Preventing necrotising enterocolitis in very preterm infants: current evidence

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Abstract
Necrotising enterocolitis (NEC) is the most common serious gastrointestinal disorder affecting very preterm or very low birth weight infants. The risk is inversely proportional to gestational age and weight at birth. Fetal growth restriction and compromise may be additional specific risk factors. Postnatally, a variety of practices have been implicated in the pathogenesis of NEC including formula feeding, early and rapid advancement of enteral feed volumes, and exposure to H2-receptor antagonists. NEC, particularly severe NEC requiring surgical intervention, is associated with acute morbidity and mortality, prolonged hospital stay, and adverse long term neuro-developmental outcomes. With the exception of feeding with human milk, only limited evidence is currently available to support interventions to prevent NEC. Promising strategies that merit further evaluation in randomized controlled trials include the use of standardized feeding protocols and immuno-prophylaxis with prebiotics and probiotics.

Keywords breast milk; lactoferrin; necrotising enterocolitis; probiotics; very low birth weight

Epidemiology and outcomes
Necrotising enterocolitis (NEC) is a syndrome of acute intestinal necrosis of unknown aetiology affecting about 5% of all very preterm (less than 32 weeks) or very low birth weight (VLBW: less than 1500 g) and about 10% of all extremely preterm (less than 28 weeks) or extremely low birth weight (ELBW: less than 1000 g) infants (Table 1, Figure 1). Although treatment with antenatal corticosteroids reduces the risk of developing NEC by 50%, the overall incidence of NEC has not changed markedly in the past 20 years because of increases in early neonatal survival rates for extremely preterm and ELBW infants.

The rate of NEC-associated acute mortality is generally reported to be greater than 10% overall and more than 25% for infants with NEC severe enough to require a surgical intervention (which occurs in up to one-third of infants with NEC). Very preterm or VLBW infants who develop NEC experience more on-going morbidity than gestation-comparable infants who do not develop NEC. Infants with NEC have a higher incidence of nosocomial infections, lower levels of nutrient intake, grow more slowly, and have longer durations of intensive care and hospital stay. NEC, particularly severe NEC requiring surgical intervention, is also associated with a higher incidence of long-term neurological disability which may be a consequence of infection and under-nutrition during a critical period of brain development.

Pathogenesis and risk factors
NEC is a postnatal condition. There are no reports of fetal NEC. The risk of developing NEC and the severity of the disease is inversely related to gestational age and weight at birth. The other putative major risk factor is intrauterine growth restriction secondary to suboptimal placental support. Although our understanding of the disease process remains incomplete, the currently accepted unifying model for the pathogenesis of NEC includes contribution and interaction of three key components:

- a susceptible immature gastro-intestinal tract.
- a precarious intestinal vascular supply (secondary to compensatory diversion to other vital organs).
- additional luminal factors secondary to milk feeding; increased intestinal metabolic demand, alteration of mucosal integrity, disturbance of optimal microbiological ecological balance, locally invasive gastrointestinal infection.

Genetic contribution
Studies comparing the concordance of disease in monozygotic versus dizygotic twins suggest that familial factors may contribute to the risk of NEC. To date, association studies (usually small scale studies focussing on inflammatory mediators) have not detected any specific and substantial genetic risk factors. Much larger family-based whole genome screening studies would be required to define important genetic contributions to NEC susceptibility. However, because NEC is a sporadic disease which occurs infrequently in individual centres, this sort of investigation would require a co-ordinated multinational effort to achieve recruitment of sufficient participants to provide a meaningful analysis.

Enteral feeding
Since most VLBW infants who develop NEC have received some milk feeds, it has long been postulated that differences between enteral feeding practices contribute to inter-unit variation in the incidence of NEC (Figure 2).

Breast milk versus formula milk
Large observational studies have demonstrated that infants who received their mother’s expressed breast milk as their primary source of enteral nutrition are much less likely to develop NEC than infants fed with cow milk formula. However, because these studies did not randomly allocate infants to breast milk versus formula, the possibility that the effect on NEC was due to other known or unknown confounding factors could not be excluded. Infants who did not receive maternal expressed milk may have
had other risk factors for NEC such as intrauterine growth restriction secondary to maternal ill health.

There have not been any randomized controlled trials of maternal breast milk versus formula milk feeding in VLBW infants. Such trials are now unlikely to be conducted because of lack of clinical and ethical equipoise. Several trials of feeding very preterm infants with donated expressed breast milk versus formula milk, either as the sole enteral diet or as a supplement when maternal breast milk is not available, have been conducted. Meta-analysis of these trials demonstrates a significantly higher risk of NEC in formula-fed infants (Figure 3).

As expressed breast milk generally contains fewer calories, protein and other nutrients than maternal milk, additional nutrient supplementation (typically with a commercially-available cow milk multi-nutrient fortifier) is usually used to promote growth and development. A North American multi-centre randomized controlled trial has demonstrated that use of a human milk multi-nutrient fortifier extracted from donated breast milk (rather than a cow milk fortifier) as an adjunct to feeding with maternal or donated expressed breast milk has an additional, synergistic effect in reducing risk of NEC in very preterm infants.

The key question raised by these studies is whether the substantial capital and opportunity costs of supplying donated breast milk would be better invested in promoting evidence-based practices to ensure mothers are optimally supported to express their own breast milk. Several evidence-based interventions are available to support mothers wishing to express breast milk for their babies (Table 2, Figure 4). The challenge is to ensure that these are implemented consistently and broadly, and especially to vulnerable and socially-disadvantaged women who are less likely to provide expressed breast milk. Supporting mothers to express breast milk for their very preterm infants may be one of the most effective (and cost-effective) interventions currently available for reducing the incidence of NEC. Infant feeding practices in neonatal units, including the use of expressed breast milk, should be included in audit and benchmarking processes.

**Timing of introduction and rate of advancement of enteral feeds**

After accounting for availability of breast milk, the incidence of NEC tends to be higher in neonatal units where enteral feeding is introduced earlier and feeding volumes are advanced quickly. However, the data currently available from randomized controlled trials do not suggest that any specific feeding strategy affects the risk of very preterm or VLBW infants developing NEC (Figure 5) and a lack of blinding may have led to an over-estimation of the size of any effect.

**Competing outcomes**

Given the paucity of high-level evidence, the recommended and implemented strategies for early enteral feeding of very preterm...
Infants in the UK tend to be conservative and promote delayed introduction and slow progression of enteral feed volumes. However, enteral fasting during the early neonatal period may have important disadvantages for very preterm infants. Because gastrointestinal hormone secretion and motility are stimulated by milk, delayed feeding could diminish the functional adaptation of the immature gastrointestinal tract with consequent intestinal dysmotility, feed intolerance, and further delay in establishing enteral feeding independently of parenteral nutrition. Prolonging the use of parenteral nutrition may be associated with infectious and metabolic complications that have adverse consequences for survival, duration of hospital stay, growth, and development.

Although NEC is a devastating condition in the 5% of very preterm infants affected, preventative strategies, including conservative feeding regimens, need to be applied to all at risk infants. It has been argued that the ‘fear of NEC’ should not be considered in isolation of the other potential clinical outcomes in determining feeding policies and practice for very preterm infants (Figure 5).

**Intra-uterine growth-restriction**

Infants who are growth-restricted in utero are widely considered to have a higher risk of developing NEC, especially if there has been antenatal detection of ‘absent or reversed end-diastolic flow velocity’ (AREDFV) in Doppler studies of the fetal aorta or umbilical artery (Figure 6). However, observational studies that have examined the association of AREDFV with NEC have reported inconsistent findings. This may be related to differences in study design, especially with regard to accounting for the confounding variables of gestational age and birth weight. Studies that matched case and control infants for gestational age and birth weight have generally not found significant associations of AREDFV with NEC.

Despite the potential for exacerbating nutritional disadvantage, enteral feeding for growth restricted infants is often delayed for a week or more after birth. Paradoxically, until recently this population of infants has usually been specifically excluded from participating in randomized controlled trials of early enteral feeding practices. The large, pragmatic UK multicentre ‘Antenatal Doppler Enteral Prescription Trial’ (ADEPT) examined the effect of delayed introduction of progressive enteral feeds on the risk of NEC and other morbidities in 400 infants with severe growth restriction and evidence of circulatory redistribution. ADEPT did not find any statistically significant effects on the risk of NEC or all-cause mortality. Infants who had delayed introduction of enteral feeds took longer to establish full enteral feeding (median difference 2–4 days).

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**Interventions for lactation support for mothers of preterm infants**

- ‘Kangaroo’ skin-to-skin contact between mother and infant
- Simultaneous expression of milk from both breasts (using electric pump)
- Peer support in hospital and community
- Multidisciplinary staff training and continuous professional development to maintain skilled professional support
- Unicef ‘Baby Friendly’ accreditation of the associated maternity hospital

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**Table 2**

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Risk Ratio M-H, Fixed, 95% CI</th>
<th>Total (95% CI)</th>
<th>Heterogeneity: Chisq = 1.68, df = 5 (P = 0.89); I² = 0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cristofalo 2013</td>
<td>6.04 [0.76, 48.26]</td>
<td>2.77 [1.40, 5.46]</td>
<td>Test for overall effect: Z = 2.94 (P = 0.003)</td>
</tr>
<tr>
<td>Gross 1983</td>
<td>4.73 [0.52, 43.09]</td>
<td></td>
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<tr>
<td>Lucas 1984a</td>
<td>4.37 [0.50, 38.23]</td>
<td></td>
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<tr>
<td>Lucas 1984b</td>
<td>2.46 [0.48, 12.49]</td>
<td></td>
<td></td>
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<tr>
<td>Schanler 2005</td>
<td>1.77 [0.63, 4.96]</td>
<td></td>
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<tr>
<td>Tyson 1983</td>
<td>2.53 [0.11, 60.39]</td>
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**Figure 3** Formula vs. donor breast milk: meta-analysis of risk of NEC.

**Figure 4** Skin-to-skin care to facilitate expression of maternal breast milk.
"SIFT"

The success of ADEPT in assessing the effect of delayed versus early enteral feeding for growth-restricted infants has generated interest and enthusiasm for further trials to assess enteral feeding strategies in very preterm or VLBW infants (Figure 7). In the UK and Ireland, the ‘Speed of Increasing Feeds Trial’ (SIFT) Group, a collaboration of service-user representatives, clinicians and trial unit experts, is undertaking a pragmatic randomized controlled trial in which 2500 very preterm or VLBW infants are being enrolled. The trial compares advancing enteral feeds at 30 ml/kg/day or 18 ml/kg/day. To enhance generalisability, human milk-fed and formula-fed infants are eligible to participate. The primary outcome is death or moderate or severe disability at 2 years post-term and the trial is also powered to assess meaningful effects on in-hospital mortality and major morbidity, antibiotic usage and duration of hospital stay (see: www.npeu.ox.ac.uk/sift).

**Standardised feeding protocols**

Despite the lack of robust evidence that specific methods of delivering enteral nutrition to very preterm infants affects important outcomes, a systematic review of observational studies has shown that the use of a feeding protocol (irrespective of its specific recommendations) may of itself reduce the risk of NEC. However, the epoch-comparison (‘before-after’) studies included in this review were unable to exclude other plausible reasons for the apparent decrease in the risk of NEC during the intervention (adoption of feeding protocol) phase. Better quality evidence from randomized or cluster-randomized trials is needed to assess this approach.

"Immuno-nutrition"

One of the potential mechanisms by which feeding with human breast milk reduces the risk of NEC is the delivery of immuno-protective factors such as secretory immunoglobulin-A and lactoferrin to the immature small intestine. Another putative mechanism is that breast milk is ‘prebiotic’, that is, it contains substances that promote the growth of a non-pathogenic ‘probiotic’ intestinal microflora. These probiotic bacteria, predominantly *Lactobacilli* spp. and bifidobacteria, in turn generate ‘postbiotic’ substances such as butyric acid that limits the growth of potential pathogens. It is feasible that enteral supplementation with these immuno-protective substances may reduce the risk of NEC in infants for whom human breast milk is not available.

**Immunoglobulin**

Randomized controlled trials of enteric immunoglobulin supplementation for very preterm or VLBW infants have not found
any evidence of an effect on the incidence of NEC. However, most trials have used immunoglobulin-G whereas enteral immunoglobulin-A prophylaxis (which is more costly) is likely to be the more biologically appropriate intervention.

**Probiotics**

Some randomized controlled trials and meta-analyses of all of the available trials have found evidence that enteral administration of non-pathogenic probiotic species such as lactobacilli and bifidobacteria reduces the incidence of NEC, nosocomial infection, and mortality in very preterm infants. Some commentators have suggested that the current evidence-base is sufficient to adopt this intervention of a standard of care and that further controlled trials are unnecessary and unethical. Others advocate a more cautious and stepwise approach for several reasons. Firstly, although the pooled estimate from meta-analyses does indicate a substantially reduced risk of NEC and death, concern exists that these estimates are biased by various methodological weaknesses in the primary trials. Secondly, careful inspection of the distribution of effect size estimates of the included trials suggests that the size of the pooled estimate is due to small study and publication bias. Finally, there remains concern that questions of safety, including the risk of invasive infection with probiotic bacteria, have not been addressed and the optimal strains and dose regimens have yet to be defined. On balance it seems appropriate and ethical at this stage to continue to support the large, good-quality, multicentre trials of probiotic supplementation that are on-going internationally and to await a more precise and less biased estimate of effect size before introducing probiotic supplementation for very preterm infants as a routine practice.

**Prebiotics**

Prebiotics, typically a mixture of galacto- and fructooligosaccharides, support the growth of probiotic lactobacilli and bifidobacteria in the bowel. Prebiotic substances are naturally present in breast milk and are resistant to gastric acid digestion. Feeding very preterm infants with formula milk supplemented with prebiotic substance stimulates the growth of an intestinal microflora that is similar to that found in humans fed with human milk. To date, there have not been any large randomized controlled trials that have assessed the effect of prebiotics on the risk of NEC in very preterm infants but several such trials are being developed.

**Lactoferrin supplementation**

Lactoferrin, an antimicrobial glycoprotein present in colostrum and breast milk, is a key component of the mammalian innate response to infection. Lactoferrin has broad microbicial activity against Gram-positive cocci, Gram-negative bacilli, and Candida species. Lactoferrin also has prebiotic properties, creating an enteric environment for the growth of beneficial bacteria and reducing colonization with pathogenic species.

Very preterm infants have low lactoferrin levels and this deficiency is exacerbated by delay in establishing enteral feeding. An Italian multi-centre trial examined whether enteral supplementation with exogenous (bovine) lactoferrin for up to 6 weeks, either alone or in combination with a probiotic lactobacillus, reduced the risk of NEC and invasive nosocomial infection in very preterm infants. This good-quality trial found evidence that lactoferrin supplementation reduced the incidence of invasive nosocomial infection by two thirds compared with controls. However, the incidence of NEC was decreased in the lactoferrin plus probiotic group only. It is plausible that a more modest independent effect of lactoferrin on the risk of NEC may still exist and further large trials are proposed to investigate this possibility.

**The ELFIN trial**

The pre-clinical data, studies using animal models, and the results of the Italian trial have generated considerable interest and enthusiasm for further trials to assess lactoferrin immune-prophylaxis in very preterm infants. Several international groups are planning large trials. In the UK, the ‘Enteral Lacto-Ferrin In Neonates’ (ELFIN) Trial Group (a collaboration of service-user representatives, clinicians, and trial unit experts) is undertaking a simple and pragmatic randomized controlled trial in which 2200 very preterm infants will be able to participate. Late-onset invasive infection is the pre-specified primary outcome. The trial will also be powered to assess meaningful effects on mortality, major morbidity, antibiotic usage and duration of hospital stay. The trial data will contribute to a prospective meta-analysis collaboration with similar trials planned in Australasia and North America. An economic evaluation will be conducted to assess whether the intervention is likely to be cost-effective (see: www.npeu.ox.ac.uk/elfin).

**Immuno-nutrition: glutamine and arginine**

Very preterm or VLBW infants who develop NEC have lower plasma levels of the amino acids arginine and glutamine compared with gestation-comparable infants who do not develop NEC.

Glutamine is a ‘conditionally essential’ amino acid and is the preferred respiratory fuel for rapidly proliferating cells such as enterocytes. In animal models of experimental enteroocolitis, glutamine supplementation reduces mucosal damage and lowers the risk of invasive infection and death. Glutamine is abundant in human milk but present only in much lower levels in cow milk formula and absent in standard parenteral nutrition solutions. Despite this biological plausibility, a Cochrane review and meta-analysis of good quality randomized controlled trials did not find any evidence that the routine use of glutamine supplementation affects important clinical outcomes including the risk of developing NEC for VLBW infants. Current research efforts have moved to assessing the potential of glutamine supplementation as a rescue therapy for accelerating recovery in infants with established NEC.

Arginine is involved in the generation of nitric oxide, a key mediator of intestinal vasomotor tone. It has been suggested that enteral arginine supplementation may enhance endothelial nitric oxide generation and thereby improve intestinal perfusion. The only trial to have examined this intervention found a reduced incidence of NEC in infants who received arginine. A large multicentre trial is needed to confirm this finding.

**Other modifiable risk factors**

**Umbilical arterial catheterization**

Umbilical artery catheters lying within the aorta could potentially disrupt intestinal perfusion directly or via micro-thrombotic emboli. The available data do not suggest that enteral feeding
while an umbilical arterial catheter is in place affects superior mesenteric blood flow, the risk of feed intolerance, or the incidence of NEC.

**Patent ductus arteriosus and non-steroidal anti-inflammatory agents**
The presence of a patent ductus arteriosus has been inconsistently reported as a risk factor for development of NEC in VLBW infants. No evidence exists that withholding enteral feeds in infants with a patent ductus arteriosus affects clinical outcomes including the risk of NEC. Furthermore, meta-analyses of good-quality randomized controlled trials of non-steroidal anti-inflammatory agents for patent ductus arteriosus closure have not detected any significant effects on the incidence of NEC.

**Gastric acidity suppression**
Innate gastrointestinal immunity provided by gastric acid may be important in preventing the cascade of infectious and inflammatory events leading to NEC. A small randomized controlled trial found that moderate acidification of milk with hydrochloric acid reduced the incidence of NEC in VLBW infants. Conversely, evidence exists that the use of histamine-receptor type 2 (H2)-blockers to suppress gastric acidity is associated with a higher risk of NEC (and nosocomial infection) in VLBW infants. Given lack of evidence that gastro-oesophageal reflux is a cause of apnoea in preterm infants, it is recommended that use of H2-blockers should be restricted until robust evidence that benefits outweigh harmful effects is obtained.

**Red blood cell transfusion**
Although several case reports have described infants developing NEC during red blood cell transfusion, a large randomized controlled trial of high versus low thresholds for red blood cell transfusion in ELBW infants did not find an effect on the incidence of NEC. The currently available evidence is insufficient to conclude that adopting a policy of enteral fasting during blood transfusion causes more benefit than harm.

**Conclusion**
The currently identified major modifiable risk factors for the development of NEC in VLBW infants relate to enteral feeding practices. Evidence exists that feeding preterm infants with human breast milk rather than formula milk can reduce the risk of NEC. Substantial clinical uncertainty remains about the effect of strategies such as delaying the introduction of enteral feeds or slowly advancing feed volumes on the risk of NEC as well as other ‘competing’ outcomes, principally nosocomial infection secondary to prolonged use of parenteral nutrition. Other promising strategies for minimising NEC that merit further evaluation include the use of prebiotics and probiotics, the avoidance of H2-receptor blockers, and the use of arginine supplementation. Large multi-centre trials within collaborative networks will be needed to address these questions.

**Further Reading**


**Practice points**
- The risk of necrotising enterocolitis is inversely related to gestational age and weight at birth.
- Only limited evidence exists to suggest that conservative feeding strategies reduces the risk of NEC, and these may be associated with other adverse (competing) outcomes including nosocomial infection.
- Injudicious use of gastric acid suppressants may be risk factor for NEC.
- Promising new interventions to prevent NEC include probiotics and prebiotics but these require further evaluation in randomized controlled trials before being adopted as routine practice.