jew, R., Owen, D., Kaufman, D. & Balmer, D. (1997). *Nutrition in Clinical Practice*, 12, 158-163).

Reviewer Comments:

6). Pg. 17, line 13, I don't think that this represents a 'potentially new theory' as it is understood that the incidence of NEC is much lower in stable infants, i.e. those who are on full feedings.

Comment #6

Page 18, lines 8-10 has been revised to read: "Thus, the findings of this study **indicate that the risk** of NEC is lower in infants who receive all of their daily nutrition volume from enteral feedings and have progressed along the enteral feeding regimen with enough success to a point where nutritional fortification is added to their breast milk or infant formula."

Reviewer Comments:

7). pg 18, lines 1-1-4, I don't know of a NICU where such protocols aren't already in place.

Comment #7

I am aware of NICUs where clinical protocols pertaining to the preterm enteral feeding regimen are not in place. In NICUs where protocols do exist, it is my understanding that these protocols are not always uniformly implemented.

Page 18, lines 21-22 have been revised to read: "Clinical protocols aimed at monitoring for signs of feeding intolerance and poor growth **must be well understood and consistently implemented by the NICU team** as part of NEC surveillance and prevention throughout the enteral feeding regimen, but especially during the time of advancement to full feeding volume."

Reviewer Comments:

8). Pg 19, lines 5-6, this is much too strong of a statement and seems to contradict your discussion that the fortification is related to feeding progression and as such isn't an independent indicator of risk of NEC

Comment #8

Page 19, lines 6-9 have been revised to read: "As a result of the findings reported, neonatal clinicians will have a heightened awareness for NEC when an infant requires an increase in

respiratory support or **is in the early stages of enteral feeding prior to achieving full volume feedings with supplemental nutritional fortification.**”

REVIEWER #3

Reviewer Comments:

The authors mentioned on page 13 that "variables were highly correlated were collapsed accordingly", what is the criteria for "judging highly correlated"? Which variables were combined based on this? In addition, at the results section, the authors entered three enteral feeding variables in the logistic regression model and reported one feeding variable being significant predictor. I thought those variables might have high correlation and multicollinearity needs to be addressed. Even though the authors argued that correlation matrix were assessed prior to the logistic regression and no correlation coefficients bigger than 0.49. I disagree with the authors' claim that this represents little if any relationship at all (page 13, line 15). Often, in this type of research, r around 0.3 or 0.4 does represent a small to medium relationship, model stability need to be concerned. It is better if the authors present a correlation table for the readers.

Reviewer #3 highlights an important issue regarding correlation between variables included in this study, particularly the variables related to the enteral feeding regimen. *r* values greater than .69 were initially determined to indicate variables highly correlated to one another. This decision was made based on Munro, 2004. Since all of the correlations were less than .69, none of the variables were omitted from the regression analysis. The statement “*Variables that were highly correlated to one another were collapsed accordingly*” has been omitted from the text of the manuscript as it was misleading to the reader. The correlation matrix is included below.

Correlations for predictor variables included in logistic regression

	Increased respiratory support	Increased perfusion support	Fortified breast milk	Fortified infant formula	Change in fortification
Increased respiratory support	1.000				
Increased hemodynamic support	.349	1.000			
Fortified breast milk	-.017	-.201	1.000		
Fortified infant formula	.009	-.138	.065	1.000	
Change in fortification	-.040	.247	-.664	-.610	1.000

Upon further analysis of the correlation table and the comments of Reviewer #3, I believe that there is a greater than initially anticipated relationship between variables with *r* values less than .69,

specifically the variables related to enteral feeding (i.e fortified breast milk, fortified infant formula, and change in fortification). Because there is at least a moderate, if not strong, relationship between the change in fortification variable and fortified enteral feeding variable (breast milk or infant formula), the regression analysis was rerun without the change in fortification variable. This decision was made based on the r values and because it is likely that the change in fortification variable is a proxy for the two more specific variables pertaining to fortification of the specific type of enteral feeding (breast milk or infant formula). As a result of this revision to the regression analysis, the model indicated three significant predictors for NEC: increase in respiratory support needs, presence of nutritional fortification in breast milk and in infant formula. All findings reported in the text, as well as the results reported in the tables have been revised accordingly. See page 13 to 14 for a discussion of this revision in the analysis.

I thank Reviewer #3 for his or her insights and comments regarding this issue. The findings reported in the paper as revised are stronger and as a result, they make a greater contribution to the developing science of necrotizing enterocolitis and neonatal enteral feeding regimens.

Thank you for your review and consideration of this manuscript for publication in *Nursing Research*.