

## **Una visión general de las revisiones sistemáticas de ensayos controlados aleatorios para prevenir Enterocolitis Necrotizante en recién nacidos prematuros.**

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### Resumen

La enterocolitis necrotizante (NEC) sigue siendo una causa importante de mortalidad y morbilidad neonatal en neonatos prematuros.

El propósito de esta revisión fue resumir la evidencia de las revisiones sistemáticas de los ensayos controlados aleatorios (ECA) con respecto a las intervenciones prenatales y postnatales para prevenir la ECN. PubMed, EMBASE, las bases de datos de la Biblioteca Cochrane,

En la Base de datos de resúmenes de revisiones de efectos, y Biblioteca Campbell, se buscaron metaanálisis en los que se informó NEC como resultado después de estrategias prenatales o postnatales. Se utilizó el instrumento AMSTAR para evaluar la calidad de los comentarios incluidos. La calificación de las recomendaciones, evaluación, desarrollo y evaluación de evaluación se utilizó para evaluar la certeza de la evidencia. Identificamos 98 metaanálisis de ECA. La calidad de las revisiones incluidas fue adecuada, mientras que la certeza de la evidencia fue moderada a muy baja.

La evidencia de certeza moderada mostró una reducción en NEC luego de la administración de una combinación de especies de probióticos, probióticos (cualquiera), corticosteroides prenatales en mujeres embarazadas en riesgo de parto prematuro e ibuprofeno versus indometacina para el tratamiento del conducto arterioso permeable (PDA).

Para el NEC quirúrgico, la evidencia de certeza moderada mostró un

mayor riesgo con un objetivo de saturación de oxígeno más bajo (85-89%), en comparación con niveles de objetivo con una mayor saturación de oxígeno (91-95%).

En conclusión, se observa una disminución del riesgo de ECN con probióticos, corticosteroides prenatales para mujeres en riesgo de parto prematuro e ibuprofeno versus indometacina para tratamiento de PDA.

Apuntar a bajas saturaciones de oxígeno puede aumentar el riesgo de NEC quirúrgico, en comparación con la focalización de mayores saturaciones.

# An Overview of Systematic Reviews of Randomized-Controlled Trials for Preventing Necrotizing Enterocolitis in Preterm Infants

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## Keywords

Necrotizing enterocolitis · Overview ·  
Randomized-controlled trial · Preterm · Neonate

## Abstract

Necrotizing enterocolitis (NEC) remains a major cause for neonatal mortality and morbidity in preterm infants. The purpose of this review was to summarize evidence from systematic reviews of randomized-controlled trials (RCTs) regarding antenatal and postnatal interventions for preventing NEC. PubMed, EMBASE, the Cochrane Library Databases, Database of Abstracts of Reviews of Effects, and Campbell Library were searched for meta-analyses in which NEC was reported as an outcome after antenatal or postnatal strategies. The AMSTAR instrument was used to evaluate quality of included reviews. Grading of Recommendations, Assessment, Development and Evaluation assessment was used to evaluate certainty of evidence. We identified 98 meta-analyses of RCTs. The quality of included reviews was adequate, whereas the certainty of evidence was moderate to very low. Moderate certainty evidence showed a reduction in NEC fol-

lowing administration of a combination of species of probiotics, probiotics (any), antenatal corticosteroids in pregnant women at risk of preterm birth, and ibuprofen versus indomethacin for treatment of patent ductus arteriosus (PDA). For surgical NEC, moderate certainty evidence showed an increased risk with lower (85–89%) oxygen saturation target levels, compared with higher (91–95%) oxygen saturation target levels. In conclusion, decreased risk of NEC is observed with probiotics, antenatal corticosteroids for women at risk of preterm birth, and ibuprofen versus indomethacin for treatment of PDA. Targeting lower oxygen saturations may increase the risk of surgical NEC, compared to targeting higher saturations.

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## Introduction

Necrotizing enterocolitis (NEC) is a devastating gastrointestinal disease of preterm infants [1]. Despite advances in neonatal care, morbidity and mortality related to NEC have not declined for decades [2]. The incidence

of NEC ranges between 5 and 10% in very low birthweight infants (birth weight <1,500 g), and mortality is high (about 25%) [3]. Up to half of infants with NEC require surgical intervention, and survivors may suffer from short-gut syndrome, total parenteral nutrition dependency, cholestatic liver disease related to total parenteral nutrition administration, and neurodevelopmental delay among others [2]. The etiology of NEC is not clear but likely to be multifactorial. Demonstrated risk factors for NEC include gut immaturity, formula feeding, microbial dysbiosis, and infection [4–6]. Studies on the microbiome of the gut preceding NEC have shown an increase in phylum Proteobacteria and decrease in phyla Firmicutes and Bacteroidetes [7]. Health-care costs in the United States due to NEC are high, and the additional expenditure has been calculated to be USD 74,004 for NEC and USD 198,040 for surgical NEC per infant, respectively (based on 2011 estimations) [8].

Since the pathogenesis of NEC is multifactorial, many interventions to prevent NEC targeting various aspects of NEC etiopathogenesis have been studied. The interventions for preventing NEC include optimal feeding strategies including donor human milk [4, 9–11], microbial optimization strategies (probiotics, prebiotics, synbiotics, and antibiotics) [12–14], immunomodulatory strategies [15–17] (lactoferrin, pentoxifylline, oral immunoglobulins), and nutritional strategies [18] (glutamine and arginine supplementation) among others. The wide range of interventions reflects the need to address the multifactorial etiology of NEC.

NEC is the targeted primary outcome for NEC prevention studies but may also be an important secondary outcome for interventions focused on other disease conditions (such as surfactant for prevention of respiratory distress syndrome) [19]. The impact of antenatal interventions on NEC is often not studied or reported, for example, interventions to prevent preterm birth in women with preterm labor [20] or prolonged rupture of membranes [21]. Considering the large numbers of systematic reviews that have been published on prevention of NEC, an overview of existing systematic reviews is justified to provide an overall perspective for the bedside clinician and researchers.

## Methods

### Search Strategy

Two reviewers (T.X. and M.P.) searched PubMed, EMBASE, the Cochrane Database of Systematic Reviews, Database of Abstracts of Reviews of Effects, and Campbell Library from inception to August

4, 2018, for meta-analyses of randomized control trials (RCTs) for preventing NEC in preterm infants. We used the following search strategy: (necrotizing enterocolitis OR necrotizing enterocolitis OR NEC) AND (systematic review OR meta-analysis) as subject word and random word for all fields (online suppl. Table 1; for all online suppl. material, see [www.karger.com/doi/10.1159/000504371](http://www.karger.com/doi/10.1159/000504371)). Two reviewers (T.X. and M.P.) independently screened the titles and abstracts and selected articles for full-text review and potentially eligible articles were retrieved for perusal in full text. We resolved disagreements through discussion, or, if required, we consulted a third member (A.E.-S.) of the review team.

### Selection Criteria

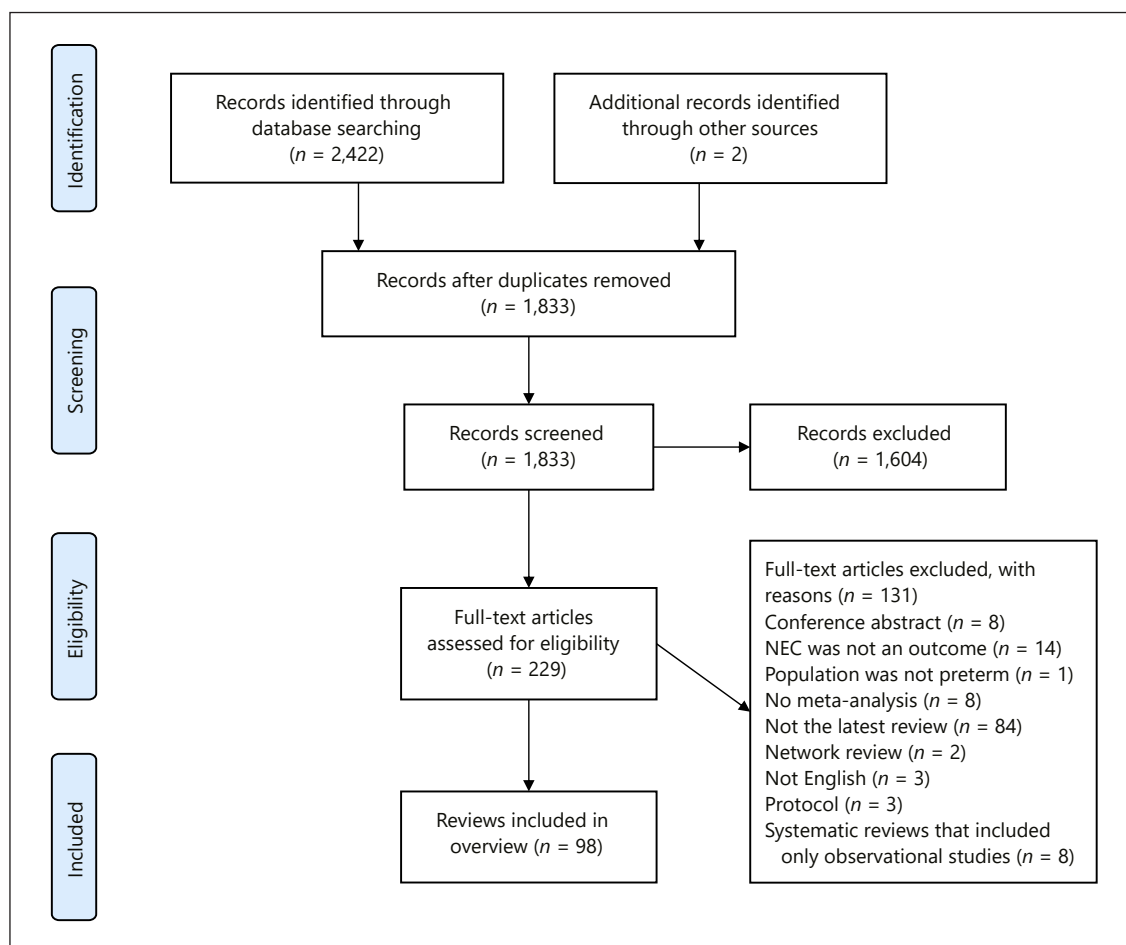
We followed the editorial decision tree proposed by the “Cochrane Comparing Multiple Interventions methods Group” to establish whether our review would better fit an overview format or an intervention review format [22]. We did not aim to compare multiple interventions to obtain their comparative effectiveness or rank the interventions, and the overview format was the best strategy (online suppl. Fig. 1).

We included meta-analyses of RCTs where a summary estimate (risk ratio [RR], OR, or risk difference with 95% CI) were reported. If a systematic review included both observational studies and RCTs, we included data from RCTs only. If 2 or more systematic reviews were published for the same prevention and same outcome, we selected the one with the highest number of RCT studies and the highest AMSTAR score. For updated Cochrane reviews, we selected the latest update.

We included reviews if the target population is at least one of the following: (1) preterm (<37 completed weeks of gestation) neonates (<28 days) or (2) low birth weight (<2,500 g) (including very low birthweight [<1,500 g] infants and extremely low birthweight [<1,000 g] infants). We considered all types of antenatal and postnatal interventions compared with placebo, no treatment, or an alternative intervention that reported NEC as a primary or a secondary outcome. We included both pharmacological and non-pharmacological interventions. Our primary outcome was incidence of NEC (stage II or above; any stage) based on modified Bell’s criteria [23] during hospital stay. Secondary outcomes were (1) surgical NEC (i.e., Bell’s stage III NEC, including placement of peritoneal drains), (2) “all-cause mortality” of infants during hospital stay (only from reviews that NEC was primary outcome), and (3) NEC-related mortality. We only included systematic reviews published in English. We also included interventions that increased the risk of NEC to emphasize avoidance of those interventions. We excluded network meta-analyses, where direct head to head comparison of different interventions was performed.

### Data Collection and Analysis

T.X. and M.P. independently extracted data from eligible systematic reviews. From each meta-analysis, we extracted (1) the first author, year of publication, (2) number of trials and neonates and their characteristics, (3) quality of the included trials (as reported by the review authors), (4) interventions and comparisons relevant to this overview, (5) outcomes relevant to this overview (including definitions), (6) summary estimate of the intervention effects, (7) heterogeneity index including  $Tau^2$ ,  $p$  value for chi-square and  $I^2$ , and (8) any other characteristics required to assess AMSTAR and Grading of Recommendations, Assessment, Development and Evaluation (GRADE) score.



**Fig. 1.** Flowchart of selection of studies for inclusion in umbrella review (PRISMA 2009 flow diagram). NEC, necrotizing enterocolitis.

### Quality of Included Reviews

The methodological quality of each systematic review was evaluated using a structured tool, the AMSTAR (A Measurement Tool to Assess Systematic Reviews) instrument [24]. AMSTAR evaluates the systematic review using 11 distinct criteria. Each criterion of AMSTAR has 4 choices, namely, “yes” (clearly done), “no” (clearly not done), “cannot answer,” or “not applicable.” We graded a review that met all of the 11 criteria to be the highest quality; achieved scores of 8–11 as high quality; scores of 4–7 as medium quality; and scores of 0–3 as low quality [25].

### Certainty of Studies Included within Reviews

Two authors (T.X. and M.P.) independently assessed the certainty of evidence and used GradePro software to enter data and assess GRADE. In the GRADE approach, outcomes from RCTs start as high-certainty evidence [26]. Relevant evidence can be rated down for high risk of bias of included studies, indirectness, imprecision, inconsistency, and publication bias. The GRADE tool assesses the certainty of evidence for each outcome by categorizing evidence into “high,” “moderate,” “low,” or “very low” certainty [26]. There was an inherent subjectivity in assessing the certainty

of evidence, and the original review authors may overstate the evidence [27]. Although some review authors have provided the GRADE assessment, we reassessed the certainty of evidence by information provided by original systematic review and derived GRADE certainty of evidence for the specified outcomes of this overview, which made a more consistent assessment for certainty of evidence.

### Data Synthesis

We reported summary estimates of the interventions from included reviews qualitatively without pooling data from the included meta-analyses. For better visual presentation, we constructed forest plots using Microsoft Excel from the extracted relative effect and 95% CIs for the 4 specified outcomes.

According to the certainty of evidence (GRADE) and the summary estimates reported by the review, we graded interventions for NEC into 5 categories [25]: (1) effective interventions: high-certainty evidence of benefit, (2) possibly effective interventions (more evidence needed): moderate-certainty evidence of benefit, (3) ineffective interventions: high-certainty evidence with lack of benefit, or harm, (4) probably ineffective interventions (more evi-

**Table 1.** Interventions for prevention of NEC with moderate certainty evidence

Intervention	Author, year	Studies	Setting	Events/ intervention Events/control	Summary of measure	Forest plot	Estimate (95% CI)	Tau <sup>2</sup>	<i>p</i> value for chi- square	<i>I</i> <sup>2</sup> , %	Amstar score	Certainty of evidence (GRADE)
Probiotics <sup>a</sup> (a combination of species)	Sawh et al. [12], 2016	18	<37 weeks or <2,500 g	55/2,357 134/2,293	RR Random		0.41 (0.29–0.56)	0	0.8	0	9	Moderate
Antenatal corticosteroids for accelerating fetal lung maturation <sup>b</sup>	Roberts et al. [28], 2017	10	Women at risk of preterm birth	27/2,380 53/2,322	RR Fixed		0.50 (0.32–0.78)	No info	0.7	0	11	Moderate
Probiotics <sup>a</sup> (Any)	Sawh et al. [12], 2016	38	<37 weeks or <2,500 g	170/5,304 311/5,216	RR Random		0.53 (0.42–0.66)	0.04	0.29	11	9	Moderate
Ibuprofen for the treatment of PDA <sup>b,c</sup> (Ibuprofen vs. indomethacin)	Ohlsson et al. [29], 2015	16	<37 weeks and/or <2,500 g with PDA	38/489 58/459	RR Fixed		0.64 (0.45–0.93)	No info	0.87	0	10	Moderate
Early (<8 days) postnatal corticosteroids for preventing chronic lung disease <sup>b</sup>	Doyle et al. [30], 2014	23	Preterm infants at risk of developing chronic lung disease	142/1,764 161/1,743	RR Fixed		0.87 (0.70–1.08)	No info	0.9	0	10	Moderate
Early erythropoietin for preventing red blood cell transfusion <sup>b</sup>	Ohlsson and Aher [31], 2014	11	<37 weeks and/or low <2,500 g, <8 days of age	52/678 45/669	RR Fixed		1.07 (0.73–1.57)	No info	0.77	0	10	Moderate
Prophylactic intravenous indomethacin <sup>b</sup> (prophylactic indomethacin vs. control)	Fowlie Peter et al. [32], 2010	12	<37 weeks	84/1,187 77/1,214	RR Fixed		1.09 (0.82–1.46)	No info	0.88	0	9	Moderate

NEC, necrotizing enterocolitis; PDA, patent ductus arteriosus; RR, risk ratio; GRADE, Grading of Recommendations, Assessment, Development and Evaluation. <sup>a</sup> Primary outcome; <sup>b</sup> secondary outcome; <sup>c</sup> NEC at any stage (stages 1, 2, 3).

dence needed): moderate-certainty evidence with lack of benefit, or harm, and (5) no conclusions possible: low or very low certainty evidence, or insufficient evidence to comment on the effectiveness of an intervention.

## Results

The process of including reviews for our overview is depicted in Figure 1. The search yielded 2,422 articles from databases and 2 from other sources. Discarding the duplicates and excluding unrelated articles by title and abstract, we had 229 reviews for which full texts of the articles were obtained. Finally, we included 98 reviews after excluding 131 reviews. NEC was targeted as a primary outcome in 22 reviews and as a secondary outcome in 76 reviews. NEC was reported as an outcome in 110 interventions, surgical NEC in 10 interventions, “all-cause mortality” during hospital stay in 21 interventions, and NEC-related mortality in 4 interventions.

### Effect of Interventions

Outcome 1: NEC (Stage II or Above; Any Stage)

- (1) Effective interventions: None.
- (2) Possibly effective interventions: (Table 1):

Moderate certainty evidence showed a reduction in NEC following administration of a combination of species of probiotics (RR 0.41, 95% CI 0.29–0.56; 18 trials; 4,650 infants) [12]; antenatal corticosteroids for accelerating fetal lung maturation in pregnant women at risk of preterm birth (RR 0.50, 95% CI 0.32–0.78; 10 trials; 4,702 infants) [28]; probiotics (any; RR 0.53, 95% CI 0.42–0.66; 38 trials; 10,520 infants) [12]; and ibuprofen compared to indomethacin for the treatment of patent ductus arteriosus (PDA; RR 0.64, 95% CI 0.45–0.93; 16 trials; 948 infants) [29].

(3) Ineffective interventions: None.

(4) Probably ineffective interventions: (Table 1):

A. Harm effect: None.

B. Lack of effectiveness:

Moderate certainty evidence showed lack of effectiveness in NEC by early (<8 days) postnatal corticosteroids for preventing chronic lung disease (RR 0.87, 95% CI 0.70–1.08; 23 trials; 3,507 infants) [30]; early erythropoietin for preventing red blood cell transfusion (RR 1.07, 95% CI 0.73–1.57; 11 trials; 1,347 infants) [31]; and prophylactic intravenous indomethacin (RR 1.09, 95% CI 0.82–1.46; 12 trials; 2,401 infants) [32].

(5) No conclusions possible because of low (online suppl. Fig. 2) or very low certainty evidence (online suppl. Fig. 3) in 103 preventative interventions for NEC.



**Table 2.** Interventions for prevention of surgical NEC

Intervention	Author year	Studies	Setting	Events/ intervention events/control	Summary of measure	Forest plot	Estimate (95% CI)	Tau <sup>2</sup>	p value for chi- square	I <sup>2</sup> , %	Amstar score	Certainty of evidence (GRADE)
Oxygen Saturation Target Levels <sup>b</sup> (lower [85–89%] vs. higher [91–95%] SPO <sub>2</sub> )	Askie et al. [33], 2018	5	<28 weeks within 24 h of birth	227/2,464 170/2,465	RR Fixed		1.33 (1.10–1.61)	No info	no info	0	5	Moderate
Arginine supplementation <sup>a</sup>	Shah et al. [36], 2017	3	<37 weeks	1/140 8/145	RR Fixed		0.13 (0.02–1.03)	No info	no info	0	10	Low
Donor-banked milk vs. formula <sup>a</sup>	Silano et al. [37], 2018	4	<37 weeks and/or <2,500 g	10/469 25/484	OR Random		0.45 (0.19–1.09)	No info	0.29	20.68	8	Low
Probiotics <sup>a</sup> (Any)	Rees et al. [38], 2017	14	Premature infants	48/1,989 74/1,986	RR Random		0.74 (0.51–1.05)	No info	0.73	0	9	Low
Antistaphylococcal immunoglobulins to prevent staphylococcal infection <sup>b</sup>	Shah et al. [39], 2009	2	Very low birth weight infants	27/1,341 24/1,147	RR Fixed		0.80 (0.46–1.40)	No info	0.57	0	9	Low
N-acetylcysteine supplementation in parenterally fed neonates <sup>b</sup>	Soghier et al. [40], 2006	1	Neonates ≤ minimal enteral nutrition	17/194 16/197	RR		1.08 (0.56–2.07)	n/a	n/a	n/a	9	Low
Human milk feeding <sup>a</sup> (exclusive human milk vs. exclusive preterm formula)	Miller et al. [41], 2018	1	≤28 weeks and/or ≤1,500 g	0/29 4/24	RR Random		0.09 (0.01–1.64)	n/a	n/a	n/a	8	Very low
Human milk feeding <sup>a</sup> (unpasteurised vs. pasteurised human milk)	Miller et al. [41], 2018	1	≤28 weeks and/or ≤1,500 g	0/151 4/152	RR Random		0.11 (0.01–2.06)	n/a	n/a	n/a	8	Very low
Oral immunoglobulin <sup>a</sup>	Foster et al. [17], 2016	2	<37 weeks or <2,500 g	0/153 4/158	RR		0.21 (0.02–1.75)	No info	0.7	0	9	Very low
Human milk feeding <sup>a</sup> (higher vs. lower dose human milk)	Miller et al. [41], 2018	2	≤28 weeks and/or ≤1,500 g	6/321 12/259	RR Random		0.36 (0.06–2.04)	1.02	0.09	66	8	Very low

NEC, necrotizing enterocolitis; RR, risk ratio; GRADE, Grading of Recommendations, Assessment, Development and Evaluation. <sup>a</sup> Primary outcome; <sup>b</sup> secondary outcome.

## Outcome 2: Surgical NEC (Including Placement of Peritoneal Drains; Table 2)

- (1) Effective interventions: None.
- (2) Possibly effective interventions: None.
- (3) Ineffective interventions: None.
- (4) Probably ineffective interventions:

### A. Harm effect:

Moderate certainty evidence showed an increase in surgical NEC by lower (85–89%) vs. higher (91–95%) oxygen saturation target levels (RR 1.33, 95% CI 1.10–1.61; 5 trials; 4,929 infants) [33].

### B. Lack of effectiveness: None.

- (5) No conclusions possible:

No conclusion could be drawn because of low to very low certainty evidence for arginine supplementation, donor-banked milk versus formula, probiotics (any), antistaphylococcal immunoglobulins to prevent staphylococcal infection, N-acetylcysteine supplementation in parenterally fed neonates, exclusive human milk versus exclusive preterm formula, unpasteurized versus pasteur-

ized human milk, oral immunoglobulin, and higher versus lower dose human milk.

## Outcome 3: “All-Cause Mortality” during Hospital Stay (Table 3)

- (1) Effective interventions: None.
- (2) Possibly effective interventions:

Moderate certainty evidence review showed a reduction in mortality following a combination of species of probiotics (RR 0.41, 95% CI 0.29–0.56; 18 trials; 4,650 infants) and probiotics (any; RR 0.79, 95% CI 0.68–0.93; 29 trials; 9,507 infants) [12].

- (3) Ineffective interventions: None.
- (4) Probably ineffective interventions:

### A. Harm effect: None.

### B. Lack of effectiveness: None.

- (5) No conclusions possible

No conclusion could be drawn for probiotics (single species *Lactobacillus*), arginine supplementation; antibiotics for preterm rupture of membranes (any antibiotic

**Table 3.** Effect of interventions for prevention of NEC as primary outcome on “all-cause mortality” during hospital stay

Intervention	Author year	Studies	Setting	Events/ intervention Events/control	Summary of measure	Forest plot	Estimate (95% CI)	Tau <sup>2</sup>	p value for chi- square	I <sup>2</sup> , %	Amstar score	Certainty of evidence (GRADE)
Probiotics <sup>a</sup> (a combination of species)	Sawh et al. [12], 2016	18	<37 weeks or <2,500 g	55/2,357 134/2,293	RR Random		0.41 (0.29–0.56)	0	0.8	0	9	Moderate
Probiotics <sup>a</sup> (any)	Sawh et al. [12], 2016	29	<37 weeks or <2,500 g	264/4,761 341/4,746	RR Random		0.79 (0.68–0.93)	0	0.59	0	9	Moderate
Probiotics <sup>a</sup> ( <i>Lactobacillus</i> )	Sawh et al. [12], 2016	8	<37 weeks or <2,500 g	32/1,299 53/1,297	RR Random		0.61 (0.40–0.95)	0	0.84	0	9	Low
Arginine supplementation <sup>a</sup>	Shah et al. [36], 2017	3	<37 weeks	13/140 18/145	RR Fixed		0.77 (0.41–1.45)	No info	0.18	42	9	Low
Antibiotics for preterm rupture of membranes <sup>a</sup> (any antibiotic vs. placebo)	Kenyon et al. [42], 2013	12	Women with preterm rupture of membranes	276/4,315 138/1,986	RR Random		0.93 (0.76–1.14)	0	0.65	0	10	Low
Probiotics <sup>a</sup> ( <i>Bifidobacterium</i> )	Sawh et al. [12], 2016	6	<37 weeks or <2,500 g	69/1,027 103/1,029	RR Random		0.37 (0.14–0.97)	0.73	0.01	69	9	Very low
Lactoferrin plus probiotics vs. placebo <sup>a</sup>	Pammi et al. [7], 2017	1	<37 weeks	9/238 18/258	RR Fixed		0.54 (0.25–1.18)	n/a	n/a	n/a	9	Very low
Early trophic feeding vs. enteral fasting <sup>a</sup>	Morgan et al. [11], 2013	8	<1,500 g or <32 weeks	23/278 37/280	RR Fixed		0.66 (0.41–1.07)	No info	0.76	0	9	Very low
Enteral antibiotics <sup>a</sup>	Bury et al. [14], 2001	2	<2,500 g and/or <37 weeks	12/106 18/109	RR Fixed		0.67 (0.34–1.32)	No info	0.25	24	9	Very low
Antibiotics for preterm rupture of membranes <sup>a</sup> (antibiotics vs. no antibiotic [no placebo])	Kenyon et al. [42], 2013	6	Women with preterm rupture of membranes	23/289 34/282	RR Random		0.69 (0.41–1.14)	0	0.7	0	10	Very low
Probiotics <sup>a</sup> ( <i>Saccharomyces boulardii</i> )	Sawh et al. [12], 2016	2	<37 weeks or <2,500 g	11/186 13/171	RR Random		0.72 (0.33–1.54)	0	0.63	0	9	Very low
Omega-3 long-chain polyunsaturated fatty acids <sup>a</sup>	Zhang et al. [43], 2014	6	<37 weeks	17/1,838 26/1,911	RR Fixed		0.76 (0.42–1.38)	No info	0.11	44	10	Very low
Colostrum <sup>a</sup>	Sadeghirad et al. [2], 2018	7	<37 weeks within the first week of life	11/168 12/162	RR Random		0.84 (0.39–1.82)	No info	0.604	0	10	Very low
Prophylactic surgical ligation of PDA <sup>a</sup>	Mosalli et al. [44], 2009	1	<1,000 g	16/40 20/44	RR		0.88 (0.53–1.45)	n/a	n/a	n/a	8	Very low
Lactoferrin vs. placebo <sup>a</sup>	Pammi et al. [7], 2017 <sup>b</sup>	10	<37 weeks	127/2,535 136/2,561	RR Fixed		0.94 (0.75–1.19)	No info	0.16	32	9	Very low
Slow (up to 24 mL/kg/days) vs. faster rates of advancement of enteral feed volumes <sup>a</sup>	Oddie et al. [4], 2017	9	Very preterm or <1,500 g	147/1,796 127/1,780	RR Fixed		1.15 (0.93–1.42)	No info	0.33	13	10	Very low
Delayed (≥4 days) vs. early introduction of progressive enteral feeding (24 mL/kg/day) <sup>a</sup>	Morgan et al. [11], 2013	7	<1,500 g or <32 weeks	33/464 29/503	RR Fixed		1.18 (0.75–1.88)	No info	0.71	0	9	Very low
Red blood cell transfusions <sup>a</sup> (liberal [greater volume and/or number of RBC transfusions] vs. restrictive transfusion)	Keir et al. [45], 2016	8	<37 weeks	65/475 53/476	RR Fixed		1.24 (0.89–1.72)	No info	0.95	0	7	Very low
Glycerin enemas and suppositories <sup>a</sup>	Livingston et al. [46], 2015	3	<32 weeks and/or <1,500 g	12/93 8/86	RR Random		1.34 (0.58–3.11)	0	0.82	0	9	Very low
Dobutamine vs. dopamine <sup>a</sup>	Osborn et al. [47], 2007	1	<37 weeks with low blood flow	14/22 9/20	RR Fixed		1.41 (0.79–2.52)	n/a	n/a	n/a	9	Very low
Adjunctive antibiotic treatment in preterm labor <sup>a</sup>	Egarter et al. [48], 1996	4	Preterm labor before 37 weeks	8/272 2/277	OR Fixed		3.25 (0.93–11.38)	No info	No info	No info	4	Very low

NEC, necrotizing enterocolitis; RR, risk ratio; GRADE, Grading of Recommendations, Assessment, Development and Evaluation; PDA, patent ductus arteriosus. <sup>a</sup> Primary outcome. <sup>b</sup> Updated with unpublished data.



**Table 4.** Effect of interventions for prevention of NEC on NEC-related mortality

Intervention	Author, year	Studies	Setting	Events/ intervention Events/control	Summary of measure	Forest plot	Estimate (95% CI)	Tau <sup>2</sup>	p value for chi- square	I <sup>2</sup> , %	Amstar score	Certainty of evidence (GRADE)
Arginine supplementation <sup>a</sup>	Shah et al. [36], 2017	3	<37 weeks	1/140 8/145	RR Fixed		0.18 (0.03–1.00)	No info	0.68	0	9	Low
Probiotics <sup>a</sup> (any)	Rees et al. [38], 2017	13	<37 weeks	No info	RR Random		0.56 (0.34–0.93)	No info	0.85	0	9	Low
Enteral antibiotics <sup>a</sup> (aminoglycoside)	Bury et al. [14], 2001	3	<2,500 g and/or <37 weeks	3/126 12/131	RR Fixed		0.32 (0.10–0.96)	No info	0.66	0	9	Very low
Oral immunoglobulin <sup>a</sup>	Foster et al. [17], 2016	3	<37 weeks or <2,500 g	10/921 9/919	RR Fixed		1.10 (0.47–2.59)	No info	0.31	14	9	Very low

NEC, necrotizing enterocolitis; RR, risk ratio; GRADE, Grading of Recommendations, Assessment, Development and Evaluation. <sup>a</sup> Primary outcome.

vs. placebo), probiotics (single species *Bifidobacterium*), lactoferrin plus probiotics versus placebo; early trophic feeding versus enteral fasting, enteral antibiotics, antibiotics for preterm rupture of membranes (antibiotics vs. no antibiotics), probiotics (single species *Saccharomyces boulardii*), omega-3 long-chain polyunsaturated fatty acids, colostrum, prophylactic surgical ligation of PDA, lactoferrin versus placebo, slow (up to 24 mL/kg/day) versus faster rates of advancement of enteral feeds, delayed ( $\geq 4$  days) versus early introduction of progressive enteral feeding (24 mL/kg/day), red blood cell transfusions (liberal [greater volume and/or number of RBC transfusions] versus restrictive transfusion), glycerin enemas and suppositories, dobutamine versus dopamine, adjunctive antibiotic treatment in preterm labor.

#### Outcome 4: NEC-Related Mortality

- (1) Effective interventions: None.
- (2) Possibly effective interventions: None.
- (3) Ineffective interventions: None.
- (4) Probably ineffective interventions: None.
- (5) No conclusions possible (Table 4) for arginine supplementation, probiotics (any), enteral antibiotics (aminoglycoside), and oral immunoglobulin.

#### Heterogeneity of Included Studies

We extracted heterogeneity index provided by the included reviews. About 4.5% (5 of 110) of interventions had significant heterogeneity ( $I^2 > 50\%$ ) for NEC, 10% (1 of 10) interventions had significant heterogeneity for surgical NEC, 4.7% (1 of 21) interventions had significant heterogeneity for all-cause mortality; and none of 4 interventions had significant heterogeneity for NEC-related mortality.

#### AMSTAR Score for Quality of Included Reviews

The quality of included reviews was adequate. Six included reviews were judged to be of the highest quality (with scores 11 out of 11), 79 were judged to be of “high quality” (with scores ranging from 8 to 10 out of 11), and 13 to be of “medium quality” (with scores ranging from 4 to 7 out of 11). Inappropriate combining of studies and presence or suspicion of publication bias were the 2 most common reasons for downgrading quality of the review. We downgraded any meta-analysis that used a fixed-effects model when high heterogeneity existed ( $I^2 > 50\%$ ,  $p$  for chi square  $< 0.10$ ). Assessing publication bias was not applicable if a review had  $< 10$  RCTs. Online supplementary Table 2 provides a breakdown of AMSTAR scores.

#### GRADE Assessment of Certainty of Evidence

In all, 10 of 145 interventions provided evidence of moderate certainty for the specified outcomes with the GRADE classification. Other 135 interventions provided evidence of low or very-low certainty and none evidence of high certainty for specified outcomes. In terms of certainty of evidence for each outcome, 7 of 110 interventions, 1 of 10 interventions, and 2 of 21 interventions were rated as moderate certainty for NEC, surgical NEC and all-cause mortality, respectively. No intervention provided moderate certainty for NEC-related mortality. Online supplementary Tables 3–8 represent summary of finding tables of GRADE assessment for each outcome. Although all the interventions were from RCTs, the certainty of evidence was downgraded because of risk of bias, inconsistency, imprecision, or publication bias.

## Discussion

### *Principal Findings and Possible Explanations*

Many interventions for preventing NEC have been evaluated, but a comprehensive overview to inform the bedside clinician for evidence-based practice does not exist. This is the first overview of systematic reviews of RCTs of interventions to prevent NEC. We summarized existing evidence from 98 systematic reviews of RCTs, which included antenatal and postnatal interventions that reported NEC as a primary or secondary outcome in preterm infants (online suppl. Table 9). Moderate certainty evidence from our overview showed that the risk of NEC was decreased by probiotics (a combination of species), antenatal corticosteroids for women at risk of preterm birth, probiotics (any), and ibuprofen versus indomethacin for the treatment of PDA. For surgical NEC, lower (85–89%) oxygen saturation target levels increased the risk, compared with higher (91–95%) target ranges. A combination of species of probiotics and any probiotics reduced the risk of “all-cause mortality” during hospital stay. No conclusions could be drawn for NEC-related mortality due to lack of data.

Overabundance of pathogenic organisms and lack of microbial diversity in gastrointestinal tract of preterm infants have been reported as key causative factors of NEC. Our findings imply that administration of probiotics decreases NEC and “all cause” mortality possibly by adjusting the microbiome imbalance. The reduced risk of NEC associated with probiotics may also be due to increasing breastfeeding in the probiotics arm of the trials. The certainty of evidence for the use of single species of probiotics is low (*Lactobacillus*, online suppl. Fig 2) or very low (*Bifidobacterium* or *Saccharomyces boulardii*, online suppl. Fig. 3). Unanswered questions for the use of probiotics in preterm infants include selecting the optimal probiotic agent or their combinations, time of initiation, dose, and duration of use.

The certainty of evidence for feeding strategies and type of milk is low to very low including exclusive human milk versus exclusive preterm formula, higher versus lower dose human milk intake, formula milk (term or preterm) versus donor breast milk (unfortified or fortified), fortified breast milk versus unfortified breast milk, hydrolyzed versus nonhydrolyzed formula, protein supplementation of human milk, unpasteurized versus pasteurized human milk, higher versus lower protein intake in formula-fed, slow (up to 24 mL/kg/day) versus faster rates of advancement of enteral feeds, delayed ( $\geq 4$  day) versus early introduction of progressive enteral feeding

(24 mL/kg/day), transpyloric versus gastric tube feeding, early trophic feeding versus enteral fasting, and continuous nasogastric milk feeding versus intermittent bolus milk feed (online suppl. Fig. 2, 3).

The outcomes of surgical NEC, “all-cause” mortality, and NEC-related mortality were reported only in a few of the included reviews. Relative effects for substantial interventions were often from only 1 or 2 trials and hence it was difficult to draw firm conclusions for most interventions due to the low or very low certainty of evidence.

### *Strengths and Limitations of this Overview*

The AMSTAR score is widely accepted as an effective and reliable tool for assessing the quality of systematic reviews. In this overview, the majority of included reviews were of “highest quality” or “high quality.” This is partly because majority of included systematic reviews (66 of 98) were published by the Cochrane Collaboration. Notably, high quality of reviews does not equate to high certainty of the evidence of outcome or high quality of the original studies.

We rated the GRADE certainty of evidence ourselves for consistency even if the original review reported it. The certainty of evidence was at best moderate with the majority of evidence being low or very low certainty. Most frequent reasons for downgrading the certainty were high risk of selection bias (lack of blinding and allocation concealment), imprecision (small sample sizes and/or wide CIs), and suspicion or presence of publication bias. Low to very low certainty of evidence call for more methodologically sound, high-quality studies with multicenter collaboration (if possible international), prospective meta-analysis of ongoing trials, and facilitating publication of trials with negative results. This will help in not downgrading the certainty for high risk of selection bias (lack of blinding and allocation concealment), imprecision (small sample sizes and/or wide CIs), and suspicion or presence of publication bias.

An important limitation of this overview is the definition of NEC, which is based on Bell’s staging based on clinical and radiologic criteria rather than intestinal pathology or etiology. Since other diseases may have similar clinical or radiological presentation as NEC, an accurate diagnosis is not possible in all patients. For example, clinical and radiological signs of stage I NEC are nonspecific and may be confused with sepsis or feeding intolerance. Surgical NEC is difficult to differentiate from spontaneous intestinal perforation in infants with intraperitoneal air. Even for abdominal X-rays findings in stage II NEC, there is high observer variability to distinguish pneuma-

tosis in the intestinal wall from fecal bubbles [34]. The assessment of “all-cause mortality,” especially where NEC is reported as the secondary outcome should be also be interpreted with caution as other factors including the primary condition may contribute to the mortality.

Our umbrella review is a secondary review of systematic reviews, where we summarize existing evidence rather than creating new evidence and has the limitations of secondary analyses of evidence. We strived to minimize potential biases in this review process. First, an overview protocol was registered with the website PROSPERO (Reg. No. CRD42018105634), [https://www.crd.york.ac.uk/PROSPERO/display\\_record.php?RecordID=105634](https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=105634)). Second, 2 authors (T.X. and M.P.) independently searched the databases, collected data, and assessed quality of the included systematic reviews by AMSTAR SCORE (T.X. and A.E.-S.), and assessed certainty of evidence for the outcomes by GRADE (T.X. and M.P.). This provided consistency and reliability of our overview. A potential source of bias is one overview author (M.P.) is the author of one included reviews [15]. A.E.-S., who was not an author of this review, performed the assessment instead of M.P. We only included systematic reviews published in English and we may have missed reviews published in other languages.

Our assessment for the certainty of interventions using the GRADE tool was based on relatively stringent set of criteria. If there were <10 trials for a particular intervention, a high likelihood of publication bias was presumed. Our approach was consistent with the recommendations of GRADEprofiler, which advocates that authors of systematic reviews consider downgrading the certainty of evidence by one level when they are not certain about the magnitude of the effect due to selective publication of studies. Our overview targeted systematic reviews that included RCTs as these studies are likely to have the least bias as compared to reviews of observational studies. To get the maximum and up-to-date evidence, we included not only Cochrane reviews but also other reviews containing RCTs. Alternative or emerging strategies for prevention of NEC may not yet have been assessed in reviews of RCTs.

Risk factors of NEC may include antenatal interventions [35]. One strength of our overview is including antenatal interventions that may influence the outcome of NEC. We found that antenatal corticosteroids, prescribed for accelerating fetal lung maturation in women at risk of preterm birth, reduced the risk of NEC (moderate certainty evidence) [28]. Interestingly, nonpharmacological intervention such as oxygen saturation target levels influ-

enced the outcome of surgical NEC [33]. Considering the multifactorial etiology of NEC, a multipronged strategy including pharmacological and nonpharmacological interventions is likely to have the best outcome.

#### *Implications for Practice and Research*

Despite a large number of interventions, none had high certainty of evidence for NEC prevention. Decreased risk of NEC was observed with probiotics (combination of species), by antenatal corticosteroids for women at risk of preterm birth, and ibuprofen versus indomethacin for the treatment of PDA. Lower (85–89%) oxygen saturation target levels may increase the risk of surgical NEC, compared with higher (91–95%) level. Probiotics, a combination of species or any, decreases the risk of all-cause mortality during hospital stay. We highlight that for most interventions the evidence was of low or very low certainty and statistically significant summary estimates are more likely with such low certainty evidence.

An important consideration is that most studies on NEC are underpowered to detect a clinically significant difference. Studies on NEC (Bell stage >2), especially on surgical NEC (Bell stage >3), require large sample sizes to achieve adequate power. The optimal information size that is required for a definite conclusion to be drawn for an intervention to prevent NEC (assuming a baseline NEC incidence of 5%, for 80% power, alpha of 0.05) for a 20% relative risk reduction, the number of patients required is over 10,000 (Trial sequential analysis software, Copenhagen Trial Unit, Centre for clinical intervention and research, Copenhagen, Denmark, <http://www.ctu.dk/tools-and-links/trial-sequential-analysis.aspx>). The large sample sizes call for large multicenter trials, prospective registry and international collaboration for definite conclusions on effectiveness of these interventions.

#### **Statement of Ethics**

The authors have no ethical conflicts to disclose.

#### **Disclosure Statement**

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## Author Contribution

All authors contributed substantially to this systematic review. T.X. and M.P.: conceptualized the overview of systematic review, performed the search, screened reviews for inclusion, assessed the full text, extracted the data, performed the analysis, and drafted

and revised the manuscript. A.E.-S.: assisted in the searches and performed methodological assessment of the included systematic reviews. J.N. and A.M.: provided critical intellectual input during the design of the overview, analysis, drafting, and revising the manuscript. All authors have seen and approved the final version of the report.

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