Cochrane Library

**Cochrane** Database of Systematic Reviews

# Synbiotics for preventing necrotising enterocolitis in preterm infants (Protocol)

Sharif S, Heath PT, Oddie SJ, McGuire W

Sharif S, Heath PT, Oddie SJ, McGuire W.
Synbiotics for preventing necrotising enterocolitis in preterm infants (Protocol).
*Cochrane Database of Systematic Reviews* 2021, Issue 5. Art. No.: CD014067.
DOI: 10.1002/14651858.CD014067.

**www.cochranelibrary.com**




WILEY

# TABLE OF CONTENTS

[Intervention Protocol]

# Synbiotics for preventing necrotising enterocolitis in preterm infants

Sahar Sharif[1], Paul T Heath[2], Sam J Oddie[3], William McGuire[1]

[1]Centre for Reviews and Dissemination, University of York, York, UK. [2]Division of Child Health and Vaccine Institute, St. George's, University of London, London, UK. [3]Bradford Neonatology, Bradford Teaching Hospitals NHS Foundation Trust, Bradford, UK

**Contact address:** William McGuire, william.mcguire@york.ac.uk.

**Editorial group:** Cochrane Neonatal Group.
**Publication status and date:** New, published in Issue 5, 2021.

**Citation:** Sharif S, Heath PT, Oddie SJ, McGuire W. Synbiotics for preventing necrotising enterocolitis in preterm infants (Protocol). *Cochrane Database of Systematic Reviews* 2021, Issue 5. Art. No.: CD014067. DOI: 10.1002/14651858.CD014067.



## ABSTRACT

### Objectives

This is a protocol for a Cochrane Review (intervention). The objectives are as follows:

To evaluate the effect of enteral supplementation with synbiotics (versus placebo or no treatment, or versus probiotics or prebiotics alone) on the risk of necrotising enterocolitis and associated morbidity and mortality in very preterm or very low birth weight infants.

## BACKGROUND

This review will assess the trial evidence for the effectiveness of enteral synbiotics (combinations of probiotic micro-organisms and prebiotic oligosaccharides) for preventing necrotising enterocolitis (NEC) in very preterm or very low birth weight (VLBW) infants. Other Cochrane Reviews assess the evidence for prebiotics alone or probiotics alone (Sharif 2020).

### Description of the condition

Necrotising enterocolitis is a syndrome of acute intestinal necrosis which affects about one in twenty very preterm (born before 32 weeks' gestation) or VLBW (birth weight less than 1500 g) infants (Horbar 2012). The risk factors for NEC include being extremely preterm (born before 28 weeks' gestation) or extremely low birth weight (ELBW; birth weight less than 1000 g), and intrauterine growth restriction or compromise indicated by absent or reversed end-diastolic flow velocities in antenatal Doppler studies of the umbilical artery or fetal aorta (Samuels 2017). Infants who develop NEC experience more infections, have lower levels of nutrient intake, grow more slowly, and have longer durations of intensive care and hospital stay than gestation-comparable infants who do not (Battersby 2018; Berrington 2012). The associated mortality rate is about 20%. Infants who survive NEC, especially if associated with bloodstream infections, have a higher risk of neurodevelopmental problems and disability compared with their peers (Hickey 2018).

The pathogenesis of NEC is not fully understood but is speculated to involve intestinal dysbiosis, infection and inflammation (Eaton 2017; Mara 2018; Stewart 2016). Evidence exists that the pattern, diversity and stability of the intestinal microbiome is associated with the risk of developing NEC (Masi 2019; Olm 2019; Stewart 2012; Warner 2016). Feeding with human milk compared with cow milk formula reduces the risk of NEC in very preterm or VLBW infants (Cleminson 2015; Quigley 2019). A putative mechanism for this protective effect is that “prebiotic” human milk oligosaccharides promote the growth of non-pathogenic probiotic micro-organisms, such as lactobacilli and bifidobacteria, that modulate the intestinal microbiome and promote mucosal barrier functions (Embleton 2017; Granger 2020; Walsh 2019). Compared with human milk-fed term infants, however, very preterm or VLBW infants tend to harbour fewer intestinal probiotic micro-organisms, and more potential pathogens, which might be due to dysbiotic effects of enteral fasting and antibiotic exposure (Stewart 2017).

### Description of the intervention

#### Synbiotics (probiotic-prebiotic combinations)

Synbiotics are combinations of probiotics and prebiotics. The prebiotic content is intended to enhance probiotic growth and intestinal colonisation (Nolan 2020).

#### *Probiotics*

Probiotics are live micro-organisms (predominantly bifidobacteria and lactobacilli) that benefit the host by modulating the intestinal microbiome and promoting mucosal barrier functions and resistance to pathogens (Berrington 2019; Esaiassen 2018). Preterm infants supplemented enterally with bifidobacteria and lactobacilli establish an intestinal microbiome that is dominated by probiotics and contains fewer potential pathogens compared with non-supplemented infants (Alcon-Giner 2020). Meta-analysis of data from more than 50 randomised controlled trials (RCTs) - which used a variety of probiotic strains and multi-organism combinations - suggests that probiotic supplementation may reduce the risk of NEC, and probably reduces mortality and late-onset infection in very preterm or VLBW infants (Sharif 2020). The certainty of this evidence, however, is low because of concerns that effect estimates are inflated by methodological weaknesses in the (mainly small) trials and by publication bias. Consequently, and because of ongoing issues about safety and the availability of regulated products, probiotic supplementation has not become established as a common practice in most neonatal care facilities (Duffield 2019; Fleming 2019; Pell 2019; Vermeulen 2020).

#### *Prebiotics*

Prebiotics are a diverse family of complex glycans that promote intestinal colonisation with probiotic micro-organisms. Human milk contains numerous prebiotic substances, predominantly galacto-oligosaccharides and fructo-oligosaccharides, that influence the intestinal microbiome in preterm infants (Boehm 2008; Nolan 2020). Natural human milk oligosaccharides vary markedly between individual women, and vary temporally (depending on the stage of lactation) within individual woman. The quantity and types of oligosaccharides affect the pattern of the probiotic microbiome of a woman’s colostrum (Aakko 2017; Smilowitz 2013). Newborn infants do not digest human milk oligosaccharides. Rather, these are primarily nutrient sources for intestinal probiotic micro-organisms, particularly bifidobacteria (Alcon-Giner 2020; Jost 2015). There is emerging evidence about how specific human milk oligosaccharides promote probiotic predominance and reduce intestinal dysbiosis in very preterm infants (Masi 2020; Underwood 2015).

Manufactured (synthetic) prebiotic oligosaccharides are less heterogeneous than natural human milk oligosaccharides, typically consisting of short chains of galactose or fructose, usually with a terminal glucose monomer (Johnson-Henry 2016). Evidence exists that giving supplemental synthetic prebiotic oligosaccharides to formula-fed very preterm infants stimulates the growth of an intestinal microflora that is similar to that found in infants fed with maternal milk (Autran 2018; Boehm 2008; Kapiki 2007; Veereman-Wauters 2011). RCTs, however, have not provided evidence of their effectiveness in preventing NEC or associated morbidity or mortality (Chi 2019; Johnson-Henry 2016; Srinivasjois 2013).

### How the intervention might work

It is postulated that administering supplemental prebiotic oligosaccharides enhances both exogenous and endogenous probiotic growth and intestinal colonisation (Nolan 2020; Underwood 2019). Probiotic bacteria and fungi use prebiotic oligosaccharides as a major nutrient source (Alcon-Giner 2020). Bifidobacteria and lactobacilli ferment prebiotic oligosaccharides to produce short-chain fatty acids that inhibit adhesion of pathogenic bacteria and modulate intestinal epithelial integrity and barrier function (Johnson-Henry 2016). Synbiotic combinations, therefore, may be more effective than supplementation with either a probiotic or prebiotic alone (Zmora 2018). A recent RCT involving more than 4500

newborn infants (birth weight at least 2000 grams or gestation greater than 34 weeks) in rural India showed that enteral synbiotic supplementation (*Lactobacillus plantarum* plus fructo-oligosaccharide) was associated with a reduced risk of neonatal sepsis (Panigrahi 2017).

### Why it is important to do this review

Necrotising enterocolitis and associated complications, particularly infection, are the commonest causes of mortality and serious morbidity beyond the early neonatal period in very preterm or VLBW infants (Berrington 2012). Current trial data do not provide high certainty evidence that probiotic or prebiotic supplementation alone reduces the risk of NEC (Quigley 2019). Given the plausibility that synbiotics might have an advantage over probiotics or prebiotics alone, appraising and synthesising the trial evidence about the effectiveness and safety of synbiotic supplementation could inform practice, policy and research.

## OBJECTIVES

To evaluate the effect of enteral supplementation with synbiotics (versus placebo or no treatment, or versus probiotics or prebiotics alone) on the risk of necrotising enterocolitis and associated morbidity and mortality in very preterm or very low birth weight infants.

## METHODS

### Criteria for considering studies for this review

#### Types of studies

Randomised or quasi-randomised controlled trials (including cluster-randomised controlled trials). Cross-over studies will not be eligible for inclusion.

#### Types of participants

Very preterm (< 32 weeks' gestation) or VLBW (< 1500 grams) infants.

#### Types of interventions

##### *Intervention*

Enteral synbiotics: any combination or dose of probiotic organisms and prebiotic oligosaccharides, commenced within 14 days of birth and continued (at least) daily for (at least) one week. Probiotics and prebiotics need not be given simultaneously, but should be given (at least) on the same day.

#### Types of outcome measures

##### *Primary outcomes*

- NEC confirmed at surgery or autopsy or using standardised clinical and radiological criteria (VON 2020):
  - at least one of: bilious gastric aspirate or emesis; or abdominal distention; or blood in stool; *and*
  - at least one of: abdominal radiograph showing pneumatosis intestinalis; or gas in the portal venous system; or free air in the abdomen.
- All-cause mortality before discharge from hospital.

##### *Secondary outcomes*

- Late-onset invasive infection, as determined by culture of bacteria or fungus from blood or cerebrospinal fluid or from a normally sterile body space (> 48 hours after birth).
- Late-onset infection with the supplemented probiotic micro-organism.
- Duration of hospitalisation since birth.
- Neurodevelopmental impairment assessed by a validated test after 12 months' post-term: neurological evaluations, developmental scores, and classifications of disability, including cerebral palsy and auditory and visual impairment.

### Search methods for identification of studies

We will use the criteria and standard methods of Cochrane Neonatal.

#### Electronic searches

We will search the Cochrane Central Register of Controlled Trials (CENTRAL, 2020, current issue), Ovid MEDLINE (1946 to date), OVID Embase (1974 to present), OVID Maternity & Infant Care Database (1971 to present), and the Cumulative Index to Nursing and Allied Health Literature (1982 to present) using a combination of text words and MeSH terms described in Appendix 1. We will limit the search outputs with the relevant search filters for clinical trials as recommended in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2020). We will not apply any language restrictions.

We will search clinical trials registries for ongoing or recently completed trials (clinicaltrials.gov; the World Health Organization's International Trial Registry and Platform, and the ISRCTN Registry).

#### Searching other resources

We will examine the reference lists of any articles selected for inclusion in this review.

### Data collection and analysis

We will use the standard methods of Cochrane Neonatal.

#### Selection of studies

Two review authors (SS, PTH or SO) will independently screen the title and abstract of all studies and assess the full articles for all potentially relevant trials. We will exclude those studies that do not meet all of the inclusion criteria and we will state the reason(s) for exclusion. We will discuss any disagreements until consensus is achieved, with referral to WM for final decision as necessary.

#### Data extraction and management

Two authors (SS, PTH, SO or WM) will extract data independently, using a form to aid extraction of information on design, methodology, participants, interventions, outcomes and treatment effects from each included study. We will discuss any disagreements until we reach a consensus. If data from the study reports are insufficient, we will contact the report authors for further information.

### Assessment of risk of bias in included studies

Two review authors (SS, PTH, SO or WM) will assess independently the risk of bias (low, high or unclear) of all included trials using the Cochrane 'Risk of bias' tool (Higgins 2011), for these domains:

- sequence generation (selection bias);
- allocation concealment (selection bias);
- blinding of participants and personnel (performance bias);
- blinding of outcome assessment (detection bias);
- incomplete outcome data (attrition bias);
- selective reporting (reporting bias);
- any other bias (including baseline imbalance).

We will resolve disagreements through discussion or by involving a third assessor. See Appendix 2 for a description of risk of bias for each domain.

### Measures of treatment effect

We will analyse the treatment effects in the individual trials and report risk ratio (RR) and risk difference (RD) for dichotomous data and mean difference (MD) for continuous data, with respective 95% confidence intervals (CI). We will determine the number needed to treat for an additional beneficial outcome (NNTB) or an additional harmful outcome (NNTH) for analyses with a statistically significant difference in the RD.

### Unit of analysis issues

The unit of analysis will be the participating infant in individually randomised trials and the neonatal unit (or sub-unit) for cluster-randomised trials. For cluster-randomised trials, we will undertake analyses at the level of the individual while accounting for the clustering in the data using the methods recommended in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2020).

### Dealing with missing data

We will request additional data from trial investigators when data on important outcomes are missing or are reported unclearly. If unavailable, we plan to undertake sensitivity analyses to assess the potential impact of missing outcome data (> 20%).

### Assessment of heterogeneity

We will examine the treatment effects of individual trials and heterogeneity between trial results by inspecting the forest plots. We will calculate the $I^2$ statistic for each analysis to quantify inconsistency across studies and describe the percentage of variability in effect estimates that may be due to heterogeneity rather than to sampling error. If we detect high levels of heterogeneity ($I^2$ > 75%), we will explore the possible sources (for example, differences in study design, participants, interventions or completeness of outcome assessments).

### Assessment of reporting biases

If at least 10 trials are included in a meta-analysis, we will examine a funnel plot for asymmetry visually and with Harbord's modification of Egger's test (Harbord 2006).

### Data synthesis

We will use a fixed-effect model for meta-analyses. When moderate or high heterogeneity is detected, we plan to examine the potential causes in subgroup and sensitivity (by methodological quality) analyses.

### Subgroup analysis and investigation of heterogeneity

Where data are available, we will undertake subgroup analyses for the primary outcomes:

- extremely preterm (< 28 weeks' gestation) or ELBW (< 1000 grams) infants);
- genus of probiotics or combinations (*Bifidobacterium* spp. (species plural)., *Lactobacillus spp., Sacchromyces spp., Streptococcal spp.,* others, and combinations thereof);
- type of prebiotic oligosaccharide: natural versus synthetic
- type of enteral feeding permitted for participating infants (human milk versus formula versus mixed).

### Sensitivity analysis

We will undertake sensitivity analyses to determine how estimates are affected by including only studies at low risk of bias: (i) selection bias (adequate randomisation and allocation concealment), (ii) detection or performance bias (adequate masking of intervention and measurement), (iii) attrition bias (< 20% loss to follow-up for primary outcome assessment), and (iv) reporting bias (selective reporting).

### Summary of findings and assessment of the certainty of the evidence

We will use the GRADE approach to assess the certainty of evidence of these outcomes (Schünemann 2013): NEC, all-cause mortality, late-onset infection and severe neurodevelopmental impairment.

Two review authors (SS, PTH, SO or WM) will assess independently the certainty of the evidence for each of the outcomes. We will consider evidence from randomised controlled trials as high certainty but downgrade the evidence one level for serious (or two levels for very serious) limitations based upon: design (risk of bias); consistency across studies; directness of the evidence; precision of estimates; and presence of publication bias (Appendix 3).

We will use the GRADEpro GDT Guideline Development Tool to create 'Summary of findings' tables to report the certainty of the evidence.

## ACKNOWLEDGEMENTS

We thank Cochrane Neonatal: Colleen Ovelman, Managing Editor; Jane Cracknell, Assistant Managing Editor; Roger Soll, Co-coordinating editor, who provided editorial and administrative support; and Kath Wright (CRD, York) for the electronic search strategy and database management. Carol Friesen, Cochrane Neonatal Information Specialist, peer reviewed the searches.

Jeffrey Horbar and Souvik Mitra have peer reviewed and offered feedback on this protocol.

The methods section of the protocol is based on a standard template used by Cochrane Neonatal.

# REFERENCES

## Additional references


### Aakko 2017

Aakko J, Kumar H, Rautava S, Wise A, Autran C, Bode L, et al. Human milk oligosaccharide categories define the microbiota composition in human colostrum. *Beneficial Microbes* 2017;**8**(4):563-7. [DOI: 10.3920/BM2016.0185]] [PMID: 28726512]

### Alcon-Giner 2020

Alcon-Giner C, Dalby MJ, Caim S, Ketskemety J, Shaw A, Sim K, et al. Microbiota supplementation with Bifidobacterium and Lactobacillus modifies the preterm infant gut microbiota and metabolome: an observational study. *Cell Reports Medicine* 2020;**1**(5):100077. [DOI: 10.1016/j.xcrm.2020.100077] [PMID: 32904427]

### Autran 2018

Autran CA, Kellman BP, Kim JH, Asztalos E, Blood AB , Spence EC, et al. Human milk oligosaccharide composition predicts risk of necrotising enterocolitis in preterm infants. *Gut* 2018;**67**(6):1064-70. [DOI: 10.1136/gutjnl-2016-312819] [PMID: 28381523]

### Battersby 2018

Battersby C, Santhalingam T, Costeloe K, Modi N. Incidence of neonatal necrotising enterocolitis in high-income countries: a systematic review. *Archives of Disease in Childhood. Fetal and Neonatal Edition* 2018;**103**(2):F182-9. [DOI: 10.1136/archdischild-2017-313880] [PMID: 29317459]

### Berrington 2012

Berrington JE, Hearn RI, Bythell M, Wright C, Embleton ND. Deaths in preterm infants: changing pathology over 2 decades. *Journal of Pediatrics* 2012;**160**(1):49-53.e1. [DOI: 10.1016/j.jpeds.2011.06.046] [PMID: 21868028]

### Berrington 2019

Berrington JE, Zalewski S. The future of probiotics in the preterm infant. *Early Human Development* 2019;**135**:75-81. [DOI: 10.1016/j.earlhumdev.2019.05.008] [PMID: 31130262]

### Boehm 2008

Boehm G, Moro G. Structural and functional aspects of prebiotics used in infant nutrition. *Journal of Nutrition* 2008;**138**(9):1818S-28S. [DOI: 10.1093/jn/138.9.1818S] [PMID: 18716193]

### Chi 2019

Chi C, Buys N, Li C, Sun J, Yin C. Effects of prebiotics on sepsis, necrotizing enterocolitis, mortality, feeding intolerance, time to full enteral feeding, length of hospital stay, and stool frequency in preterm infants: a meta-analysis. *European Journal of Clinical Nutrition* 2019;**73**(5):657-70. [DOI: 10.1038/s41430-018-0377-6] [PMID: 30568297]

### Cleminson 2015

Cleminson J, Oddie S, Renfrew MJ, McGuire W. Being baby friendly: evidence-based breastfeeding support. *Archives of Disease in Childhood. Fetal and Neonatal Edition* 2015;**100**(2):F173-8. [DOI: 10.1136/archdischild-2013-304873] [PMID: 25293712]

### Duffield 2019

Duffield SD, Clarke P. Current use of probiotics to prevent necrotising enterocolitis. *Archives of Disease in Childhood. Fetal and Neonatal Edition* 2019;**104**(2):F228. [DOI: 10.1136/archdischild-2018-316199] [PMID: 30464004]

### Eaton 2017

Eaton S, Rees CM, Hall NJ. Current research on the epidemiology, pathogenesis, and management of necrotizing enterocolitis. *Neonatology* 2017;**111**(4):423-30. [DOI: 10.1159/000458462] [PMID: 28538238]

### Embleton 2017

Embleton ND, Berrington JE, Dorling J, Ewer AK, Juszczak E, Kirby JA, et al. Mechanisms affecting the gut of preterm infants in enteral feeding trials. *Frontiers in Nutrition* 2017;**4**:14. [DOI: 10.3389/fnut.2017.00014] [PMID: 28534028]

### Esaiassen 2018

Esaiassen E, Hjerde E, Cavanagh JP, Pedersen T, Andresen JH, Rettedal SI, et al. Effects of probiotic supplementation on the gut microbiota and antibiotic resistome development in preterm infants. *Frontiers in Pediatrics* 2018;**6**:347. [DOI: 10.3389/fped.2018.00347] [PMID: 30505830]

### Fleming 2019

Fleming PF, Berrington JE, Jacobs SE. Addressing safety concerns of probiotic use in preterm babies. *Early Human Development* 2019;**135**:72-4. [DOI: 10.1016/j.earlhumdev.2019.05.016] [PMID: 31155280]

### GRADEpro GDT [Computer program]

McMaster University (developed by Evidence Prime) GRADEpro GDT. Version accessed 12 May 2020. Hamilton (ON): McMaster University (developed by Evidence Prime). Available at gradepro.org.

### Granger 2020

Granger CL, Embleton ND, Palmer JM, Lamb CA, Berrington JE, Stewart CJ. Maternal breast milk, infant gut microbiome, and the impact on preterm infant health. *Acta Paediatrica* 2020;**110**(2):450-7. [DOI: 10.1111/apa.15534] [PMID: 33245565]

### Harbord 2006

Harbord RM, Egger M, Sterne JA. A modified test for small-study effects in meta-analyses of controlled trials with binary endpoints. *Statistics in Medicine* 2006;**25**(20):3443-57. [DOI: 10.1002/sim.2380] [PMID: 16345038]

### Hickey 2018

Hickey M, Georgieff M, Ramel S. Neurodevelopmental outcomes following necrotizing enterocolitis. *Seminars in Fetal and Neonatal Medicine* 2018;**23**(6):426-32. [DOI: 10.1016/j.siny.2018.08.005] [PMID: 30145060]

**Higgins 2011**

Higgins JP, Altman DG, Sterne JA, on behalf of the Cochrane Statistical Methods Group and the Cochrane Bias Methods Group. Chapter 8: Assessing risk of bias in included studies. In: Higgins JP, Green S, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updated March 2011). The Cochrane Collaboration, 2011. Available from handbook.cochrane.org.

**Higgins 2020**

Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, et al (editors). Cochrane Handbook for Systematic Reviews of Interventions version 6.1 (updated September 2020). Cochrane, 2020. Available from www.training.cochrane.org/handbook.

**Horbar 2012**

Horbar JH, Carpenter JH, Badger GJ, Kenny MJ, Soll RF, Morrow KA, et al. Mortality and neonatal morbidity among infants 501 to 1500 grams from 2000 to 2009. *Pediatrics* 2012;**129**(6):1019-26. [DOI: 10.1542/peds.2011-3028] [PMID: 22614775]

**Johnson-Henry 2016**

Johnson-Henry KC, Abrahamsson TR, Wu RY, Sherman PM. Probiotics, prebiotics, and synbiotics for the prevention of necrotizing enterocolitis. *Advances in Nutrition* 2016;**7**(5):928-37. [DOI: 10.3945/an.116.012237] [PMID: 27633108]

**Jost 2015**

Jost T, Lacroix C, Braegger C, Chassard C. Impact of human milk bacteria and oligosaccharides on neonatal gut microbiota establishment and gut health. *Nutrition Reviews* 2015;**73**(7):426-37. [DOI: 10.1093/nutrit/nuu016] [PMID: 26081453]

**Kapiki 2007**

Kapiki A, Costalos C, Oikonomidou C, Triantafyllidou A, Loukatou E, Pertrohilou V. The effect of a fructo-oligosaccharide supplemented formula on gut flora of preterm infants. *Early Human Development* 2007;**83**:335-9. [DOI: 10.1016/j.earlhumdev.2006.07.003]] [PMID: 16978805]

**Mara 2018**

Mara MA, Good M, Weitkamp JH. Innate and adaptive immunity in necrotizing enterocolitis. *Seminars in Fetal Neonatal Medicine* 2018;**23**:394-9. [DOI: 10.1016/j.siny.2018.08.002] [DOI: 30146477]

**Masi 2019**

Masi AC, Stewart CJ. The role of the preterm intestinal microbiome in sepsis and necrotising enterocolitis. *Early Human Development* 2019;**138**:104854. [DOI: 10.1016/j.earlhumdev.2019.104854] [PMID: 31481262]

**Masi 2020**

Masi AC, Embleton ND, Lamb CA, Young G, Granger CL, Najera J, et al. Human milk oligosaccharide DSLNT and gut microbiome in preterm infants predicts necrotising enterocolitis. Gut 2020 [Epub ahead of print]. [DOI: 10.1136/gutjnl-2020-322771] [PMID: 33328245]

**Nolan 2020**

Nolan LS, Rimer JM, Good M. The role of human milk oligosaccharides and probiotics on the neonatal microbiome and risk of necrotizing enterocolitis: a narrative review. *Nutrients* 2020;**12**(10):3052. [DOI: 10.3390/nu12103052]] [PMID: 33036184]

**Olm 2019**

Olm MR, Bhattacharya N, Crits-Christoph A, Firek BA, Baker R, Song YS, et al. Necrotizing enterocolitis is preceded by increased gut bacterial replication, Klebsiella, and fimbriae-encoding bacteria. *Science Advances* 2019;**5**(12):eaax5727. [DOI: 10.1126/sciadv.aax5727] [PMID: 31844663]

**Panigrahi 2017**

Panigrahi P, Parida S, Nanda NC, Satpathy R, Pradhan L, Chandel DS, et al. A randomized synbiotic trial to prevent sepsis among infants in rural India. *Nature* 2017;**548**(7668):407-12. [DOI: 10.1038/nature23480] [PMID: 28813414]

**Pell 2019**

Pell LG, Loutet MG, Roth DE, Sherman PM. Arguments against routine administration of probiotics for NEC prevention. *Current Opinions in Pediatrics* 2019;**31**(2):195-201. [DOI: 10.1097/MOP.0000000000000730] [PMID: 30624281]

**Quigley 2019**

Quigley M, Embleton ND, McGuire W. Formula versus donor breast milk for feeding preterm or low birth weight infants. *Cochrane Database of Systematic Reviews* 2019, Issue 7. Art. No: CD002971. [DOI: 10.1002/14651858.CD002971.pub5] [PMID: 31322731]

**Samuels 2017**

Samuels N, Van de Graaf RA, de Jonge RC, Reiss IK, Vermeulen MJ. Risk factors for necrotizing enterocolitis in neonates: a systematic review of prognostic studies. *BMC Pediatrics* 2017;**17**(1):105. [DOI: 10.1186/s12887-017-0847-3] [PMID: 28410573]

**Schünemann 2013**

Schünemann H, Brożek J, Guyatt G, Oxman A, editor(s). Handbook for grading the quality of evidence and the strength of recommendations using the GRADE approach (updated October 2013). GRADE Working Group, 2013. Available from gdt.guidelinedevelopment.org/app/handbook/handbook.html.

**Sharif 2020**

Sharif S, Meader N, Oddie SJ, Rojas-Reyes MX, McGuire W. Probiotics to prevent necrotising enterocolitis in very preterm or very low birth weight infants. *Cochrane Database of Systematic Reviews* 2020, Issue 10. Art. No: CD005496. [DOI: 10.1002/14651858.CD005496.pub5]

**Smilowitz 2013**

Smilowitz JT, O'Sullivan A, Barile D, German JB, Lönnerdal B, Slupsky CM. The human milk metabolome reveals diverse oligosaccharide profiles. *Journal of Nutrition* 2013;**143**(11):1709-18. [DOI: 10.3945/jn.113.178772] [PMID: 24027187]

**Srinivasjois 2013**

Srinivasjois R, Rao S, Patole S. Prebiotic supplementation in preterm neonates: updated systematic review and meta-analysis of randomised controlled trials. *Clinical Nutrition* 2013;**32**(6):958-65. [DOI: 10.1016/j.clnu.2013.05.009] [PMID: 23786897]

**Stewart 2012**

Stewart CJ, Marrs EC, Magorrian S, Nelson A, Lanyon C, Perry JD, et al. The preterm gut microbiota: changes associated with necrotizing enterocolitis and infection. *Acta Paediatrica* 2012;**101**(11):1121-7. [DOI: 10.1111/j.1651-2227.2012.02801.x] [PMID: 22845166]

**Stewart 2016**

Stewart CJ, Embleton ND, Marrs EC, Smith DP, Nelson A, Abdulkadir B, et al. Temporal bacterial and metabolic development of the preterm gut reveals specific signatures in health and disease. *Microbiome* 2016;**4**(1):67. [DOI: 10.1186/s40168-016-0216-8] [PMID: 28034304]

**Stewart 2017**

Stewart CJ, Embleton ND, Marrs EC, Smith DP, Fofanova T, Nelson A, et al. Longitudinal development of the gut microbiome and metabolome in preterm neonates with late onset sepsis and healthy controls. *Microbiome* 2017;**5**(1):75. [DOI: 10.1186/s40168-017-0295-1] [PMID: 28701177]

**Underwood 2015**

Underwood MA, Gaerlan S, De Leoz ML, Dimapasoc L, Kalanetra KM, Lemay DG, et al. Human milk oligosaccharides in premature infants: absorption, excretion, and influence on the intestinal microbiota. *Pediatric Research* 2015;**78**(6):670-7. [DOI: 10.1038/pr.2015.162] [PMID: 26322410]

**Underwood 2019**

Underwood MA. Probiotics and human milk oligosaccharides in premature infants. *Neoreviews* 2019;**20**(1):e1-1. [DOI: 10.1542/neo.20-1-e1] [PMID: 31261069]

**Veereman-Wauters 2011**

Veereman-Wauters G, Staelens S, Van de Broek H, Plaskie K, Wesling F, Roger LC, et al. Physiological and bifidogenic effects of prebiotic supplements in infant formulae. *Journal of Pediatric Gastroenterology and Nutrition* 2011;**52**(6):763-71. [DOI: 10.1097/MPG.0b013e3182139f39] [PMID: 21593649]

**Vermeulen 2020**

Vermeulen MJ, Luijendijk A, Van Toledo L, Van Kaam AH, Reiss IK. Quality of probiotic products for preterm infants: contamination and missing strains. *Acta Paediatrica* 2020;**109**(2):276-9. [DOI: 10.1111/apa.14976] [PMID: 31423636]

**VON 2020**

Vermont Oxford Network. Manual of Operations. Data Definitions & Infant Data Booklets 2020;**Part 2**(Release 25.0).

**Walsh 2019**

Walsh V, McGuire W. Immunonutrition for preterm infants. *Neonatology* 2019;**115**(4):398-405. [DOI: 10.1159/000497332] [PMID: 30974431]

**Warner 2016**

Warner BB, Deych E, Zhou Y, Hall-Moore C, Weinstock GM, Sodergren E, et al. Gut bacteria dysbiosis and necrotising enterocolitis in very low birthweight infants: a prospective case-control study. *Lancet* 2016;**387**(10031):1928-36. [DOI: 10.1016/S0140-6736(16)00081-7] [PMID: 26969089]

**Zmora 2018**

Zmora N, Zilberman-Schapira G, Suez J, Mor U, Dori-Bachash M, Bashiardes S, et al. Personalized gut mucosal colonization resistance to empiric probiotics is associated with unique host and microbiome features. *Cell* 2018;**174**(6):1388-405. [DOI: 10.1016/j.cell.2018.08.041] [PMID: 30193112]

# APPENDICES

## Appendix 1. Electronic search strategy

Indicative strategy developed and tested for Cochrane Register of Controlled Trials (CENTRAL)

[To be adapted for MEDLINE, Embase, Maternity & Infant Care Database (MIDIRS), and CINAHL Plus]

#1 MeSH descriptor: [Probiotics] explode all trees

#2 (probiotic*):ti,ab,kw (Word variations have been searched)

#3 MeSH descriptor: [Bifidobacterium] explode all trees

#4 (bifidobacterium*):ti,ab,kw (Word variations have been searched)

#5 MeSH descriptor: [Lactobacillus] explode all trees

#6 (lactobacill*):ti,ab,kw (Word variations have been searched)

#7 MeSH descriptor: [undefined] explode all trees

#8 MeSH descriptor: [Saccharomyces boulardii] this term only

#9 (Saccharomyces):ti,ab,kw (Word variations have been searched)

#10 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9

#11 MeSH descriptor: [Prebiotics] explode all trees

#12 (prebiotic*):ti,ab,kw (Word variations have been searched)

#13 MeSH descriptor: [Oligosaccharides] explode all trees

#14 (oligosaccharide*):ti,ab,kw (Word variations have been searched)

#15 MeSH descriptor: [Inulin] explode all trees

#16 (inulin*):ti,ab,kw (Word variations have been searched)

#17 ((fructooligosaccharide* or fructo-oligosaccharide* or FOS or FOSs or galacto-oligosaccharide* or galactooligosaccharide*)):ti,ab,kw (Word variations have been searched)

#18 MeSH descriptor: [Lactoferrin] explode all trees

#19 (lactoferrin*):ti,ab,kw (Word variations have been searched)

#20 MeSH descriptor: [Lactulose] explode all trees

#21 (lactulose*):ti,ab,kw

#22 #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 or #20 or #21

#23 MeSH descriptor: [Synbiotics] explode all trees

#24 (synbiotic*):ti,ab,kw (Word variations have been searched)

#25 (((probiotic* and prebiotic*) NEAR/4 combin*)):ti,ab,kw (Word variations have been searched)

#26 #23 OR #24 OR #25

#27 #10 AND #22 AND #26

#28 MeSH descriptor: [Infant, Newborn] explode all trees

#29 MeSH descriptor: [Premature Birth] explode all trees

#30 neonat*:ti,ab,kw (Word variations have been searched)

#31 neo-nat*:ti,ab,kw (Word variations have been searched)

#32 newborn or new born* or newly born*:ti,ab,kw (Word variations have been searched)

#33 preterm or preterms or (pre term) or (pre terms):ti,ab,kw (Word variations have been searched)

#34 preemie* or premie or premies:ti,ab,kw (Word variations have been searched)

#35 prematur* near/3 (birth* or born or deliver*):ti,ab,kw (Word variations have been searched)

#36 low near/3 (birthweight* or birth weight*):ti,ab,kw (Word variations have been searched)

#37 lbw or vlbw or elbw:ti,ab,kw (Word variations have been searched)

#38 infan* or baby or babies:ti,ab,kw (Word variations have been searched)

#39 #28 or #29 or #30 or #31 or #32 or #33 or #34 or #35 or #36 or #37 or #38

#40 #27 AND #39

---

## Appendix 2. 'Risk of bias' tool

***Sequence generation (checking for possible selection bias). Was the allocation sequence adequately generated?***

For each included study, we will categorise the method used to generate the allocation sequence as:

- low risk (any truly random process, e.g. random number table; computer random number generator);
- high risk (any non-random process, e.g. odd or even date of birth; hospital or clinic record number); or
- unclear risk.

***Allocation concealment (checking for possible selection bias). Was allocation adequately concealed?***

For each included study, we will categorise the method used to conceal the allocation sequence as:

- low risk (e.g. telephone or central randomisation; consecutively numbered sealed opaque envelopes);
- high risk (open random allocation; unsealed or non-opaque envelopes, alternation; date of birth); or
- unclear risk.

***Blinding of participants and personnel (checking for possible performance bias). Was knowledge of the allocated intervention adequately prevented during the study?***

For each included study, we will categorise the methods used to blind study participants and personnel from knowledge of which intervention a participant received. Blinding will be assessed separately for different outcomes or class of outcomes. We will categorise the methods as:

- low risk, high risk or unclear risk for participants; and
- low risk, high risk or unclear risk for personnel.

***Blinding of outcome assessment (checking for possible detection bias). Was knowledge of the allocated intervention adequately prevented at the time of outcome assessment?***

For each included study, we will categorise the methods used to blind outcome assessment. Blinding will be assessed separately for different outcomes or class of outcomes. We will categorise the methods as:

- low risk for outcome assessors;
- high risk for outcome assessors; or
- unclear risk for outcome assessors.

***Incomplete outcome data (checking for possible attrition bias through withdrawals, dropouts, protocol deviations). Were incomplete outcome data adequately addressed?***

For each included study and for each outcome, we will describe the completeness of data including attrition and exclusions from the analysis. We will note whether attrition and exclusions were reported, the numbers included in the analysis at each stage (compared with the total randomised participants), reasons for attrition or exclusion where reported, and whether missing data were balanced across groups or were related to outcomes. Where sufficient information was reported or supplied by the trial authors, we will re-include missing data in the analyses. We will categorise the methods as:

- low risk (< 20% missing data);
- high risk (≥ 20% missing data); or
- unclear risk.

***Selective reporting bias. Are reports of the study free of suggestion of selective outcome reporting?***

For each included study, we will describe how we investigated the possibility of selective outcome reporting bias and what we found. For studies in which study protocols were published in advance, we will compare prespecified outcomes versus outcomes eventually reported in the published results. If the study protocol was not published in advance, we will contact study authors to gain access to the study protocol. We will assess the methods as:

- low risk (where it is clear that all of the study's prespecified outcomes and all expected outcomes of interest to the review have been reported);
- high risk (where not all the study's prespecified outcomes have been reported; one or more reported primary outcomes were not prespecified outcomes of interest and are reported incompletely and so cannot be used; study fails to include results of a key outcome that would have been expected to have been reported); or
- unclear risk.

***Other sources of bias. Was the study apparently free of other problems that could put it at a high risk of bias?***

For each included study, we will describe any important concerns we had about other possible sources of bias (for example, whether there was a potential source of bias related to the specific study design or whether the trial was stopped early due to some data-dependent process). We will assess whether each study was free of other problems that could put it at risk of bias as:

- low risk;
- high risk;
- unclear risk.

If needed, we will explore the impact of the level of bias through undertaking sensitivity analyses.

### Appendix 3. GRADE

GRADE considers that evidence from randomised controlled trials is of high certainty, but that assessment may be downgraded based on consideration of any of five areas.

- Design (risk of bias).
- Consistency across studies.
- Directness of evidence.
- Precision of estimates.
- Presence of publication bias.

Usually, the quality rating will be downgraded by one level for each factor, up to a maximum of three levels for all factors. If there are very severe problems for any one factor (e.g. when assessing limitations in design and implementation, all trials were unconcealed, unmasked, and lost over 50% of their participants to follow-up), trial evidence may be downgraded by two levels due to that factor alone.

This results in an assessment of the certainty of a body of evidence in one of four grades.

**High certainty:** we are very confident that the true effect lies close to that of the estimate of the effect.
**Moderate certainty:** we are moderately confident in the effect estimate; the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
**Low certainty:** our confidence in the effect estimate is limited; the true effect may be substantially different from the estimate of the effect.
**Very low certainty:** we have very little confidence in the effect estimate; the true effect is likely to be substantially different from the estimate of effect.

## HISTORY

Protocol first published: Issue 5, 2021

## CONTRIBUTIONS OF AUTHORS

All authors contributed to the development of the protocol.

## DECLARATIONS OF INTEREST

SS has declared that they have no conflict of interest.

PTH has declared that they have no conflict of interest.

SJO has declared that they have no conflict of interest.

WM has declared that they have no conflict of interest.

## SOURCES OF SUPPORT

### Internal sources

- Centre for Reviews and Dissemination, University of York, UK

### External sources

- Vermont Oxford Network, USA

  Cochrane Neonatal Reviews are produced with support from Vermont Oxford Network, a worldwide collaboration of health professionals dedicated to providing evidence-based care of the highest quality for newborn infants and their families.

- National Institute for Health Research (NIHR), UK

  This report is independent research funded by a UK NIHR Cochrane Programme Grant (16/114/03). The views expressed in this publication are those of the review authors and are not necessarily those of the National Health Service, the NIHR, or the UK Department of Health.