Supplementary material

Table a Systematic review protocol

Review title	Early versus delayed fortification of human milk in very low birth weight preterm infants: a systematic review
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Reviewers team	Prof Christine A. Edwards Dr Judith Simpson Dr Ada L. Garcia Dr Helen Mactier
Background	Nutrition in early postnatal life can predict the risk of short-term morbidities and long-term outcomes. It is crucial to optimize feeding practice for preterm infants in a timely manner. Expressed breast milk (EBM) confers numerous advantages for preterm infants, especially those born with a very low birth weight (VLBW). EBM is commonly supplemented with commercially available human milk fortifier (HMF) to meet the additional nutritional needs of preterm infants. The timing at which to introduce fortification is unknown. In this review, we will aim to compare the outcomes of early versus delayed introduction of HMF to enteral feeding for VLBW preterm infants and explore research priorities.
Outcomes	**Primary outcomes:** • Short-term growth parameters [length, head growth, and weight gain]. • Feeding intolerance. • Length of hospital stay. • Post-menstrual age at discharge (i.e. gestational age plus chronological age). **Secondary outcomes:** • Necrotizing enterocolitis • Sepsis.
Search Strategy	**Search methods** • Electronic and manual search will be used. Five databases will be searched using search terms tailored to different databases. Clinical trials registration websites will be searched for ongoing and completed studies. Details of search strategy is available in Table b. • The reference lists of the most relevant papers and reviews will be searched manually to identify any relevant study not detected by the electronic search. • Search limits English language and no date restriction.
Inclusion criteria	• **Participants:** VLBW preterm infants, Infants are fed exclusively or predominantly fed (>50% of enteral feeding) with EBM. • **Settings:** Neonatal intensive care units or special care baby Unit. • **Study design:** Randomized controlled trials and quasi-randomized controlled trials. Designed to compare early versus delayed EBM fortification. • **Interventions or exposures:** Early versus delayed fortification of EBM indicated as enteral feeds volume or age in days, using multinutrient fortifier (mothers' own milk or donated human milk). • **Outcomes measurement:** at least one of the primary or secondary outcomes is measured.
Exclusion Criteria	• **Participants:** infants born with congenital problems. • **Study designs:** Observational studies or case reports. • **Outcomes measurement:** primary or secondary outcome was measured
Review method	• **Study selection:** screening of studies will be done by the first reviewer (WA) and then by the other three reviewers independently (JS, CE & AG). The first reviewer (WA) will do abstract screening and assessment of full texts. Two reviewers (CE & AG) will check excluded studies. Inclusion criteria will be applied using eligibility checklist (Appendix 1). • **Data extraction:** WA will extract the data in details from the studies meeting the inclusion criteria using data extraction form (Appendix 2). Data items: Authors and publication date, methods, information on intervention baseline subject's

	characteristics, nutrition management, outcomes definitions and outcomes measurement.
	• **Risk of bias:** Three reviewers (WA, CE and AG) will independently assess the risk of bias Using concepts and criteria adopted from Cochrane Collaboration tool. • **Quality assessment:** A scoring tool that we developed will be used for quality assessment. Three reviewers (WA, CE & AG) will independently assess evidence quality for the outcome of interest. • **Data synthesis:** Statistical analysis using RevMan 5.3 for the outcomes if the intervention and participants are comparable. The model for meta-analysis will be fixed-effect model. • **Analysis of subgroups:** Based on data availability, subgroups analysis will be done for following categories: - Type of milk fortified: Mothers' own milk versus donated human milk. - Origin of fortifier: human milk based versus bovine based. - Birth weight: very low birth weight (<1500g) versus extremely low birth weight (<1000 g).
Results presentation	Flow chart, tables and forest plots

Table b Search strategy

Database	Search terms	Websites
PubMed	Human milk[ti] AND fortif*[ti] food, fortified[mesh] AND milk, human[mesh]	www.ncbi.nlm.nih.gov/pubmed/
	("Infant, Premature/growth and development"[MAJR]) AND "Food, Fortified"[MAJR]	
	("Infant, Premature/growth and development"[MAJR]) AND "Food, Fortified"[MAJR] OR Human milk[ti] AND fortif*[ti] OR food, fortified[mesh] AND milk, human[mesh]	
Ovid Medline	Human milk AND Food, fortified	http://ovidsp.tx.ovid.com.ezproxy.lib.gla.ac.uk/sp-3.33.0b/ovidweb.cgi
	Human milk and fortif*	
	Infant, Premature/gd, mo [Growth & Development, Mortality] AND Food, Fortified/	
	breast milk and fortif*	
Web of science	Human milk AND fortif*	https://apps.webofknowledge.com/UA_GeneralSearch_input.do?product=UA&search_mode=GeneralSearch&SID=E25G8tft7FzzkZi8sU5&preferencesSaved=
	Breast milk AND fortif*	
Cochrane library	Human milk AND fortified food	http://www.cochrane.org/
	Premature infant AND fortified food	
	Human milk AND fortification	
	Breast milk AND fortif*	
Ovid Embase	Milk, Human AND Food, Fortified/	http://ovidsp.tx.ovid.com.ezproxy.lib.gla.ac.uk/sp-3.33.0b/ovidweb.cgi
	Human milk and fortif*	
	Low birth weight AND breast milk AND food fortified or diet supplementation	
	Breast milk AND fortif*	
Clinical trial registry	human milk fortification	www.clinicaltrial.gov
	Expressed breast milk	
	Fortification	

Database	Search terms	Websites
Current Controlled Trials	human milk fortification Expressed breast milk Fortification	www.isrctn.com

Table c Criteria for assessing risk of bias

Type of bias	Criteria for judgment		
	Low risk	**High risk**	**Unclear risk**
Random sequence generation	Described clearly the method used of random sequence generation	Used non-random methods for randomisation	Not reported
Allocation concealment	Allocation sequence was concealed	Inadequate concealment of allocations prior to assignment.	Not reported
Blinding of participants and personnel	Personnel were blinded of the allocated intervention during the study.	Personnel was not blinded of the allocated intervention during the study.	Not reported
Blinding of outcome assessment	Outcome evaluator was blinded for outcome measurement	Outcome evaluator was not blinded for outcome measurement	Not reported
Incomplete outcome data	Describe the completeness of outcome data for each main outcome, including attrition and exclusions from the analysis	Attrition bias due to amount, nature or handling of incomplete outcome data	Not reported
Selective reporting	All outcomes were reported	Selective outcome reporting	Not reported

Table d Quality assessment tool

Criteria	Score
Multicentre	
One	0
Two	1
More than two	2
Sample size	
< 50	0
≥ 50	1
≥ 100	2
Description of Intervention	
Not defined	0
Not clear	1
Clear	2
Baseline characteristics comparable	0,1
Outcomes defined	0,1
Outcomes measurement described	0,1
Confounding factors	0,1
Decision	
Low quality	≤ 3
Moderate quality	4 - 6
High quality	≥ 7

Table e Definition of outcomes in the included studies

	Shah et al		Alizadeh et al	
Study outcomes	**Outcome**	**Definition**	**Outcomes**	**Definition**
Primary outcome	Time to reach full feeding	Dumber of days to reach full feeding from birth and from feeds initiation (i.e. > than 140 ml/kg/d).	Growth	Weight, head circumference and length change.
Secondary outcomes	Feeding intolerance	EN stopped: EN held for at least 24 hours as a result of feeding intolerance	Feeding intolerance	Signs of feeding intolerance: Emesis, abdominal distention, or gastric residual > 20-30 % of total EN in the last feeding.
	NEC	Stage II or greater using Bell criteria	NEC	• Clinical diagnosis criteria: Sepsis like syndrome, abdominal distention, feeding intolerance, bloody stool, abdominal tenderness, abdominal skin erythema, apnea, and shock. • Laboratory diagnosis criteria: Changed in complete blood count and arterial blood gases including metabolic acidosis and thrombocytopenia. • Radiologic diagnosis criteria: Bowel distention, pneumatosis intestinalis, air in the biliary ducts and pneumoperitoneum.
	Late-onset sepsis	clinical signs of sepsis associated with positive blood culture after 3 days of age	Sepsis	Signs of sepsis. Clinical syndrome of bacteremia with systemic signs and symptoms of infection in the first weeks of life.
Other secondary outcomes	• Weight velocity • days on parenteral nutrition • Length of stay.			