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Early fortification of human milk versus late fortification to promote growth in preterm infants (Review)

Thanigainathan S, Abiramalatha T

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[Intervention Review]

Early fortification of human milk versus late fortification to promote growth in preterm infants

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ABSTRACT

Background

Uncertainty exists about the optimal point at which multi-component fortifier should be added to human milk for promoting growth in preterm infants. The most common practice is to start fortification when the infant's daily enteral feed volume reaches 100 mL/kg body weight. Another approach is to commence fortification earlier, in some cases as early as the first enteral feed. Early fortification of human milk could increase nutrient intake and growth rates but may increase the risk of feed intolerance and necrotising enterocolitis (NEC).

Objectives

To assess effects on growth and safety of early fortification of human milk versus late fortification in preterm infants

To assess whether effects vary based upon gestational age (≤ 27 weeks; 28 to 31 weeks; ≥ 32 weeks), birth weight (< 1000 g; 1000 to 1499 g; ≥ 1500 g), small or appropriate for gestational age, or type of fortifier (bovine milk-based human milk fortifier (HMF); human milk-based HMF; formula powder)

Search methods

We used the standard strategy of Cochrane Neonatal to search the Cochrane Central Register of Controlled Trials (CENTRAL; 2019, Issue 8); OVID MEDLINE (R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions (R) (1946 to 15 August 2019); MEDLINE via PubMed (1 August 2018 to 15 August 2019) for the previous year; and the Cumulative Index to Nursing and Allied Health Literatue (CINAHL) (1981 to 15 August 2019). We searched clinical trials databases and reference lists of included studies.

Selection criteria

We included randomised controlled trials that compared early versus late fortification of human milk in preterm infants. We defined early fortification as fortification started at < 100 mL/kg/d enteral feed volume or < 7 days postnatal age, and late fortification as fortification started at ≥ 100 mL/kg/d feeds or ≥ 7 days postnatal age.

Data collection and analysis

Both review authors assessed trial eligibility and risk of bias and independently extracted data. We analysed treatment effects in individual trials, and we reported risk ratio (RR) for dichotomous data and mean difference (MD) for continuous data, with respective 95% confidence intervals (CIs). We used the GRADE approach to assess the certainty of evidence.

Main results

We included two trials with a total of 237 infants. All participants were very low birth weight infants (birth weight < 1500 g). Early fortification was started at 20 mL/kg/d enteral feeds in one study and 40 mL/kg/d in the other study. Late fortification was started at 100 mL/kg/d feeds in both studies. One study used bovine milk-based fortifier, and the other used human milk-based fortifier.

Meta-analysis showed that early fortification may have little or no effect on growth outcomes including time to regain birth weight (MD -0.06 days, 95% CI -1.32 to 1.20 days), linear growth (MD 0.10 cm/week, 95% CI -0.03 to 0.22 cm/week), or head growth (MD -0.01 cm/week, 95% CI -0.07 to 0.06 cm/week) during the initial hospitalisation period. Early fortification may have little or no effect on the risk of NEC (MD -0.01, 95% CI -0.07 to 0.06). The certainty of evidence was low for these outcomes due to risk of bias (lack of blinding) and imprecision (small sample size).

Early fortification may have little or no effect on incidence of surgical NEC, time to reach full enteral feeds, extrauterine growth restriction at discharge, proportion of infants with feed interruption episodes, duration of total parenteral nutrition (TPN), duration of central venous line usage, or incidence of invasive infection, all-cause mortality, and duration of hospital stay. The certainty of evidence was low for these outcomes due to risk of bias (lack of blinding) and imprecision (small sample size).

We did not have data for other outcomes such as subsequent weight gain after birth weight is regained, parenteral nutrition-associated liver disease, postdischarge growth, and neurodevelopmental outcomes.

Authors' conclusions

Available evidence is insufficient to support or refute early fortification of human milk in preterm infants. Further large trials would be needed to provide data of sufficient quality and precision to inform policy and practice.

PLAIN LANGUAGE SUMMARY

Earlier compared to later addition of human milk fortifier to human milk to promote growth in preterm infants

Review question

Does adding human milk fortifier (HMF) early promote growth and improve outcomes in preterm infants compared to adding it late?

Background

Uncertainty exists about the optimal point at which HMF should be added to human milk for promoting growth in preterm infants. The most common practice is to start HMF when the infant's daily feed volume reaches 100 mL/kg body weight. Another approach is to commence HMF earlier, in some cases as early as the first feed. Adding HMF early could increase nutrient intake and growth rates but may increase the risk of feed intolerance and necrotising enterocolitis.

Study characteristics

Evidence is up-to-date as of August 2019. We identified two randomised controlled trials that evaluated the effects of adding HMF early for preterm infants.

Key results

We found only limited data from two trials. There is uncertainty as to whether adding HMF early for preterm infants has an effect on important outcomes such as growth during hospital stay, necrotising enterocolitis, death before discharge, presence of growth failure at discharge, and length of hospital stay.

Certainty of evidence

The available evidence is insufficient to support or refute early addition of HMF to human milk to promote growth in preterm infants. More trials are needed to examine whether adding HMF early is beneficial or harmful for preterm infants.

SUMMARY OF FINDINGS

Summary of findings 1. Early versus late fortification of human milk in preterm infants

Early versus late fortification of human milk in preterm infants

Patient or population: preterm infants
Settings: neonatal unit
Intervention: early fortification
Comparison: late fortification

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No. of participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Late fortification	Early fortification				
Time to regain birth weight (days)		Mean time to regain birth weight (days) in the intervention groups was **0.06 lower** (1.32 lower to 1.2 higher)		237 (2 studies)	⊕⊕⊖⊖ **low** [a,b]	
Linear growth (cm/week)		Mean linear growth (cm/week) in the intervention groups was **0.1 higher** (0.03 lower to 0.22 higher)		237 (2 studies)	⊕⊕⊖⊖ **low** [a,b]	
Increase in head circumference (cm/week)		Mean increase in head circumference (cm/week) in the intervention groups was **0.01 lower** (0.07 lower to 0.06 higher)		237 (2 studies)	⊕⊕⊖⊖ **low** [a,b]	
Necrotising enterocolitis stage 2 or 3	**Study population**		**RR 1.36** (0.44 to 4.16)	237 (2 studies)	⊕⊕⊖⊖ **low** [a,b]	
	43 per 1000	**58 per 1000** (19 to 178)				
	Moderate					
	42 per 1000	**57 per 1000** (18 to 175)				

Time to reach full enteral feeds		Mean time to reach full enteral feeds in the intervention groups was **0.27 higher** (3.48 lower to 4.02 higher)		237 (2 studies)	⊕⊕⊝⊝ **low** [a,b]
Extrauterine growth restriction at discharge	**Study population**		**RR 1.06** (0.81 to 1.39)	237 (2 studies)	⊕⊕⊝⊝ **low** [a,b]
	342 per 1000	**362 per 1000** (277 to 475)			
	Moderate				
	385 per 1000	**408 per 1000** (312 to 535)			
Proportion of infants with feed interruption episodes	**Study population**		**RR 0.99** (0.73 to 1.34)	237 (2 studies)	⊕⊕⊝⊝ **low** [a,b]
	402 per 1000	**398 per 1000** (293 to 538)			
	Moderate				
	389 per 1000	**385 per 1000** (284 to 521)			

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: confidence interval; RR: risk ratio.

GRADE Working Group grades of evidence.
High quality: further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: we are very uncertain about the estimate.

[a]Lack of blinding.
[b]Small sample size.

BACKGROUND

Description of the condition

Preterm birth places the infant at risk of nutritional deprivation and results in interruption of growth. It is a challenge to sustain in utero growth velocity in preterm infants due to difficulty in maintaining adequate protein–energy supplementation and due to their catabolic state secondary to postnatal illnesses such as sepsis, necrotising enterocolitis (NEC), chronic lung disease, need for assisted ventilation, and exposure to postnatal steroids (Lima 2014).

Extremely low birth weight (ELBW) infants take 16 ± 7 days to regain birth weight (Steward 2002). The rate of extrauterine growth restriction (EUGR) at discharge is unacceptably high, ranging from 23% in infants born at 34 weeks' gestation to 71% in those born at 23 weeks' gestation (Clark 2003). Moreover, being small for gestational age (SGA) at birth increases the likelihood of EUGR at discharge by six times (Freitas 2016). Growth failure can continue even after discharge. Data from the National Institute of Child Health and Human Development (NICHD) cohort in the USA showed that 40% of ELBW-SGA infants had weight, length, and head circumference less than the 10th percentile at 18 to 22 months' corrected age (Dusick 2003). Growth restriction in early infancy has long-term consequences such as stunting, neurodevelopmental impairment, and early onset of adult diseases such as hypertension, diabetes, obesity, and hypercholesterolaemia (Barker 1989; Cooke 2004; Lucas 1994; Lucas 2004).

Early aggressive nutrition is the norm in the management of preterm infants. The nutritional requirement in the first few days is met by total parenteral nutrition (TPN), which is started soon after birth and is continued until adequate enteral feeds are established. However, TPN administration is technically demanding (need for trained staff, laminar flow, laboratory backup, and appropriate equipment) and expensive. It may cause adverse effects such as azotaemia, metabolic acidosis, hyperlipidaemia, cholestasis, and catheter-related complications (Calkins 2014). Further, each day without enteral nutrition increases the likelihood of EUGR by 8% (Freitas 2016). Hence, enteral feeds should be started early, and full enteral feeds should be achieved as soon as possible.

Description of the intervention

Human milk is the best enteral food for preterm infants. However, unfortified human milk may not provide adequate protein to support growth and lean body mass accretion in very low birth weight infants (Morales 2007). The amount of calcium and phosphorus provided by unfortified human milk is too low to match the in utero accretion rate (Boyd 2007; Lucas 1996). The method most commonly used to increase enteral supplementation of protein, calories, and minerals consists of adding multi-component human milk fortifier (HMF) to human milk. On average, unfortified human milk provides 67 kcal and 1.1 g protein per 100 mL, while human milk with HMF provides 80 kcal and 2 g protein per 100 mL. A recent Cochrane meta-analysis showed that fortification of human milk with multi-component HMF improved in-hospital growth rates; however, there was no significant difference in other major clinical outcomes (Brown 2016).

Definitive guidelines on when to start HMF are not available. The common practice is to start fortification when enteral feed volume reaches around 100 mL/kg/d (Berseth 2004; Gathwala 2007; Mukhopadhyay 2007). Fortification is delayed because of clinicians' concern about the risk of NEC and feed intolerance. However, some studies have started fortification earlier, as early as the first feed (Alizadeh 2017; Maas 2013; Mimouni 2017; Shah 2016; Sullivan 2010; Tillman 2012).

How the intervention might work

Early fortification of human milk could improve protein, calorie, and mineral intake in preterm infants (Shah 2016; Tillman 2012). This may avoid the dip in nutrition and reduce the time needed to regain birth weight. It may also improve further postnatal growth and decrease the risk of EUGR (Steward 2002). Early fortification may be especially important for infants who receive pasteurised donor milk, which contains lower levels of protein, energy, and minerals than mother's own milk (Arslanoglu 2010).

On the other hand, because most of the available HMFs have cow's milk as the base, and because HMF increases the osmolarity of feeds, early fortification may increase the risk of feed intolerance and NEC. This may result in interruption of feeds and delay in reaching full enteral feeds, which in turn may increase the duration of TPN and risk of parenteral nutrition-associated liver disease (Calkins 2014). It may also increase the number of days of central venous line (CVL) usage, along with the risk of late-onset sepsis and other CVL-related complications (Hermansen 2005).

Why it is important to do this review

Given the potential use of early fortification of human milk to improve postnatal growth and other outcomes, as well as the possible risks, we performed this systematic review and meta-analysis to identify and appraise data from RCTs with the goal of providing a synthesis of evidence to inform practice and research.

OBJECTIVES

To assess effects on growth and safety of early fortification of human milk versus late fortification in preterm infants

To assess whether effects vary based upon gestational age (≤ 27 weeks; 28 to 31 weeks; ≥ 32 weeks), birth weight (< 1000 g; 1000 to 1499 g; ≥ 1500 g), small or appropriate for gestational age, or type of fortifier (bovine milk-based human milk fortifier (HMF); human milk-based HMF; formula powder)

METHODS

Criteria for considering studies for this review

Types of studies

We included randomised controlled trials (RCTs) in the review.

Types of participants

We included preterm infants (< 37 weeks' gestation).

Types of interventions

Intervention

Early fortification of human milk, started at < 100 mL/kg/d enteral feed volume or at < 7 days' postnatal age.

Comparison

Late fortification, started at ≥ 100 mL/kg/d feeds or at ≥ 7 days' postnatal age.

Fortification should be done with a multi-component fortifier containing carbohydrate, protein, lipid, and micro-nutrients. The fortifier could be bovine milk-based HMF, human milk-based HMF, or formula powder.

Types of outcome measures

Primary outcomes

1. Time to regain birth weight (days) and subsequent rate of weight gain (g/kg/d), linear growth (cm/week), and increase in head circumference (cm/week) during the initial hospitalisation period
2. Incidence of necrotising enterocolitis (NEC) stage 2 or 3 (modified Bell's staging; Walsh 1986)

Secondary outcomes

1. Incidence of surgical NEC
2. Time to reach full enteral feeds ≥ 150 mL/kg/d
3. Incidence of extrauterine growth restriction at discharge (number of infants with weight < 10th percentile for the index population)
4. Proportion of infants with ≥ 1 episode of feed interruption lasting ≥ 12 hours
5. Duration of total parenteral nutrition (TPN) (days)
6. Incidence of parenteral nutrition-associated liver disease
7. Duration of central venous line (CVL) usage (days)
8. Incidence of invasive infection as determined by culture of bacteria or fungus from blood, cerebrospinal fluid, urine, or a normally sterile body space
9. All-cause mortality before discharge or up to 44 weeks' postmenstrual age
10. Duration of hospital stay (days)
11. Growth measures following discharge from hospital to latest follow-up (weight, length, and head circumference)
12. Neurodevelopmental outcomes assessed after 12 months' corrected age: neurological evaluations; developmental scores; and classifications of disability, including auditory and visual disability. We defined neurodevelopmental impairment as the presence of one or more of the following: non-ambulant cerebral palsy; developmental quotient more than 2 standard deviations below the population mean; blindness (visual acuity < 6/60), or deafness (any hearing impairment requiring or not improved by amplification)

Search methods for identification of studies

We used the criteria and standard methods of Cochrane and Cochrane Neonatal (see the Cochrane Neonatal search strategy for specialized register). We searched for errata or retractions from included studies published in full text on PubMed (www.ncbi.nlm.nih.gov/pubmed).

Electronic searches

We conducted a comprehensive search including the Cochrane Central Register of Controlled Trials (CENTRAL; 2019, Issue 8), in the Cochrane Library; OVID MEDLINE Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions (R) (1946 to 15 August 2019); MEDLINE via PubMed (1 August 2018 to 15 August 2019) for the previous year; and the Cumulative Index to Nursing and Allied Health Literature (CINAHL; 1981 to 15 August 2019). We have presented the search strategies for each database in Appendix 1. We did not apply language restrictions.

We searched clinical trial registries for ongoing and recently completed trials. We searched the World Health Organization's International Clinical Trials Registry Platform (ICTRP) (www.who.int/ictrp/search/en/), as well as the US National Library of Medicine's clinical trials registry (clinicaltrials.gov), via Cochrane CENTRAL. Additionally, we searched the ISRCTN Registry for any unique trials not found through Cochrane CENTRAL.

Searching other resources

We also searched the reference lists of any articles selected for inclusion in this review to identify additional relevant articles. Trials reported only as abstracts were eligible if sufficient information was available from the report, or through contact with study authors, to fulfil the inclusion criteria.

Data collection and analysis

We used the standard methods of Cochrane Neonatal and Cochrane (Higgins 2017).

Selection of studies

Both review authors (ST and TA) screened the title and abstract of all studies identified by the search strategy and independently assessed the full-text articles for potentially relevant trials. We excluded those studies that did not meet all inclusion criteria, and we stated the reasons for exclusion. We discussed disagreements until consensus was achieved.

We recorded the selection process in sufficient detail to complete a Characteristics of excluded studies table and a PRISMA flow diagram (Moher 2009).

Data extraction and management

Both review authors (ST and TA) extracted data independently using a data collection form to aid extraction of information on design, methods, participants, interventions, outcomes, and treatment effects from each included study. We assessed each identified trial for methodological quality with respect to (a) inclusiveness of the population, (b) masking of allocation, (c) masking of intervention, (d) completeness of follow-up, and (e) masking of outcome assessment. If data from the trial reports were insufficient, we contacted the trialists for further information. We sought clarification from at least one author of each trial considered for selection. We discussed any disagreements until we reached a consensus.

Assessment of risk of bias in included studies

Both review authors (ST and TA) independently assessed the risk of bias (low, high, or unclear) of all included trials using the Cochrane 'Risk of bias' tool for the following domains (Higgins 2017).

1. Sequence generation (selection bias).
2. Allocation concealment (selection bias).

3. Blinding of participants and personnel (performance bias).
4. Blinding of outcome assessment (detection bias).
5. Incomplete outcome data (attrition bias).
6. Selective reporting (reporting bias).
7. Any other bias.

We discussed disagreements until we reached a consensus. See Appendix 2 for a more detailed description of risk of bias for each domain.

Measures of treatment effect

We analysed treatment effects in individual trials using Review Manager 2014, and we reported the risk ratio (RR) for dichotomous data and the mean difference (MD) for continuous data, along with respective 95% confidence intervals (CIs).

Unit of analysis issues

The unit of analysis was the participating infant in individually randomised trials. We combined study results where there was little heterogeneity between study designs, and we considered interactions between effects of the intervention and the choice of randomisation unit to be unlikely.

Dealing with missing data

We requested and obtained from trial investigators additional data on important outcomes that were missing.

Assessment of heterogeneity

We examined the treatment effects of individual trials and heterogeneity between trial results by inspecting the forest plots. We calculated the I^2 statistic for each RR or MD analysis to quantify inconsistency across studies, and we described the percentage of variability in effect estimates that might be due to heterogeneity rather than to sampling error.

Assessment of reporting biases

Because only two trials were included in the meta-analysis, we could not examine a funnel plot for possible publication bias.

Data synthesis

We analysed all infants randomised on an intention-to-treat basis and treatment effects in individual trials using a fixed-effect model to combine the data. We calculated RRs for meta-analyses of categorical outcomes and MDs for continuous outcomes, each with 95% CIs.

Certainty of evidence

We used the GRADE approach, as outlined in the GRADE Handbook to assess the certainty of evidence for the following clinically relevant outcomes (Schünemann 2013).

1. Time to regain birth weight (days).
2. Linear growth (cm/week) during hospital stay.
3. Increase in head circumference (cm/week) during hospital stay.
4. Incidence of necrotising enterocolitis (NEC) stage 2 or 3.
5. Time to reach full enteral feeds (days).
6. Incidence of extrauterine growth restriction at discharge.
7. Proportion of infants with ≥ 1 episode of feed interruption lasting ≥ 12 hours.

Both review authors (ST and TA) independently assessed the certainty of evidence for each outcome. We considered evidence from RCTs as high certainty but downgraded the evidence one level for serious (or two levels for very serious) limitations based upon the following: design (risk of bias), consistency across studies, directness of the evidence, precision of estimates, and the presence of publication bias. We used the GRADEpro GDT Guideline Development Tool to create Summary of findings 1 to report the certainty of evidence.

The GRADE approach results in an assessment of the certainty of a body of evidence as one of four grades.

1. High certainty: further research is very unlikely to change our confidence in the estimate of effect.
2. Moderate certainty: further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
3. Low certainty: further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
4. Very low certainty: we are very uncertain about the estimate.

Subgroup analysis and investigation of heterogeneity

We planned to do subgroup analysis based on:

1. gestational age: ≤ 27 weeks; 28 to 31 weeks; ≥ 32 weeks' gestation;
2. birth weight: < 1000 g; 1000 to 1499 g; ≥ 1500 g;
3. small for gestational age or appropriate for gestational age infants (classified using birth weight relative to the reference population); and
4. type of human milk fortifier (HMF) (bovine milk-based HMF; human milk-based HMF; formula powder).

We did not perform the above mentioned subgroup analyses due to inadequate data. We assessed statistical heterogeneity using the I^2 statistic.

Sensitivity analysis

We planned to undertake sensitivity analyses to determine if findings were affected by including only studies reporting adequate methods (low risk of bias), defined as reporting adequate randomisation and allocation concealment, blinding of intervention and measurement, and less than 10% loss to follow-up. However, we did not conduct sensitivity analyses because it was not required.

RESULTS

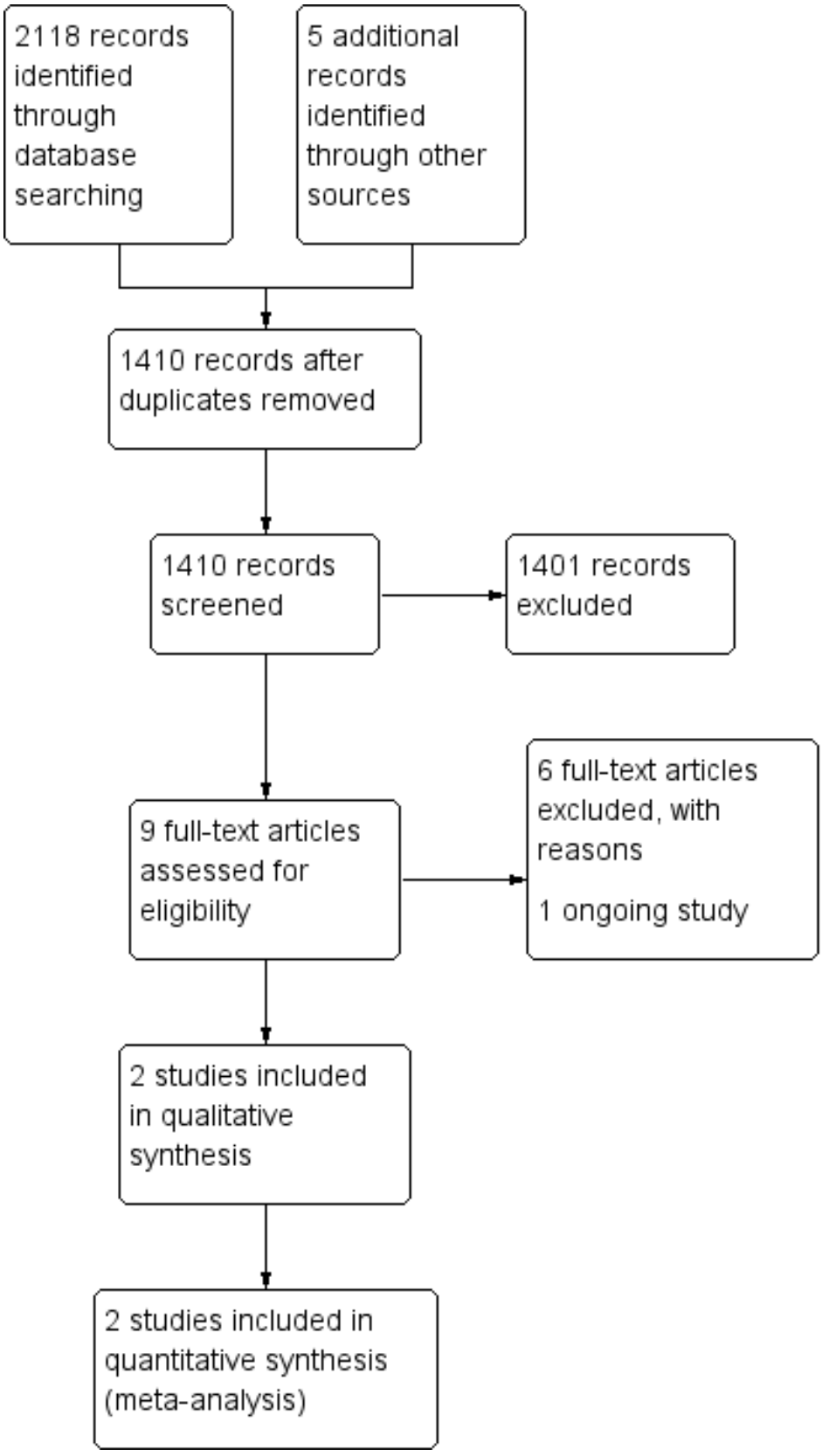
Description of studies

Results of the search

We screened 2118 titles and abstracts that were identified via the search strategy. We carried out full-text review of nine articles. We excluded six studies, and we reported details of the excluded studies. We identified one ongoing study. We identified two eligible

studies for inclusion in qualitative and quantitative synthesis. See Figure 1.

Figure 1. Study flow diagram.



Included studies

Two studies satisfied our inclusion criteria (Shah 2016; Sullivan 2010). Early fortification was started from 20 mL/kg/d feed volume in Shah 2016 and 40 mL/kg/d feed volume in Sullivan 2010. Late fortification was started from 100 mL/kg/d feed volume in both studies (Shah 2016; Sullivan 2010). One study used bovine milk-based HMF (Shah 2016), and the other used human milk-based HMF (Sullivan 2010). (See Characteristics of included studies.)

Shah 2016 was performed in the USA. Trialists randomised 100 infants with birth weight < 1500 g into the early fortification group (fortification starting from 20 mL/kg/d feeds) or the late fortification group (fortification starting from 100 mL/kg/d feeds). Fortification was done with bovine milk-based liquid HMF (Enfamil); 5 mL HMF was added to 25 mL human milk to increase caloric density to 24 kcal/oz. Infants were given only trophic feeds for one to three days depending on their birth weight, followed by a gradual increase in feed volume. Feeds were delivered continuously (three hours on and one hour off). TPN was given until infants reached sufficient enteral feeds. Primary outcome was time to reach full enteral feeds (> 140 mL/kg/d). Secondary outcomes were feeding intolerance, NEC, daily weight gain, protein and caloric intake for the first four weeks of life, weight velocity at four weeks after birth and at 36 weeks' postmenstrual age, TPN days, length of hospital stay, metabolic acidosis, late-onset sepsis, ventilation days, chronic lung disease, postnatal steroid treatment, patent ductus arteriosus, severe intraventricular haemorrhage (grade III and IV), periventricular leukomalacia, and retinopathy of prematurity.

Sullivan 2010 was a multi-centre study done at 12 neonatal intensive care units - 11 in the USA and 1 in Austria. Trialists included 207 infants with 500 to 1250 g birth weight. Infants were randomised into three arms: human milk-based HMF fortification starting at 100 mL/kg/d feed volume (HM100), human milk-based HMF fortification starting at 40 mL/kg/d feed volume (HM40), and bovine milk-based HMF fortification or preterm formula feeding group (BOV). We have included only HM100 (late fortification) and HM40 (early fortification) groups in our analysis. Human milk-based HMF (Prolact+ H2MF) was used and calorie density was 24 kcal/oz. Trophic feeds were given for five days, followed by a gradual increase in feed volume up to a maximum of 160 mL/kg/d. The primary outcome was duration of TPN. Secondary outcomes were growth indices, late-onset sepsis, NEC stage 2 or 3, feed intolerance, bronchopulmonary dysplasia, retinopathy of prematurity, duration of CVL usage, duration of hospital day, and duration of ventilation and oxygen therapy.

Excluded studies

We excluded six studies (see Characteristics of excluded studies).

Alizadeh 2017 was an RCT comparing early fortification (fortification from the first feed) and later fortification (fortification starting from 75 mL/kg/d feed volume). The trial recruited 80 preterm infants of 28 to 34 weeks' gestational age and < 2000 g birth weight. Fortification was done with bovine milk-based HMF (Aptamil FMS HMF powder), 4.4 g for 100 mL of expressed breast milk, which gives 24 kcal/oz. We excluded this trial because late fortification was started at 75 mL/kg/d feed volume.

We excluded three studies as they were retrospective studies (Huston 2019; Maas 2013; Tillman 2012). Huston 2019 was a multi-centre retrospective study that compared neonates with 500 to 1250 g birth weight receiving early fortification (starting at < 60 mL/kg/d) or later fortification (starting at > 60 mL/kg/d). Maas 2013 enrolled preterm babies at < 32 weeks' gestation and with birth weight < 1500 g born in 2006, 2007, and 2010. Babies who were born in 2006 and 2007 received later fortification (starting at 150 mL/kg/d feed volume), and babies who were born in 2010 received early fortification (starting at 100 mL/kg/d). Tillman 2012 used a retrospective pre–post design and compared early fortification (starting with the first feeding) in infants born before June 2009 versus later fortification (starting at 50 to 80 mL/kg/d) in infants born June 2009 and after.

We excluded Ghandehari 2012, as it was a post-hoc analysis from Sullivan 2010.

We excluded Sajjadian 2014 because we found no published data and we could not obtain unpublished data from study authors.

Ongoing studies

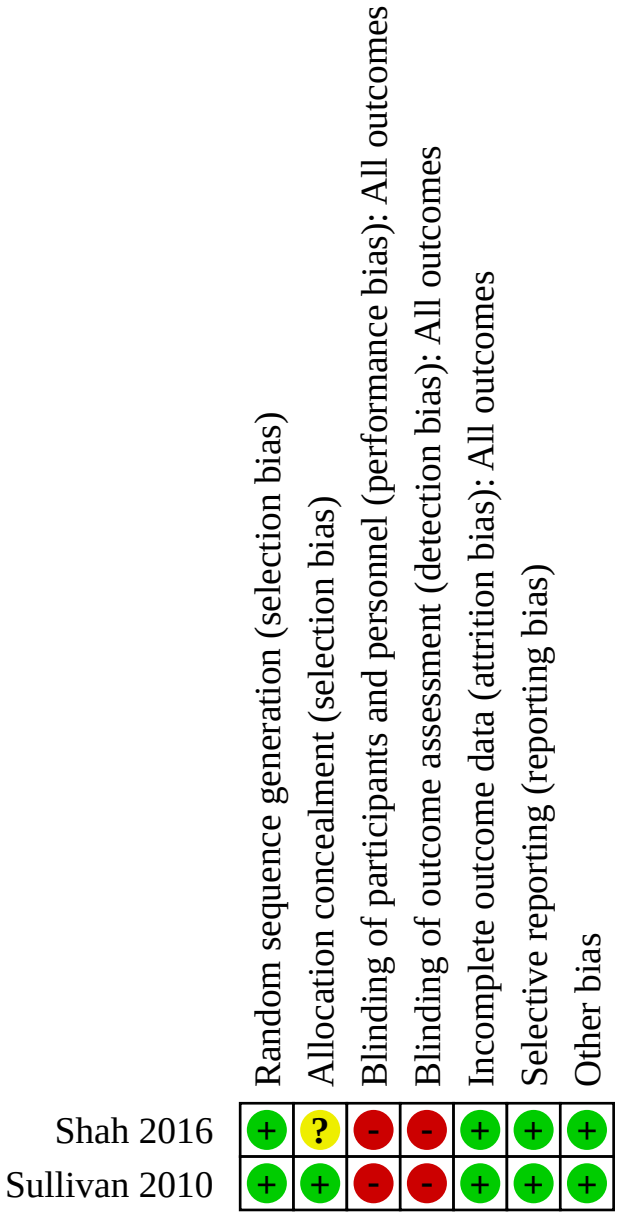
We found one ongoing study (IRCT20171030037093N3).

This trial has randomised 90 preterm infants into three groups, with fortification started at 30, 70, and 100 mL/kg/d enteral feeds. The main outcome is weight, length, and head circumference at four weeks' postnatal age. The study has been completed. However, it remains to be published. See the Characteristics of ongoing studies table.

Risk of bias in included studies

See Figure 2.

Figure 2. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.



Allocation

Both included studies have used block randomisation with a fixed block size of four. The random sequence was computer generated.

Sullivan 2010 was a multi-centre study with central randomisation. In Shah 2016, the method of allocation concealment was not mentioned. Moreover, a fixed block size of four made allocation of every fourth infant predictable, as caregivers and trial investigators were not masked.

Blinding

Both studies were open-label trials (Shah 2016; Sullivan 2010). Therefore, risk for performance bias and detection bias was high in both trials.

Incomplete outcome data

Both studies reported the outcomes of all participants (Shah 2016; Sullivan 2010).

Selective reporting

Both studies published all outcomes that were mentioned in the protocol (Shah 2016; Sullivan 2010).

Other potential sources of bias

No other potential source of bias was noted in either study (Shah 2016; Sullivan 2010).

Effects of interventions

See: **Summary of findings 1** Early versus late fortification of human milk in preterm infants

See Summary of findings 1.

We included two RCTs with 237 infants in the meta-analysis to assess the benefits and safety of early fortification versus late fortification of human milk for various outcomes in preterm infants (Shah 2016; Sullivan 2010).

Primary outcomes

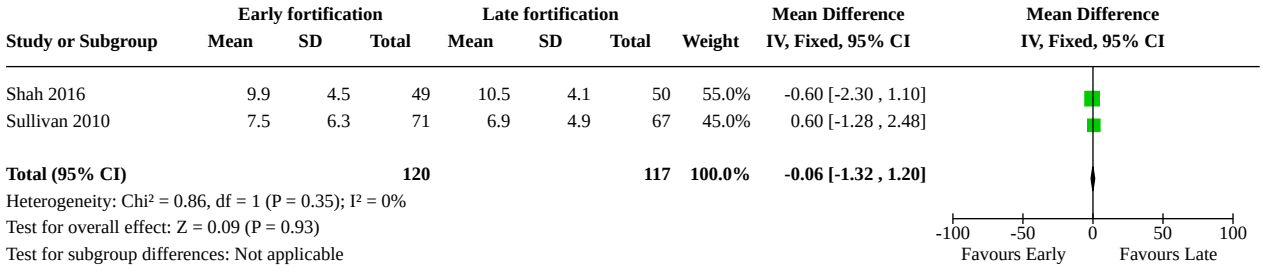
1. Time to regain birth weight (days) and subsequent rate of weight gain (g/kg/d), linear growth (cm/week), and increase in head circumference (cm/week) during the initial hospitalisation period

Data on time to regain birth weight were available from both included studies (Shah 2016; Sullivan 2010). The meta-analysis did not show a difference in time to regain birth weight between early and late fortification groups (mean difference (MD) -0.06 days, 95% confidence interval (CI) -1.32 to 1.20 days; participants = 237; studies = 2). There was no evidence of heterogeneity (I^2 = 0%) (Analysis 1.1; Figure 3). We did not find any data on subsequent rate of weight gain during the initial hospitalisation period beyond the time to regain birth weight.

Meta-analysis of data from both trials did not show a difference in linear growth between early and late fortification groups (MD 0.10 cm/week, 95% CI -0.03 to 0.22 cm/week; participants = 237; studies = 2) (Shah 2016; Sullivan 2010). There was no evidence of heterogeneity (I^2 = 0%) (Analysis 1.2).

Meta-analysis of data from both studies did not show a difference in the increase in head circumference between the two groups (MD -0.01 cm/week, 95% CI -0.07 to 0.06 cm/week; participants = 237; studies = 2) (Shah 2016; Sullivan 2010). Heterogeneity was moderate (I^2 = 27%) (Analysis 1.3).

Figure 3. Forest plot of comparison: 1 Early versus late fortification, outcome: 1.1 Time to regain birth weight (days).

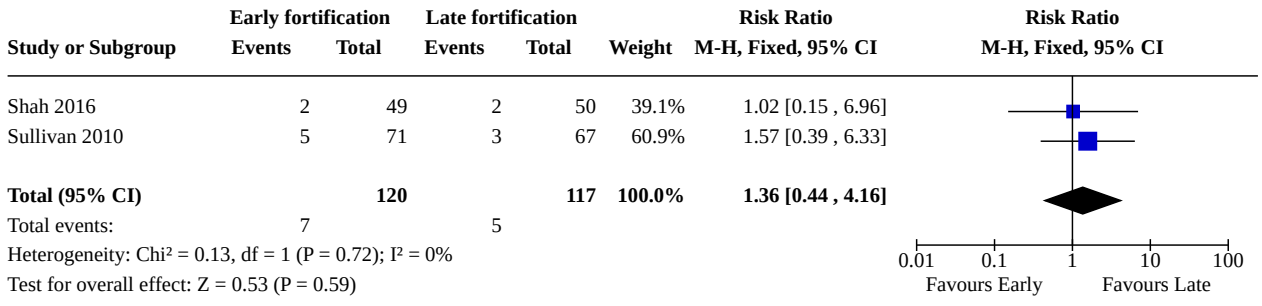


The certainty of evidence was low (downgraded for lack of blinding and small sample size) for all three outcomes.

2. Incidence of necrotising enterocolitis (NEC) stage 2 or 3

Data for analysis of this outcome were available from both trials (Shah 2016; Sullivan 2010). The estimated risk ratio for this outcome was 1.36 (95% CI 0.44 to 4.16; participants = 237; studies = 2). The meta-analysis did not show a difference in the incidence of NEC between early and late fortification groups. There was no evidence of heterogeneity (I^2 = 0%) (Analysis 1.4; Figure 4). The certainty of evidence was low, downgraded for lack of blinding and small sample size.

Figure 4. Forest plot of comparison: 1 Early versus late fortification, outcome: 1.4 Necrotising enterocolitis stage 2 or 3.



Secondary outcomes

1. Incidence of surgical NEC

Data from both trials were available for analysis of this outcome (Shah 2016; Sullivan 2010). The meta-analysis did not show a difference between the two groups in the incidence of surgical NEC (risk ratio (RR) 0.98, 95% CI 0.14 to 6.85; participants = 237; studies = 2). There was no evidence of heterogeneity (I^2 = 0%) (Analysis 1.5). The certainty of evidence was low, downgraded for lack of blinding and small sample size.

2. Time to reach full enteral feeds ≥ 150 mL/kg/d

Two RCTs including 237 participants contributed data (Shah 2016; Sullivan 2010). Early fortification did not show a difference in the time to reach full enteral feeds (MD 0.27 days, 95% CI -3.48 to 4.02 days; participants = 237; studies = 2). Heterogeneity was moderate (I^2 = 39%) (Analysis 1.6). The certainty of evidence was low, downgraded for lack of blinding and small sample size.

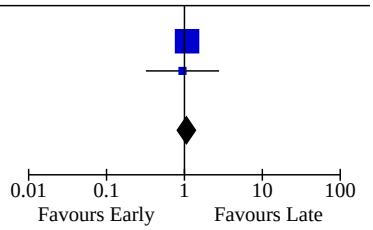
3. Incidence of extrauterine growth restriction at discharge (number of infants with weight < 10th percentile for the index population)

Meta-analysis of two trials did not show a difference between groups in the incidence of extrauterine growth restriction at discharge (RR 1.06, 95% CI 0.81 to 1.39; participants = 237; studies = 2) (Shah 2016; Sullivan 2010). There was no evidence of heterogeneity (I^2 = 0%) (Analysis 1.7; Figure 5). The certainty of evidence was low, downgraded for lack of blinding and small sample size.

Figure 5. Forest plot of comparison: 1 Early versus late fortification, outcome: 1.7 Extrauterine growth restriction at discharge.

Study or Subgroup	Early fortification Events	Total	Late fortification Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Shah 2016	36	49	34	50	84.5%	1.08 [0.84 , 1.39]
Sullivan 2010	6	71	6	67	15.5%	0.94 [0.32 , 2.78]
Total (95% CI)		**120**		**117**	**100.0%**	**1.06 [0.81 , 1.39]**
Total events:	42		40			

Heterogeneity: Chi² = 0.07, df = 1 (P = 0.80); I² = 0%
Test for overall effect: Z = 0.42 (P = 0.67)
Test for subgroup differences: Not applicable



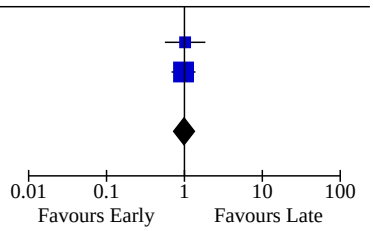
4. Proportion of infants with feed interruption episodes

Meta-analysis of two trials did not show a difference between early and late fortification groups in the proportion of infants with feed interruption episodes (RR 0.99, 95% CI 0.73 to 1.34; participants = 237; studies = 2) (Shah 2016; Sullivan 2010). There was no evidence of heterogeneity (I^2 = 0%) (Analysis 1.8; Figure 6). The certainty of evidence was low, downgraded for lack of blinding and small sample size.

Figure 6. Forest plot of comparison: 1 Early versus late fortification, outcome: 1.8 Proportion of infants with feed interruption episodes.

Study or Subgroup	Early fortification Events	Total	Late fortification Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Shah 2016	15	49	15	50	31.1%	1.02 [0.56 , 1.85]
Sullivan 2010	33	71	32	67	68.9%	0.97 [0.68 , 1.39]
Total (95% CI)		**120**		**117**	**100.0%**	**0.99 [0.73 , 1.34]**
Total events:	48		47			

Heterogeneity: Chi² = 0.02, df = 1 (P = 0.89); I² = 0%
Test for overall effect: Z = 0.08 (P = 0.94)
Test for subgroup differences: Not applicable



5. Duration of total parenteral nutrition (TPN) (days)

Meta-analysis of two trials did not show a difference between groups in the duration of TPN (MD 0.08 days, 95% CI -3.07 to 3.24 days; participants = 237; studies = 2) (Analysis 1.9) (Shah 2016; Sullivan 2010). The certainty of evidence was low, downgraded for lack of blinding and small sample size.

6. Incidence of parenteral nutrition-associated liver disease

We did not find any data on this outcome.

7. Duration of central venous line (CVL) usage (days)

Data from two studies did not show a difference between early and late fortification groups in the duration of CVL usage (MD 1.04 days, 95% CI -3.13 to 5.20; participants = 237; studies = 2) (Analysis 1.10) (Shah 2016; Sullivan 2010). The certainty of evidence was low, downgraded for lack of blinding and small sample size.

8. Incidence of invasive infection as determined by culture of bacteria or fungus from blood, cerebrospinal fluid, urine, or a normally sterile body space

Data for analysis of this outcome were available from both trials (Shah 2016; Sullivan 2010). The meta-analysis did not show a difference between groups in the incidence of invasive infection (RR 0.69, 95% CI 0.40 to 1.18; participants = 237; studies = 2). There was no evidence of heterogeneity (I^2 = 0%) (Analysis 1.11). The certainty of evidence was low, downgraded for lack of blinding and small sample size.

9. All-cause mortality before discharge or up to 44 weeks' postmenstrual age

Meta-analysis of two trials did not show a difference between groups for this outcome (RR 1.32, 95% CI 0.30 to 5.77; participants = 237; studies = 2) (Shah 2016; Sullivan 2010). There was no evidence of heterogeneity (I^2 = 0%) (Analysis 1.12). The certainty of evidence was low, downgraded for lack of blinding and small sample size.

10. Duration of hospital stay (days)

Meta-analysis of data from both studies did not show a difference between groups in the duration of hospital stay (MD 2.33, 95% CI -6.44 to 11.11; participants = 237; studies = 2) (Shah 2016; Sullivan 2010). There was no evidence of heterogeneity (I^2 = 0%) (Analysis 1.13). The certainty of evidence was low, downgraded for lack of blinding and small sample size.

11. Growth measures following discharge from hospital to latest follow-up (weight, length, and head circumference)

We did not find any data on this outcome.

12. Neurodevelopmental outcomes assessed after 12 months' corrected age: neurological evaluations; developmental scores; and classifications of disability, including auditory and visual disability

We did not find any data on this outcome.

DISCUSSION

Summary of main results

Evidence from two studies including 237 infants that contributed data to the outcomes of this review showed that early fortification of human milk compared to late fortification may have little or no effect on growth outcomes during the initial hospitalisation period nor on the incidence of necrotising enterocolitis (NEC). Similarly, the meta-analysis showed that early fortification may have little or no effect on other important outcomes such as surgical NEC, time to reach full enteral feeds, extrauterine growth restriction at discharge, proportion of infants with feed interruption episodes, duration of total parenteral nutrition (TPN), duration of central venous line usage, incidence of invasive infection, all-cause mortality, and duration of hospital stay. The quality of evidence was low for all outcomes, downgraded for lack of blinding and small sample size. We did not find any data on other important outcomes such as subsequent weight gain after birth weight was regained, parenteral nutrition-associated liver disease, postdischarge growth, and neurodevelopmental outcomes.

Overall completeness and applicability of evidence

Early fortification may improve protein, calorie, and mineral intake in preterm infants and thus may reduce extrauterine growth restriction. However, adding human milk fortifier (HMF) to human milk early may increase the risk of feed intolerance and NEC, and thus increase the time to full enteral feeds. This review identified only two studies for meta-analysis (Shah 2016; Sullivan 2010). Shah 2016 used bovine milk-based HMF, and Sullivan 2010 used human milk-based HMF, both with the same calorie density of 24 kcal/oz. Thus the results may be applicable to both commonly used fortifiers, namely, bovine milk-based and human milk-based HMF.

Both studies were done in very low birth weight infants; inclusion criteria were birth weight < 1500 g in Shah 2016 and birth weight 500 to 1250 g in Sullivan 2010. Hence, the results may not be applicable to larger preterm infants, who have a biologically more mature gastrointestinal system and lower risk of NEC.

Feed increment was started only after the first few days in both studies - after one to three days in Shah 2016, and at five days in Sullivan 2010. Only trophic feeds were given to the babies until then. Hence, even in the early fortification group, fortification was started on or after day 2 in Shah 2016, and on or after day 6 in Sullivan 2010. Hence, the results are not applicable to neonatal intensive care units (NICUs), where fortification is started on day 1 of postnatal life.

Both studies were conducted in high-income countries.

Quality of the evidence

The methodological quality of both included trials was good (Shah 2016; Sullivan 2010). Trialists used computer-generated randomisation and reported all intended outcomes, and there was no attrition. However, both were open-label trials, hence risk of performance and detection bias was high. One trial did not mention the method of allocation concealment (Shah 2016).

The certainty of evidence was low for all outcomes such as time to regain birth weight (downgraded for serious risk of bias due to lack of blinding and serious imprecision due to small sample size), linear growth (downgraded for serious risk of bias due to lack of blinding and serious imprecision due to small sample size), increase in head circumference (downgraded for serious risk of bias due to lack of blinding and serious imprecision due to small sample size), NEC stage 2 or 3 (downgraded for serious risk of bias due to lack of blinding and serious imprecision due to small sample size), time to reach full enteral feeds (downgraded for serious risk of bias due to lack of blinding and serious imprecision due to small sample size), extrauterine growth restriction at discharge (downgraded for serious risk of bias due to lack of blinding and serious imprecision due to small sample size), and feed interruption episodes among infants (downgraded for serious risk of bias due to lack of blinding and serious imprecision due to small sample size).

Potential biases in the review process

We have no financial or other conflicts of interest.

We found only two small trials for inclusion in this review. Although we conducted a comprehensive search, we cannot exclude fully the possibility of publication bias because we do not know whether other published (but not indexed) or unpublished trials have been conducted. We did not have a sufficient number of trials to explore symmetry of funnel plots as a means of identifying possible publication or reporting bias.

Agreements and disagreements with other studies or reviews

Three systematic reviews have compared early versus late fortification in preterm infants (Alyahya 2020; Godden 2019; Mimouni 2017). None of these reviews predefined early and late fortification by using a specific feed volume. They intended to include all studies that started fortification at two different feed volumes.

Two systematic reviews included only clinical trials (Alyahya 2020; Mimouni 2017); Godden 2019 included retrospective cohort studies as well. Mimouni 2017 included the two randomised controlled trials (RCTs) that were included in our study (Shah 2016; Sullivan 2010). The studies included in the other two reviews were different (Alyahya 2020; Mimouni 2017). Alyahya 2020 included two RCTs (Alizadeh 2017; Shah 2016). Godden 2019 included three RCTs (Alizadeh 2017; Shah 2016; Sullivan 2010), as well as two retrospective studies (Lapointe 2016; Tillman 2012). Two systematic reviews concluded that early fortification had no significant impact on any clinical outcomes (Alyahya 2020; Mimouni 2017). Godden 2019 concluded that early fortification is safe and well tolerated but has no impact on growth outcomes.

Alizadeh 2017 was an RCT done in 80 infants at 28 to 34 weeks' gestational age with birth weight < 2000 g. This trial compared early fortification (starting from first feed) to late fortification (starting from 75 mL/kg/d feed volume) and showed no difference in clinical outcomes between groups.

Huston 2019 is a large multi-centre retrospective study done in 394 infants with birth weight of 500 to 1250 g. Early fortification (starting at < 60 mL/kg/d) was compared to late fortification (starting at > 60 mL/kg/d). This study showed that early fortification improved weight gain velocity and head growth and decreased the occurrence of chronic lung disease without increasing the risk of NEC.

Tillman 2012 is a retrospective study done in 95 infants at < 31 weeks' gestational age. The study compared early fortification (fortification from first feed) and late fortification (fortification from 50 to 80 mL/kg/d). Early fortification did not increase the incidence of feed intolerance but did not increase weight gain at 34 weeks' postmenstrual age as well. Babies in the early fortification group had less alkaline phosphatase from 33 weeks' postmenstrual age.

Thus, the results of our review matched the results of almost all previous studies and showed that there is no difference in important clinical outcomes between early and late fortification groups, and that limited data are available.

AUTHORS' CONCLUSIONS

Implications for practice

We found only limited data from two unblinded trials on the effects on growth and safety of early fortification compared to late fortification of human milk in preterm infants. The certainty of evidence was low for all outcomes due to lack of blinding and small sample size. Hence, available evidence is insufficient to either support or refute early fortification of human milk to promote growth in preterm infants.

Implications for research

Further randomised controlled trials adequately powered to detect meaningful differences in outcomes are needed to assess whether early fortification compared to late fortification of human milk improves important clinical outcomes for preterm infants. These trials should provide more precise estimates on important outcomes such as in-hospital growth, time to reach full enteral feeds, time to regain birth weight, incidence of extrauterine growth restriction (EUGR), duration of total parenteral nutrition (TPN), incidence of parenteral nutrition-associated liver disease, duration of central venous line (CVL) usage, incidence of invasive infection, and duration of hospital stay. Trials should also provide data on postdischarge growth and neurodevelopmental outcomes. Trialists should aim to include extremely preterm infants and infants with intrauterine growth restriction, so that subgroup analyses can be planned for this population, which is at higher risk of necrotising enterocolitis (NEC).

We identified one ongoing study (IRCT20171030037093N3). This trial has randomised 90 preterm infants into three groups, with fortification started at 30, 70, and 100 mL/kg/d enteral feeds. The main outcome is weight, length, and head circumference at four weeks' postnatal age. This study has been completed, but it remains to be published. See the Characteristics of ongoing studies table.

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We are grateful to Drs Shah, Sullivan, and Lee for providing further details and data from their trials.

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As a Cochrane Neonatal Associate Editor, Dr Jon Dorling has peer-reviewed and offered feedback for this review.

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CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Shah 2016

Study characteristics	
Methods	Randomised controlled trial
Participants	A total of 100 preterm infants were recruited Inclusion criteria: infants with birth weight < 1500 g Exclusion criteria: death or expected to die within 72 hours, major congenital or chromosomal abnormalities, when mother could not provide her own milk and refused the use of donor breast milk
Interventions	Early fortification group - fortification starting from 20 mL/kg/d feeds Late fortification group - fortification starting from 100 mL/kg/d feeds Fortification was done with bovine milk-based liquid HMF (Enfamil); 5 mL HMF was added to 25 mL human milk to increase caloric density to 24 kcal/oz
Outcomes	Primary outcome: time to reach full enteral feeds (≥ 140 mL/kg/d) Secondary outcomes: feed intolerance, NEC, time to regain birth weight, daily weight gain, protein and caloric intake for the first 4 weeks of life, weight velocity at 4 weeks after birth and at 36 weeks' postmenstrual age, TPN days, length of hospital stay, metabolic acidosis, late-onset sepsis, ventilation days, chronic lung disease, postnatal steroid treatment, patent ductus arteriosus, severe intraventricular haemorrhage (grade III and IV), periventricular leukomalacia, retinopathy of prematurity
Notes	Infants were given only trophic feeds for 1 to 3 days depending on their birth weight, followed by a gradual increase in feed volume. Feeds were delivered continuously (3 hours on and 1 hour off). TPN was given until infants reached sufficient enteral feeds Definition of full-volume enteral feeds differed from our definition (≥ 140 vs ≥ 150 mL/kg/d, respectively) Definition of feed intolerance in the trial differed from our definition (≥ 24 hours of feed interruption vs ≥ 12 hours of feed interruption, respectively)

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Block randomisation was performed by computerised software. Block size was 4
Allocation concealment (selection bias)	Unclear risk	Not mentioned

Shah 2016 *(Continued)*

Blinding of participants and personnel (performance bias) All outcomes	High risk	Intervention was not blinded to allow proper handling of mother's own milk and appropriate fortification
Blinding of outcome assessment (detection bias) All outcomes	High risk	Proper handling of mother's own milk and appropriate fortification prevented masking of infants' caregivers and research investigators
Incomplete outcome data (attrition bias) All outcomes	Low risk	All participants' outcomes were analysed, except 1 infant in the early fortification group. Reason was not stated. This was not considered a significant source of bias
Selective reporting (reporting bias)	Low risk	All outcomes mentioned in the protocol were reported
Other bias	Low risk	Nil

Sullivan 2010

Study characteristics	
Methods	Randomised controlled trial
Participants	Inclusion criteria: preterm infants with birth weight 500 to 1250 g were recruited Exclusion criteria: major congenital malformations, high likelihood of transfer to a non-study institution during the study period
Interventions	Infants were randomised into 3 arms: HM100 - human milk-based HMF fortification starting at 100 mL/kg/d feed volume HM40 - human milk-based HMF fortification starting at 40 mL/kg/d feed volume BOV - bovine milk-based HMF fortification or preterm formula feeding We have included only the HM100 (late fortification) and HM40 (early fortification) groups in our analysis. Human milk-based HMF (Prolact+ H2MF) was used, and calorie density was 24 kcal/oz
Outcomes	Primary outcome: duration of TPN Secondary outcomes: growth indices (weight, length, and head circumference), late-onset sepsis, NEC stage 2 or 3, feed intolerance, bronchopulmonary dysplasia, retinopathy of prematurity, duration of CVL usage, duration of hospital day, duration of ventilation and oxygen therapy
Notes	Trophic feeds were given for 5 days, followed by a gradual increase in feed volume up to a maximum of 160 mL/kg/d As per protocol, the study was planned with 2 groups - Group 1 with 3 arms (HM100, HM40, bovine-based HMF) and Group 2 with 2 arms (HM100 vs Preterm/term formula). However, only Group 1 outcomes were published; Group 2 outcomes were not published Triple blinding was mentioned in the protocol but was not followed
Risk of bias	

Sullivan 2010 *(Continued)*

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomisation was performed in blocks of 4
Allocation concealment (selection bias)	Low risk	Separate block randomisation schemes were prepared for each of the strata and were performed centrally
Blinding of participants and personnel (performance bias) All outcomes	High risk	The need to ensure proper handling of mother's own milk precluded true blinding of infants' caregivers
Blinding of outcome assessment (detection bias) All outcomes	High risk	Not mentioned
Incomplete outcome data (attrition bias) All outcomes	Low risk	Data for all enrolled infants were published
Selective reporting (reporting bias)	Low risk	All outcomes mentioned in the protocol were published
Other bias	Low risk	Nil

BOV = bovine.
CVL = central venous line.
HMF = human milk fortifier.
NEC = necrotising enterocolitis.
TPN = total parenteral nutrition.

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Alizadeh 2017	Late fortification was started at 75 mL/kg/d feed volume.
Ghandehari 2012	Post-hoc analysis of Sullivan 2010
Huston 2019	Retrospective study
Maas 2013	Retrospective study including multiple interventions
Sajjadian 2014	No published data available
Tillman 2012	Retrospective study

Characteristics of ongoing studies *[ordered by study ID]*

IRCT20171030037093N3

Study name	Investigation and comparison of the effects of early and late breast milk enrichment in preterm infants

IRCT20171030037093N3 *(Continued)*

Methods	Clinical trial with parallel groups
Participants	Inclusion criteria: infants with gestational age of 28 to 32 weeks with birth weight less than 2000 g Exclusion criteria: presence of any congenital anomaly and formula feeding Planned to recruit 90 preterm infants
Interventions	Group 1: fortification started at 30 mL/kg/d feed volume Group 2: fortification started at 70 mL/kg/d feed volume Group 3: fortification started at 100 mL/kg/d feed volume Fortification is done with 4.4 g of Aptamil human milk fortifier in 100 mL human milk
Outcomes	Weight, length, and head circumference at 4 weeks' postnatal age
Starting date	11 November 2017
Contact information	Name of organisation: Shahre-kord University of Medical Sciences Name of responsible person: Majid Hamidi Street address: Shahrekord University of Medical Sciences, Building No. 2, University headquarters, Ayatollah Kashani Blvd City: Shahrekord Province: Chahar-Mahal-va-Bakhtiari Postal code: 8815713471 Phone: +98 38 3227 4004 Email: majid.hamidi@yahoo.com
Notes	Trial completion date: 1 November 2018 Trial results are not yet published

DATA AND ANALYSES

Comparison 1. Early versus late fortification

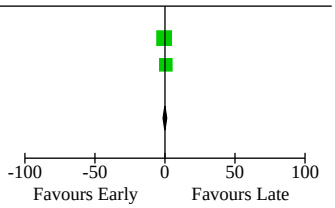
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.1 Time to regain birth weight (days)	2	237	Mean Difference (IV, Fixed, 95% CI)	-0.06 [-1.32, 1.20]
1.2 Linear growth (cm/week)	2	237	Mean Difference (IV, Fixed, 95% CI)	0.10 [-0.03, 0.22]
1.3 Increase in head circumference (cm/week)	2	237	Mean Difference (IV, Fixed, 95% CI)	-0.01 [-0.07, 0.06]
1.4 Necrotising enterocolitis stage 2 or 3	2	237	Risk Ratio (M-H, Fixed, 95% CI)	1.36 [0.44, 4.16]
1.5 Surgical NEC	2	237	Risk Ratio (M-H, Fixed, 95% CI)	0.98 [0.14, 6.85]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.6 Time to reach full enteral feeds	2	237	Mean Difference (IV, Fixed, 95% CI)	0.27 [-3.48, 4.02]
1.7 Extrauterine growth restriction at discharge	2	237	Risk Ratio (M-H, Fixed, 95% CI)	1.06 [0.81, 1.39]
1.8 Proportion of infants with feed interruption episodes	2	237	Risk Ratio (M-H, Fixed, 95% CI)	0.99 [0.73, 1.34]
1.9 Duration of TPN (days)	2	237	Mean Difference (IV, Fixed, 95% CI)	0.08 [-3.07, 3.24]
1.10 Duration of CVL usage (days)	2	237	Mean Difference (IV, Fixed, 95% CI)	1.04 [-3.13, 5.20]
1.11 Incidence of invasive infection	2	237	Risk Ratio (M-H, Fixed, 95% CI)	0.69 [0.40, 1.18]
1.12 All-cause mortality before discharge	2	237	Risk Ratio (M-H, Fixed, 95% CI)	1.32 [0.30, 5.77]
1.13 Duration of hospital stay (days)	2	237	Mean Difference (IV, Fixed, 95% CI)	2.33 [-6.44, 11.11]

Analysis 1.1. Comparison 1: Early versus late fortification, Outcome 1: Time to regain birth weight (days)

Study or Subgroup	Early fortification Mean	SD	Total	Late fortification Mean	SD	Total	Weight	Mean Difference IV, Fixed, 95% CI
Shah 2016	9.9	4.5	49	10.5	4.1	50	55.0%	-0.60 [-2.30 , 1.10]
Sullivan 2010	7.5	6.3	71	6.9	4.9	67	45.0%	0.60 [-1.28 , 2.48]
Total (95% CI)			**120**			**117**	**100.0%**	**-0.06 [-1.32 , 1.20]**

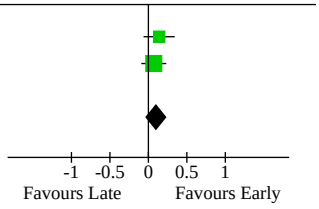
Heterogeneity: Chi² = 0.86, df = 1 (P = 0.35); I² = 0%
Test for overall effect: Z = 0.09 (P = 0.93)
Test for subgroup differences: Not applicable



Analysis 1.2. Comparison 1: Early versus late fortification, Outcome 2: Linear growth (cm/week)

Study or Subgroup	Early fortification Mean	SD	Total	Late fortification Mean	SD	Total	Weight	Mean Difference IV, Fixed, 95% CI
Shah 2016	0.76	0.4	49	0.62	0.61	50	39.0%	0.14 [-0.06 , 0.34]
Sullivan 2010	0.93	0.53	71	0.86	0.44	67	61.0%	0.07 [-0.09 , 0.23]
Total (95% CI)			**120**			**117**	**100.0%**	**0.10 [-0.03 , 0.22]**

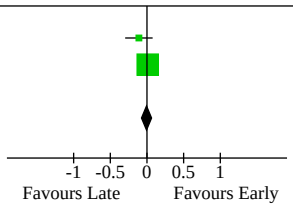
Heterogeneity: Chi² = 0.28, df = 1 (P = 0.60); I² = 0%
Test for overall effect: Z = 1.51 (P = 0.13)
Test for subgroup differences: Not applicable



Analysis 1.3. Comparison 1: Early versus late fortification, Outcome 3: Increase in head circumference (cm/week)

Study or Subgroup	Early fortification Mean	SD	Total	Late fortification Mean	SD	Total	Weight	Mean Difference IV, Fixed, 95% CI
Shah 2016	0.5	0.46	49	0.61	0.49	50	13.3%	-0.11 [-0.30 , 0.08]
Sullivan 2010	0.72	0.22	71	0.71	0.22	67	86.7%	0.01 [-0.06 , 0.08]
Total (95% CI)			**120**			**117**	**100.0%**	**-0.01 [-0.07 , 0.06]**

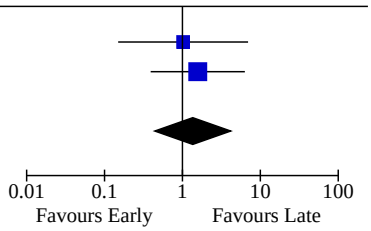
Heterogeneity: Chi² = 1.37, df = 1 (P = 0.24); I² = 27%
Test for overall effect: Z = 0.17 (P = 0.86)
Test for subgroup differences: Not applicable



Analysis 1.4. Comparison 1: Early versus late fortification, Outcome 4: Necrotising enterocolitis stage 2 or 3

Study or Subgroup	Early fortification Events	Total	Late fortification Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Shah 2016	2	49	2	50	39.1%	1.02 [0.15 , 6.96]
Sullivan 2010	5	71	3	67	60.9%	1.57 [0.39 , 6.33]
Total (95% CI)		**120**		**117**	**100.0%**	**1.36 [0.44 , 4.16]**
Total events:	7		5			

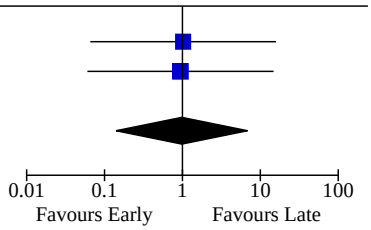
Heterogeneity: Chi² = 0.13, df = 1 (P = 0.72); I² = 0%
Test for overall effect: Z = 0.53 (P = 0.59)
Test for subgroup differences: Not applicable



Analysis 1.5. Comparison 1: Early versus late fortification, Outcome 5: Surgical NEC

Study or Subgroup	Early fortification Events	Total	Late fortification Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Shah 2016	1	49	1	50	49.0%	1.02 [0.07 , 15.86]
Sullivan 2010	1	71	1	67	51.0%	0.94 [0.06 , 14.79]
Total (95% CI)		**120**		**117**	**100.0%**	**0.98 [0.14 , 6.85]**
Total events:	2		2			

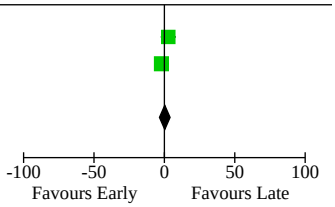
Heterogeneity: Chi² = 0.00, df = 1 (P = 0.97); I² = 0%
Test for overall effect: Z = 0.02 (P = 0.98)
Test for subgroup differences: Not applicable



Analysis 1.6. Comparison 1: Early versus late fortification, Outcome 6: Time to reach full enteral feeds

Study or Subgroup	Early fortification Mean	SD	Total	Late fortification Mean	SD	Total	Weight	Mean Difference IV, Fixed, 95% CI
Shah 2016	28.5	14.7	49	25.7	12.6	50	48.4%	2.80 [-2.60 , 8.20]
Sullivan 2010	24.4	12.7	71	26.5	18	67	51.6%	-2.10 [-7.33 , 3.13]
Total (95% CI)			**120**			**117**	**100.0%**	**0.27 [-3.48 , 4.02]**

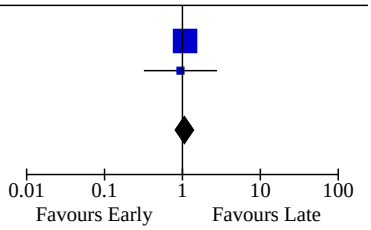
Heterogeneity: Chi² = 1.63, df = 1 (P = 0.20); I² = 39%
Test for overall effect: Z = 0.14 (P = 0.89)
Test for subgroup differences: Not applicable



Analysis 1.7. Comparison 1: Early versus late fortification, Outcome 7: Extrauterine growth restriction at discharge

Study or Subgroup	Early fortification Events	Total	Late fortification Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Shah 2016	36	49	34	50	84.5%	1.08 [0.84 , 1.39]
Sullivan 2010	6	71	6	67	15.5%	0.94 [0.32 , 2.78]
Total (95% CI)		**120**		**117**	**100.0%**	**1.06 [0.81 , 1.39]**
Total events:	42		40			

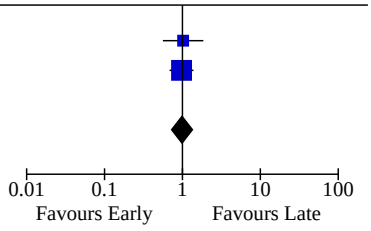
Heterogeneity: Chi² = 0.07, df = 1 (P = 0.80); I² = 0%
Test for overall effect: Z = 0.42 (P = 0.67)
Test for subgroup differences: Not applicable



Analysis 1.8. Comparison 1: Early versus late fortification, Outcome 8: Proportion of infants with feed interruption episodes

Study or Subgroup	Early fortification Events	Total	Late fortification Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Shah 2016	15	49	15	50	31.1%	1.02 [0.56 , 1.85]
Sullivan 2010	33	71	32	67	68.9%	0.97 [0.68 , 1.39]
Total (95% CI)		**120**		**117**	**100.0%**	**0.99 [0.73 , 1.34]**
Total events:	48		47			

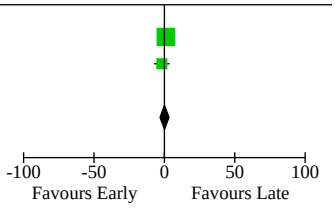
Heterogeneity: Chi² = 0.02, df = 1 (P = 0.89); I² = 0%
Test for overall effect: Z = 0.08 (P = 0.94)
Test for subgroup differences: Not applicable



Analysis 1.9. Comparison 1: Early versus late fortification, Outcome 9: Duration of TPN (days)

Study or Subgroup	Early fortification Mean	SD	Total	Late fortification Mean	SD	Total	Weight	Mean Difference IV, Fixed, 95% CI
Shah 2016	21.4	10.3	49	20.4	9.2	50	67.2%	1.00 [-2.85 , 4.85]
Sullivan 2010	23.9	16.6	71	25.7	16.4	67	32.8%	-1.80 [-7.31 , 3.71]
Total (95% CI)			**120**			**117**	**100.0%**	**0.08 [-3.07 , 3.24]**

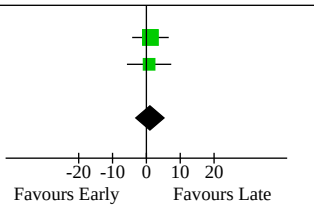
Heterogeneity: Chi² = 0.67, df = 1 (P = 0.41); I² = 0%
Test for overall effect: Z = 0.05 (P = 0.96)
Test for subgroup differences: Not applicable



Analysis 1.10. Comparison 1: Early versus late fortification, Outcome 10: Duration of CVL usage (days)

Study or Subgroup	Early fortification Mean	SD	Total	Late fortification Mean	SD	Total	Weight	Mean Difference IV, Fixed, 95% CI
Shah 2016	19.9	14.7	49	18.7	12.6	50	59.5%	1.20 [-4.20 , 6.60]
Sullivan 2010	25.4	20.8	71	24.6	18.4	67	40.5%	0.80 [-5.74 , 7.34]
Total (95% CI)			**120**			**117**	**100.0%**	**1.04 [-3.13 , 5.20]**

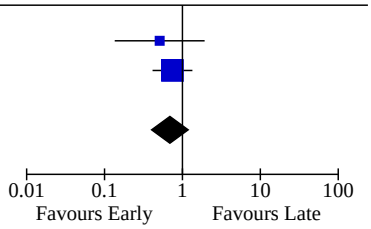
Heterogeneity: Chi² = 0.01, df = 1 (P = 0.93); I² = 0%
Test for overall effect: Z = 0.49 (P = 0.63)
Test for subgroup differences: Not applicable



Analysis 1.11. Comparison 1: Early versus late fortification, Outcome 11: Incidence of invasive infection

Study or Subgroup	Early fortification Events	Total	Late fortification Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Shah 2016	3	49	6	50	23.3%	0.51 [0.14 , 1.93]
Sullivan 2010	15	71	19	67	76.7%	0.74 [0.41 , 1.34]
Total (95% CI)		**120**		**117**	**100.0%**	**0.69 [0.40 , 1.18]**
Total events:	18		25			

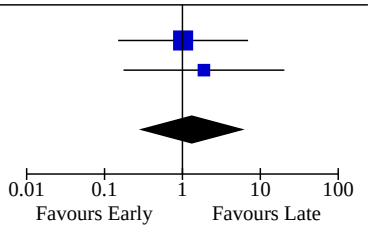
Heterogeneity: Chi² = 0.26, df = 1 (P = 0.61); I² = 0%
Test for overall effect: Z = 1.35 (P = 0.18)
Test for subgroup differences: Not applicable



Analysis 1.12. Comparison 1: Early versus late fortification, Outcome 12: All-cause mortality before discharge

Study or Subgroup	Early fortification Events	Total	Late fortification Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Shah 2016	2	49	2	50	65.8%	1.02 [0.15 , 6.96]
Sullivan 2010	2	71	1	67	34.2%	1.89 [0.18 , 20.33]
Total (95% CI)		**120**		**117**	**100.0%**	**1.32 [0.30 , 5.77]**
Total events:	4		3			

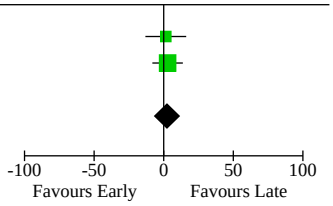
Heterogeneity: Chi² = 0.16, df = 1 (P = 0.69); I² = 0%
Test for overall effect: Z = 0.37 (P = 0.72)
Test for subgroup differences: Not applicable



Analysis 1.13. Comparison 1: Early versus late fortification, Outcome 13: Duration of hospital stay (days)

Study or Subgroup	Early fortification Mean	SD	Total	Late fortification Mean	SD	Total	Weight	Mean Difference IV, Fixed, 95% CI
Shah 2016	71.9	40.5	49	70.4	33.5	50	35.8%	1.50 [-13.16 , 16.16]
Sullivan 2010	78.2	32.7	71	75.4	32.9	67	64.2%	2.80 [-8.15 , 13.75]
Total (95% CI)			**120**			**117**	**100.0%**	**2.33 [-6.44 , 11.11]**

Heterogeneity: Chi² = 0.02, df = 1 (P = 0.89); I² = 0%
Test for overall effect: Z = 0.52 (P = 0.60)
Test for subgroup differences: Not applicable



APPENDICES

Appendix 1. Search methods

The RCT filters were created using Cochrane's highly sensitive search strategies for identifying randomised trials (Higgins 2017). The neonatal filters were created and tested by the Cochrane Neonatal Information Specialist.

Cochrane CENTRAL via CRS Web

Date searched: 15 August 2019
Terms:
1. MESH DESCRIPTOR Milk, Human EXPLODE ALL AND CENTRAL:TARGET
2. MESH DESCRIPTOR Food, Fortified EXPLODE ALL AND CENTRAL:TARGET
3. MESH DESCRIPTOR Dietary Supplements EXPLODE ALL AND CENTRAL:TARGET

4. #3 OR #2
5. #1 AND #4
6. (fortif* OR supplement* OR enrich*) ADJ4 (human OR breast OR expressed OR mother* OR maternal OR donor*) ADJ2 milk* AND CENTRAL:TARGET
7. (fortif* OR supplement* OR enrich*) ADJ4 (DHM OR HM OR breastmilk*) AND CENTRAL:TARGET
8. #5 OR #6 OR #7
9. MESH DESCRIPTOR Infant, Newborn EXPLODE ALL AND CENTRAL:TARGET
10. infant or infants or infantile or infancy or newborn* or "new born" or "new borns" or "newly born" or neonat* or baby* or babies or premature or prematures or prematurity or preterm or preterms or "pre term" or premies or "low birth weight" or "low birthweight" or VLBW or LBW or ELBW or NICU AND CENTRAL:TARGET
11. #10 OR #9 AND CENTRAL:TARGET
12. #8 AND #11

MEDLINE via Ovid

Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) 1946 to Present:
Date ranges: 1946 to 15 August 2019
Terms:
1. exp Milk, Human/
2. exp Food, Fortified/
3. exp Dietary Supplements/
4. 2 or 3
5. 1 and 4
6. (fortif* adj4 ((human or breast or expressed) adj2 milk*)).mp.
7. (fortif* adj4 ((mother* or maternal or donor*) adj2 milk*)).mp.
8. (supplement* adj4 ((human or breast or expressed) adj2 milk*)).mp.
9. (supplement* adj4 ((mother* or maternal or donor*) adj2 milk*)).mp.
10. (enrich* adj4 ((human or breast or expressed) adj2 milk*)).mp.
11. (enrich* adj4 ((mother* or maternal or donor*) adj2 milk*)).mp.
12. ((fortif* or supplement* or enrich*) adj4 DHM).mp.
13. ((fortif* or supplement* or enrich*) adj4 HM).mp.
14. ((fortif* or supplement* or enrich*) adj4 breastmilk*).mp.
15. 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14
16. 5 or 15
17. exp infant, newborn/
18. (newborn* or new born or new borns or newly born or baby* or babies or premature or prematurity or preterm or pre term or low birth weight or low birthweight or VLBW or LBW or infant or infants or infantile or infancy or neonat*).ti,ab.
19. 17 or 18
20. randomized controlled trial.pt.
21. controlled clinical trial.pt.
22. randomized.ab.
23. placebo.ab.
24. drug therapy.fs.
25. randomly.ab.
26. trial.ab.
27. groups.ab.
28. or/20-27
29. exp animals/ not humans.sh.
30. 28 not 29
31. 19 and 30
32. 16 and 31

MEDLINE via PubMed

Date ranges: 01 August 2018 to 15 August 2019
Terms: ((((("Milk, Human"[Mesh] AND ("Food, Fortified"[Mesh] OR "Dietary Supplements"[Mes2h])))) OR ((fortif*[TW] OR supplement*[TW] OR enrich*[TW]) AND (human[TW] OR breast[TW] OR expressed[TW] OR mother*[TW] OR maternal[TW] OR donor*[TW]) AND milk*[TW])) OR ((fortif*[TW] OR supplement*[TW] OR enrich*[TW]) AND (DHM[TW] OR HM[TW] OR breastmilk*[TW]))) AND (((infant, newborn[MeSH] OR newborn*[TIAB] OR "new born"[TIAB] OR "new borns"[TIAB] OR "newly born"[TIAB] OR baby*[TIAB] OR babies[TIAB] OR premature[TIAB] OR prematurity[TIAB] OR preterm[TIAB] OR "pre term"[TIAB] OR “low birth weight”[TIAB] OR "low birthweight"[TIAB] OR VLBW[TIAB] OR LBW[TIAB] OR infant[TIAB] OR infants[TIAB] OR infantile[TIAB] OR infancy[TIAB] OR neonat*[TIAB]) AND (randomized controlled trial[pt] OR controlled clinical trial[pt] OR randomized[tiab] OR placebo[tiab] OR drug therapy[sh] OR randomly[tiab] OR trial[tiab] OR groups[tiab]) NOT (animals[mh] NOT humans[mh]))) Filters activated: Publication date from 2018/08/01

CINAHL via EBSCOhost

Date ranges: 1981 to 15 August 2019
Terms:
S1MH milk, human
S2MH Food, Fortified
S3MH Dietary Supplementation
S4S2 OR S3
S5S1 AND S4
S6(fortif* OR supplement* OR enrich*) AND (human OR breast OR expressed OR mother* OR maternal OR donor*) AND milk*
S7(fortif* OR supplement* OR enrich*) AND (DHM OR HM OR breastmilk*)
S8S5 OR S6 OR S7
S9((infant or infants or infantile or infancy or newborn* or "new born" or "new borns" or "newly born" or neonat* or baby* or babies or premature or premature or prematurity or preterm or preterms or "pre term" or premies or "low birth weight" or "low birthweight" or VLBW or LBW)) AND ((randomized controlled trial OR controlled clinical trial OR randomized OR placebo OR clinical trials as topic OR randomly OR trial OR PT clinical trial))
S10S8 AND S9

ISRCTN

Date searched: 15 August 2019
Search terms:
milk AND Interventions: fortification AND Participant age range: Neonate
Retrieved: 2 (1 was retrieved in the CRS, so the remaining record was saved in the text file)
milk AND Interventions: supplementation AND Participant age range: Neonat

Appendix 2. 'Risk of bias' tool

We used the standard methods of Cochrane and Cochrane Neonatal to assess the methodological quality of trials. For each trial, we sought information regarding the method of randomisation and blinding and reporting of all outcomes of all infants enrolled in the trial. We assessed each criterion as being at low, high, or unclear risk of bias. Both review authors separately assessed each study. We resolved any disagreement by discussion. We added this information to the table 'Characteristics of included studies'. We evaluated the following issues and entered the findings into the 'Risk of bias' table.

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

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

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

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

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

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

3. 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 categorised the methods used to blind study participants and personnel from knowledge of which intervention a participant received. Blinding was assessed separately for different outcomes or class of outcomes. We categorised the methods as:

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

4. 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 categorised the methods used to blind outcome assessment. Blinding was assessed separately for different outcomes or classes of outcomes. We categorised the methods as:

1. low risk for outcome assessors;
2. high risk for outcome assessors; or
3. unclear risk for outcome assessors.

5. 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 described the completeness of data including attrition and exclusions from the analysis. We noted 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 is reported or supplied by the trial authors, we re-included missing data in the analyses. We categorised the methods as:

1. low risk (< 20% missing data);
2. high risk (≥ 20% missing data); or
3. unclear risk.

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

For each included study, we described 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 compared prespecified outcomes versus outcomes eventually reported in the published results. If the study protocol was not published in advance, we contacted study authors to gain access to the study protocol. We assessed the methods as:

1. 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);
2. high risk (where not all of 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
3. unclear risk.

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

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

1. low risk;
2. high risk; or
3. unclear risk.

HISTORY

Protocol first published: Issue 8, 2019
Review first published: Issue 7, 2020

CONTRIBUTIONS OF AUTHORS

Both authors (ST and TA) developed the protocol, screened search outputs, assessed study eligibility, and extracted and synthesised data. Both authors (ST and TA) assessed risk of bias across key domains and undertook GRADE assessment. Both authors revised the final review.

DECLARATIONS OF INTEREST

ST has no conflict of interest to declare.

TA has no conflict of interest to declare.

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DIFFERENCES BETWEEN PROTOCOL AND REVIEW

We made the following changes to the published protocol (Thanigainathan 2019).

We did not prespecify in the protocol outcomes for Summary of findings 1. We defined feed interruption as interruption for ≥ 12 hours. However, in Shah 2016, feed interruption was defined as interruption for ≥ 24 hours, which we accepted and included the outcome data in our meta-analysis.

As of July 2019, the Cochrane Neonatal Review Group no longer searches Embase for its reviews. RCTs and controlled clinical trials (CCTs) from Embase are added to the Cochrane Central Register of Controlled Trials (CENTRAL) via a robust process (see How CENTRAL is created). Cochrane Neonatal has validated its searches to ensure that relevant Embase records are found while searching CENTRAL.

Also starting in July 2019, the Cochrane Neonatal Review Group no longer searches for RCTs and CCTs from ClinicalTrials.gov nor from the World Health Organization's ICTRP (http://International Clinical Trials Registry Platform), as records from both platforms are added to CENTRAL on a monthly basis (see How CENTRAL is created). Comprehensive search strategies are executed in CENTRAL to retrieve relevant records. The ISRCTN at http://www.isrctn.com/, formerly Controlled-trials.com, is searched separately.

For the 2019 update, we developed a new search strategy, which we ran without applying date limits (Appendix 1).

INDEX TERMS

Medical Subject Headings (MeSH)

Birth Weight; Enterocolitis, Necrotizing [epidemiology]; *Food, Fortified; Head [growth & development]; Infant, Premature [*growth & development]; Infant, Very Low Birth Weight [*growth & development]; *Milk, Human; Randomized Controlled Trials as Topic; Time Factors

MeSH check words

Humans; Infant, Newborn