Office of Evidence Based Practice (EBP) – Critically Appraised Topic (CAT): Human milk – derived fortifier use in very low birth weight infants

Specific Care Question In very low birth weight infants, ≤ 1250 gm, does the use of human milk-derived fortifier (HMF) compared to bovine milk fortifier (BMF) support better outcomes for necrotizing enterocolitis (NEC), feeding tolerance, neurodevelopment, bronchopulmonary dysplasia (BPD), and retinopathy of prematurity (ROP)?

Recommendations Based on Current Literature (Best Evidence) Only

A conditional recommendation is made for the use of human milk fortifier in very low birth weight infants to decrease incidence of NEC, based on the GRADE Evidence to Decision instrument[a] and the Summary of Findings Table[a]. The overall certainty in the evidence is very low[a]. Two randomized controlled trials (RCT) and five cohort studies found that human milk fortifier added to human milk for nutritional support in VLBW infants was favorable in reducing incidences of NEC (see Summary by Outcome for substantiation of recommendations).

No recommendation can be made for or against the use of human milk fortifier to human milk for VLBW infants in decreasing feeding intolerance, based on an expert review of current literature by the subject matter expert and the Department of EBP. The overall certainty in the evidence is very low[a]. While the cohort study reported fewer incidences of feeding intolerance with HMF compared to bovine milk fortifier, the RCT found no difference in feeding tolerance.

No recommendation can be made for or against the use of human milk fortifier to human milk for VLBW infants for improved neurodevelopment, based on an expert review of current literature by the subject matter expert and the Department of EBP. The overall certainty in the evidence is very low[a]. The evidence does not demonstrate any differences in neurodevelopmental gains over the first 18 months of development with the addition of HMF compared to BMF.

No recommendation can be made for or against the use of human milk fortifier to human milk for VLBW infants to decrease incidence of BPD, based on an expert review of current literature by the subject matter expert and the Department of EBP. The overall certainty in the evidence is low[a]. While the cohort studies reported fewer incidences of BPD with HMF compared to BMF, the RCTs found no difference.

A conditional recommendation is made for the use of human milk fortifier in very low birth weight infants to decrease incidence of ROP, based on an expert review of current literature by the subject matter expert and the Department of EBP. The overall certainty in the evidence is very low[a]. The cohort studies indicated the intervention of HMF compared to BMF was favorable in reduction of incidences of ROP, but one RCT found no difference.

Based on data summarized for all outcomes, human milk fortifier may be beneficial for the outcomes of NEC, ROP, and BPD but the evidence is mixed and of low to very low certainty. Consideration of product cost and the certainty of evidence should be practiced when using this product. When there is a lack of scientific evidence, standard work should be developed, implemented, and monitored.

Literature Summary

Background

Very low birth weight (VLBW) infants, categorized as those whose birth weight is ≤ 1250 grams, have increased demands on energy stores secondary to exponential postnatal growth (Premkumar et al., 2020). Without added nutritional support, infants are at risk for decreased immunity, growth reduction, and sepsis, leading to issues with neurodevelopment, digestive health, and both lung and visual development (Taylor, 2019). Infants are provided with various means of nutrition to fill these fat and energy stores, including but not limited to, mother's own milk, donated human milk, bovine milk, and formula, which can be animal or plant-based (Brown, 2016; Premkumar et al., 2020). For nutritional support, milk alone is not enough to provide the enteral energy and protein intake needed for a preterm infant to achieve adequate growth (Ananthan et al., 2020; Hopperton et al., 2019; Premkumar et al., 2020). Both human milk-derived fortifiers and bovine milk-derived fortifiers play a supportive role in providing the higher content of protein, calcium and phosphate needed in VLBW infants. There has been a shift in care standards to use primarily the HMF due to the associated reduced risk of an exclusive human milk diet on reduction of necrotizing enterocolitis (Ananthan et al., 2020; Taylor, 2019). Mounting evidence shows that feeding an exclusive human milk diet (EHMD) to preterm infants, especially those less than 1250 gm, lowers the risk for adverse outcomes and increased length of stay compared to

those fed exclusively or partially bovine milk products (Lucas et al., 2020). Another challenge in the management and care of VLBW infants includes feeding intolerance which can delay not only nutritional support but also nutritional advancement (Sandhu, 2017). Due to the highly desirable benefits of an EHMD, including HMF, the American Academy of Pediatrics (2021), recommends breastfeeding or offering mother's own milk through a minimum of six months of age for all infants. Considering the overall benefits of an EHMD, many neonatal intensive care units are providing and supporting an EHMD, including human milk fortifier. However, the literature to compare the benefits of the addition of HMF to human milk compared to BMF to human milk for the VLBW infant is limited. This review will summarize identified literature to answer the specific care question on the topic.

Study Characteristics

The search for suitable studies was completed on April 5, 2021. A. Jones, MD, FAAP reviewed the 79 titles and/or abstracts found in the search and identified[b] 18 studies believed to answer the question. After an in-depth review of the identified 18 studies[d], nine were determined to answer the question. Three cohort studies (Box & Shakuntala, 2018; Bushati et al., 2021; Delaney Manthe et al., 2019), one systematic review (Villamor-Martínez et al., 2018), which included three cohorts (Assad et al., 2016; Colacci et al., 2017; Hair et al., 2016), and two randomized controlled trials (O'Connor et al., 2018; Sullivan et al., 2010), answered the question. For this review, human milk fortifier, or HMF, will include only fortifiers derived from human milk and bovine milk fortifier, or BMF, will include to only fortifiers derived from cow's milk.

Summary by Outcome

Necrotizing Enterocolitis

Seven studies (Box & Shakuntala, 2018; Bushati et al., 2021; Colacci et al., 2017; Delaney Manthe et al., 2019; Hair et al., 2016; O'Connor et al., 2018; Sullivan et al., 2010) measured for the incidence of necrotizing enterocolitis (NEC) (defined as Bell Stage II or greater) in VLBW infants receiving HMF versus BMF once intake reached 100 mL/kg/d, (N = 2,286). For the two reported RCTs (O'Connor et al., 2018; Sullivan et al., 2010) (n = 261), the OR = 0.32, 95% CI [0.12, 0.84], p = .02, HMF was favorable in decreasing the incidence of NEC (see Figure 2 & *Table 1*). For the five reporting cohorts (Box & Shakuntala, 2018; Bushati et al., 2021; Colacci et al., 2017; Delaney Manthe et al., 2019; Hair et al., 2016) (n = 2,025), the OR = 0.45, 95% CI [0.33, 0.60], p = .00001, HMF was also favorable in decreasing the incidence of NEC (see Figure 3 & *Table 1*). Based on the data reported within the seven studies, the use of HMF in VLBW infants will result in 19 to 113 fewer cases of NEC at stage II or greater per 1,000 VLBW infants.

Certainty Of The Evidence For Necrotizing Enterocolitis (NEC). The certainty of the body of evidence was very low based on four factors[a]: *within-study risk of bias*, *consistency among studies, directness of evidence*, and *precision of effect estimates*. The body of evidence was assessed to have serious risk of bias, serious inconsistency, and serious imprecision. Risk of bias was serious due to lack of blinding, in addition the RCTs were not powered to detect the outcome of NEC. Risk of bias was serious in the cohort studies due to number of potential confounding variables unreported. There was serious inconsistency due to judgment of moderate heterogeneity based on the I^2 of 66% in the cohort studies. Serious imprecision was found in the cohort studies due to low number of events (n = 213).

Feeding Intolerance

Four studies (Assad et al., 2016; Bushati et al., 2021; O'Connor et al., 2018; Sullivan et al., 2010) measured feeding intolerance as days of no oral intake following oral feed initiation in VLBW infants receiving HMF versus BMF once oral intake reached 100 mL/kg/d of, (N = 497). For the outcome of feeding intolerance, two of the studies (Assad et al., 2016; Sullivan et al., 2010) did not report full data to include in analysis however, the O'Connor et al. (2018) an RCT (n = 125), indicated the intervention of HMF was not different to the comparator of BMF in reducing feeding intolerance in VLBW infants OR = 0.74, 95% CI [0.34, 1.60], p = .45 (see Figure 4 & *Table 1)*. Bushati et al., 2021 (n = 64), reported a MD = -3.00, 95% CI [-4.89, -1.11], p = .002 indicating the intervention of HMF was favorable to the comparator of BMF in reducing feeding intolerance in VLBW infants (see Figure 5 & *Table 1).* Based off the data reported in the RCT study, the use of HFM in VLBW infants will result in an average of 63 (110 to 186) fewer incidences of feeding intolerance per 1,000 VLBW infants (O'Connor et al. 2018).

Certainty Of The Evidence For Feeding Intolerance. The certainty of the body of evidence was very low based on four factors[a]: *within-study risk of bias*, *consistency among studies, directness of evidence*, and *precision of effect estimates*. The body of evidence was assessed to have

serious risk of bias and very serious imprecision. Serious risk of bias was assessed for the cohort study due to clinical discretion used to determine withholding of oral feedings. Risk of bias for the RCT study was not serious. Very serious imprecision was assessed for the cohort study based on the mean number of events of 64 and in the RCT study there was a total of 37 events between the HMF and BMF groups.

Neurodevelopment

One cohort study (Colacci et al., 2017) measured neurodevelopment (cognitive, language, motor), using the Bayley-III Scales of Infant and Toddler Development of VLBW infants that had received HMF versus BMF once oral feeds reached a dosage of 100 mL/kg/d. Colacci et al. (2017) completed neurodevelopment assessments at 6 months of chronological age, (*n* = 190), at twelve months of chronological age, (*n* = 160) and at 18 months of chronological age (*n* = 165). For the outcome of cognitive development at 6 months of chronological age, the *MD* = -1.00, 95% CI [-6.48, 4.48], *p* = .72 indicated the intervention of HMF was not different to the comparator of BMF (see Figure 6 & *Table 1*). For the outcome of language development at 6 months of chronological age, the *MD* = 2.00, 95% CI [-3.39, 7.39], *p* = .47 indicated the intervention of HMF was not different to the comparator of BMF (see Figure 6 & *Table 1*). For the outcome of motor development at 6 months of chronological age, the *MD* = 2.00, 95% CI [-5.93, 9.93], *p* = .62 indicated the intervention of HMF was not different to the comparator of BMF (see Figure 6 & *Table 1*). For the outcome of cognitive development at 12 months of chronological age, the *MD* = 1.00, 95% CI [-4.02, 6.02], *p* = .70 indicated the intervention of HMF was not different to the comparator of BMF (see Figure 7 & *Table 1*). For the outcome of language development at 12 months of chronological age, the *MD* = 0.00, 95% CI [-5.00, 5.00], *p* = 1.00 indicated the intervention of HMF was not different to the comparator of BMF (see Figure 7 & *Table 1*). For the outcome of motor development at 12 months of chronological age, the *MD* = -2.00, 95% CI [-8.40, 4.40], *p* = .54 indicated the intervention of HMF was not different to the comparator of BMF (see Figure 7 & *Table 1*). For the outcome of cognitive development at 18 months of chronological age, the *MD* = 1.00, 95% CI [-6.84, 8.84]. *p* = .80, indicated the intervention of HMF was not different to the comparator of BMF (*see Figure 8 & Table 1*). For the outcome of language development at 18 months of chronological age, the *MD* = 2.00, 95% CI [-7.16, 11.16]. *p* = .67, indicated the intervention of HMF was not different to the comparator of BMF (*see Figure 8 & Table 1*). For the outcome of motor development at 18 months of chronological age, the *MD* = 3.00, 95% CI [-5.07, 11.07]. *p* = .47, indicated the intervention of HMF was not different to the comparator of BMF (*see Figure 8 & Table 1*).

Certainty Of The Evidence For Neurodevelopment. The certainty of the body of evidence was very low based on four factors[a]: *within-study risk of bias*, *consistency among studies, directness of evidence*, and *precision of effect estimates*. The body of evidence was assessed to have serious risk of imprecision due to limited number of participants. As only one study (Colacci et al., 2017) was identified to answer this question, consistency could not be assessed.

Bronchopulmonary dysplasia (BPD)

Six studies (Assad et al., 2016; Colacci et al., 2017; Delaney Manthe et al., 2019; Hair et al., 2016; O'Connor et al., 2018; Sullivan et al., 2010), measured the incidence of bronchopulmonary dysplasia, defined as the need for supplemental oxygen at 36 weeks post menstrual age in VLBW infants that received HMF versus BMF once oral feeds reached 100 mL/kg/d (*N* = 2,352). The two included RCTs (O'Connor et al., 2018; Sullivan et al., 2010 (*n* = 261), *OR* = 0.78, 95% CI [0.46, 1.31], *p* = .34, indicated the intervention of HMF was not different to the comparator of BMF in reducing incidence of BPD in VLBW infants (see Figure 9 & *Table 1*). For the four cohort studies (Assad et al., 2016; Colacci et al., 2017; Delaney Manthe et al., 2019; Hair et al., 2016) (*n* = 2,091), *OR* = 0.66, 96% CI [0.56, 0.79], *p* = .00001, indicated the intervention of HMF was favorable to the comparator of BMF in reducing incidence of BPD in VLBW infants (see Figure 10 & *Table 1*). Based off the data reported in the RCTs, the use of HMF in VLBW infants will result in 54 (range: 63 to 150) fewer cases of BPD per 1,000 VLBW infants. Data reported in the cohort studies suggests the use of HMF in VLBW infants will result in 103 (range: 59 to 143) fewer cases of BPD per 1,000 VLBW infants.

Certainty Of The Evidence For Bronchopulmonary Dysplasia (BPD). The certainty of the body of evidence was low based on four factors[a]: *within-study risk of bias*, *consistency among studies, directness of evidence*, and *precision of effect estimates*. The body of evidence was assessed to have serious risk of bias and serious imprecision. Serious risk of bias was found in the RCTs due to lack of blinding and misclassification biases found in the retrospective, cohort studies. Serious risk of imprecision was found in the RCTs due to limited number of events (*n* = 83).

Retinopathy of prematurity (ROP)

Five studies (Assad et al., 2016; Colacci et al., 2017; Delaney Manthe et al., 2019; Hair et al., 2016; O'Connor et al., 2018) recorded incidence of retinopathy of prematurity (assessed by need for intervention), in VLBW infants receiving HMF or BMF once infants reached an oral intake of 100 mL/kg/d (*N* = 2,212). For the outcome of ROP incidence O'Connor et al., (2018) a RCT study (*n* = 121), shows the *OR* = 0.14, 95% CI [0.02, 1.24], *p* = 0.08, indicating the intervention of HMF was favorable to the comparator of BMF in reducing incidence of ROP in VLBW infants, (see Figure 11 & *Table 1*). The four (Assad et al., 2016; Colacci et al., 2017; Delaney Manthe et al., 2019; Hair et al., 2016) reported cohort studies (*n* = 2091), *OR* = 0.51, 95% CI [0.38, 0.68], *p* = 0.00001, indicated the intervention of HMF was favorable to the comparator of BMF in reducing the incidence of ROP in VLBW infants, (see Figure 12 & *Table 1*). Based off the data reported in the RCTs, there was no difference of occurrence of ROP in the HMF group versus the BMF group. However, in the cohort studies, the data shows the use of HMF in VLBW infants will result in 47 to 97 fewer incidences of ROP per 1,000 VLBW infants.

Certainty Of The Evidence For Retinopathy of Prematurity. The certainty of the body of evidence was low based on four factors[a]: *within-study risk of bias*, *consistency among studies, directness of evidence*, and *precision of effect estimates*. The body of evidence was assessed to have serious risk of bias and very serious imprecision. Serious risk of bias was found in the cohort studies due to the possibility of misclassifications with retrospective studies. Very serious imprecision was determined for the RCT due to limited number of events reported (*n* = 7).

Identification of Studies

Search Strategy and Results (see Figure 1)

("Milk, Human"[Mesh] OR "exclusive human milk" OR EHMD[tiab] OR "donor human milk" OR "human donor milk" OR "donor milk" OR "milk donor" OR "human milk" OR "mother's own milk" OR "mother's milk") AND ("Bovine milk-based protein" OR "human milk-based protein" OR ((human OR bovine) AND (fortified[tiab] OR fortifier[tiab] OR fortification[tiab])) OR Prolacta) AND (infant OR newborn OR neonate OR neonatal OR premature OR preterm)

Records identified through database searching *n* = 79

Additional records identified through other sources *n* = 1

Studies Included in this Review

Citation	Study Type
*Assad et al., (2016)	Cohort
Box & Shakuntala, (2018)	Cohort
Bushati et al., (2021)	Cohort
*Colacci et al., (2017)	Cohort
Delaney Manthe et al, (2019)	Cohort
*Hair et al., (2016)	Cohort
*O'Connor et al., (2018)	RCT
*Sullivan et al., (2010)	RCT
Villamor-Martinez et al., (2018)	SR

References marked with an asterisk indicate studies included the meta-analysis

Studies Not Included in this Review with Exclusion Rationale

Citation	Reason for exclusion
Agrawal et al., (2019)	Wrong intervention
Bergner et al., (2020)	Wrong comparison
Hair et al., (2015)	Abstract only
Hopperton et al., (2019)	Wrong data format
Huston et al., (2020)	Wrong comparison

Premkumar et al., (2019)	Narrative review
Rahman et al., (2020)	Wrong comparison
Scholz & Greiner, (2019)	Wrong outcome
Taylor, S., (2019)	Narrative review

Methods Used for Appraisal and Synthesis

[a]The GRADEpro Guideline Development Tool (GDT) is the tool used to create the Summary of Findings table(s) for this analysis.

[a]GRADEpro GDT: GRADEpro Guideline Development Tool (2015). McMaster University, (developed by Evidence Prime, Inc.). [Software]. Available from gradepro.org.

[b]Rayyan is a web-based software used for the initial screening of titles and / or abstracts for this analysis (Ouzzani, Hammady, Fedorowicz & Elmagarmid, 2017).

[b]Ouzzani, M., Hammady, H., Fedorowicz, Z., & Elmagarmid, A. (2016). Rayyan-a web and mobile app for systematic reviews. *Systematic Reviews, 5*(1), 210. doi:10.1186/s13643-016-0384-4

[c]Review Manager (Higgins & Green, 2011) is a Cochrane Collaborative computer program used to assess the study characteristics as well as the risk of bias and create the forest plots found in this analysis.

[c]Higgins, J. P. T., & Green, S. e. (2011). *Cochrane Handbook for Systematic Reviews of Interventions [updated March 2011]* (Version 5.1.0 ed.): The Cochrane Collaboration, 2011.

[d]The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram depicts the process in which literature is searched, screened, and eligibility criteria is applied (Moher, Liberati, Tetzlaff, & Altman, 2009).

[d]Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097 **For more information, visit www.prisma-statement.org.**

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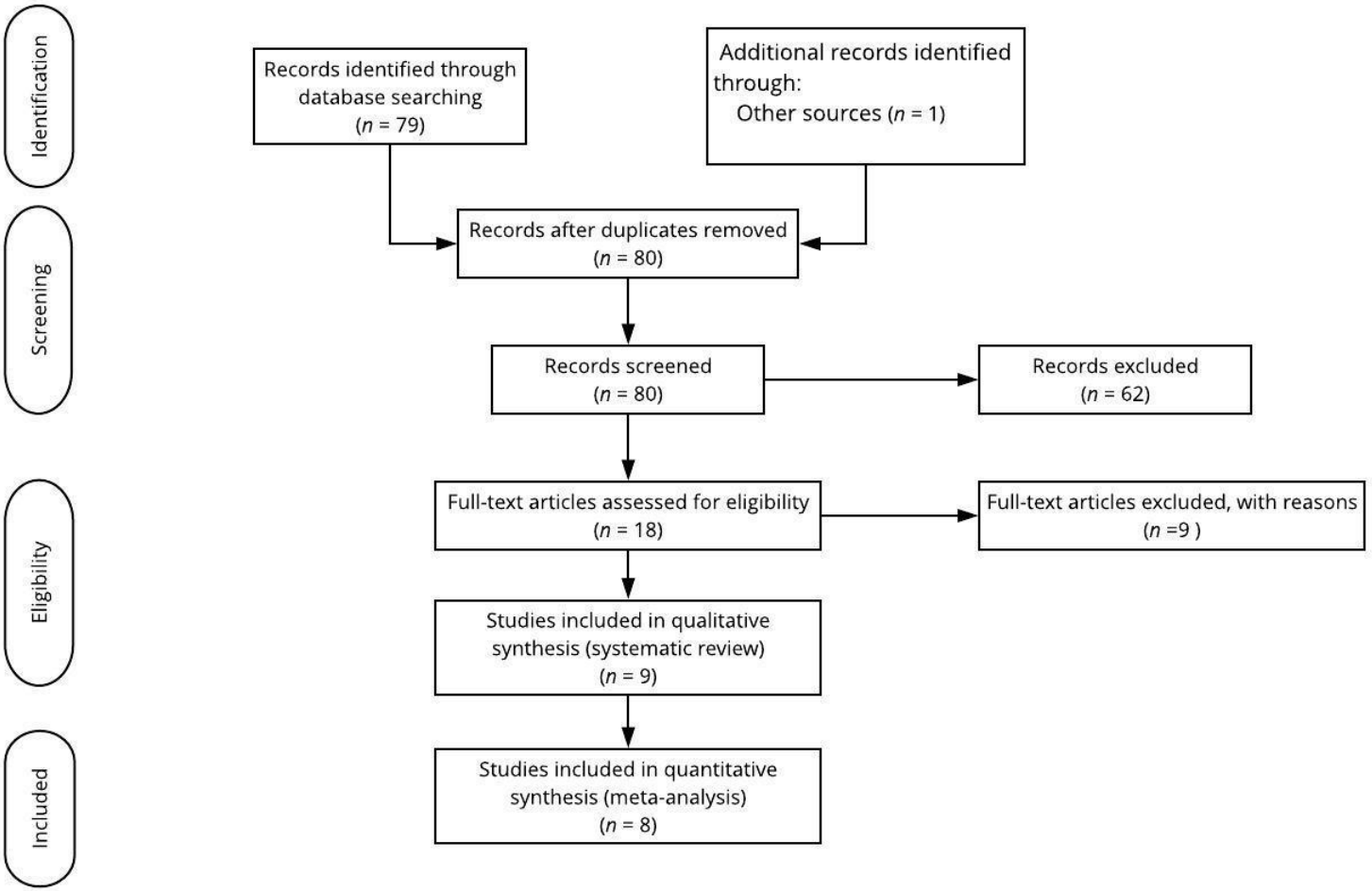
Acronyms Used in this Document

Acronym	Explanation
BMF	Bovine milk-based fortifier
BPD	Bronchopulmonary dysplasia
CAT	Critically Appraised Topic
CMF	Cow's milk fortifier
EBP	Evidence Based Practice
EHM	Exclusive human milk
EHMD	Exclusive human milk diet
GA	Gestational age
HMF	Human milk-based fortifier
MOM	Mother's Own Milk
NEC	Necrotizing Enterocolitis
PDHM	Pasteurized donor human milk
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
ROP	Retinopathy of prematurity
VLBW	Very low birth weight

Figure 1

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRIMSA)[d]



Summary of Findings Table
Table 1

Summary of Findings Table[c]: Human milk fortifier

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**human milk fortifier**	**bovine milk fortifier**	**Relative (95% CI)**	**Absolute (95% CI)**		
Necrotizing Enterocolitis (NEC) (assessed with: Bell's criteria)												
2	randomized trials	serious [a,b]	not serious	not serious	very serious [c]	none	6/131 (4.6%)	17/130 (13.1%)	**OR 0.32** (0.12 to 0.84)	**85 fewer per 1,000** (from 113 fewer to 19 fewer)	⊕◯◯◯ VERY LOW	CRITICAL
Necrotizing Enterocolitis (NEC) (assessed with: assessed with Bell's criteria)												
5	observational studies	serious [d]	serious [e]	not serious	serious [f]	publication bias strongly suspected strong association [d,g]	70/997 (7.0%)	143/1028 (13.9%)	**OR 0.45** (0.33 to 0.60)	**71 fewer per 1,000** (from 88 fewer to 51 fewer)	⊕◯◯◯ VERY LOW	CRITICAL
Feeding Intolerance												
1	randomized trials	not serious	not serious	not serious	very serious [f]	none	17/64 (26.6%)	20/61 (32.8%)	**OR 0.74** (0.34 to 1.60)	**63 fewer per 1,000** (from 186 fewer to 110 more)	⊕⊕◯◯ LOW	CRITICAL
Feeding intolerance												
1	observational studies	serious [h]	not serious	not serious	serious [f]	none	15	49	-	MD **3 lower** (4.89 lower to 1.11 lower)	⊕◯◯◯ VERY LOW	CRITICAL

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**human milk fortifier**	**bovine milk fortifier**	**Relative (95% CI)**	**Absolute (95% CI)**		
Bayley-III Development Scores at 6 months CA												
1	observational studies	not serious	not serious	not serious	serious [i]	none	92	98	-	MD **0.8 higher** (2.65 lower to 4.26 higher)	⊕◯◯◯ VERY LOW	CRITICAL
Bayley-III Development Scores at 12 months CA												
1	observational studies	not serious	not serious	not serious	serious [i]	none	76	84	-	MD **0.09 lower** (3.19 lower to 3.01 higher)	⊕◯◯◯ VERY LOW	CRITICAL
Bayley-III Development Scores at 18 months CA												
1	observational studies	not serious	not serious	not serious	serious [i]	none	86	79	-	MD **1.98 higher** (2.81 lower to 6.77 higher)	⊕◯◯◯ VERY LOW	CRITICAL
Bronchopulmonary dysplasia (BPD) (assessed with: need for oxygen at 36 weeks PMA)												
2	randomized trials	serious [a,j]	not serious	not serious	serious [f]	none	38/131 (29.0%)	45/130 (34.6%)	**OR 0.78** (0.46 to 1.31)	**54 fewer per 1,000** (from 150 fewer to 63 more)	⊕⊕◯◯ LOW	CRITICAL
Bronchopulmonary dysplasia (BPD) (assessed with: need for oxygen at 36 weeks PMA)												

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**human milk fortifier**	**bovine milk fortifier**	**Relative (95% CI)**	**Absolute (95% CI)**		
4	observational studies	serious [k]	not serious	not serious	not serious [f]	none	472/1049 (45.0%)	558/1042 (53.6%)	**OR 0.66** (0.56 to 0.79)	**103 fewer per 1,000** (from 143 fewer to 59 fewer)	⊕◯◯◯ VERY LOW	CRITICAL
Retinopathy of prematurity (assessed with: need for intervention)												
1	randomized trials	not serious	not serious	not serious	very serious [f]	none	1/62 (1.6%)	6/59 (10.2%)	**OR 0.14** (0.02 to 1.24)	**86 fewer per 1,000** (from 99 fewer to 21 more)	⊕⊕◯◯ LOW	CRITICAL
Retinopathy of prematurity (ROP) (assessed with: need of intervention)												
4	observational studies	serious [k]	not serious	not serious	not serious	none	105/1049 (10.0%)	175/1042 (16.8%)	**OR 0.51** (0.38 to 0.68)	**75 fewer per 1,000** (from 97 fewer to 47 fewer)	⊕◯◯◯ VERY LOW	CRITICAL

Notes

a. lack of blinding and limited power to look at subgroups
b. randomization based on weight, not random sequence
c. limited number of events (23 for both groups)
d. significant number of potential confounding variables unreported
e. moderate heterogeneity (Heterogeneity: $Chi^2 = 11.78$, $df = 4$ ($P = 0.02$); $I^2 = 66\%$)
f. limited number of events
g. RR is 0.49 supporting large effect
h. clinical discretion used to determine withholding of feedings orally
i. Limited number of participants
j. lack of blinding
k. retrospective studies lend to misclassification biases

Meta-analyses

Figure *2*

Comparison: Human milk fortifier versus Bovine milk fortifier, Outcome: Incidence of necrotizing enterocolitis in RCTs

Study or Subgroup	HMF Events	HMF Total	BMF Events	BMF Total	Weight	Odds Ratio M-H, Fixed, 95% CI
O'Connor 2018	3	64	6	61	36.1%	0.45 [0.11, 1.89]
Sullivan2010	3	67	11	69	63.9%	0.25 [0.07, 0.93]
Total (95% CI)		**131**		**130**	**100.0%**	**0.32 [0.12, 0.84]**
Total events	6		17			

Heterogeneity: Chi² = 0.37, df = 1 (P = 0.55); I² = 0%
Test for overall effect: Z = 2.31 (P = 0.02)

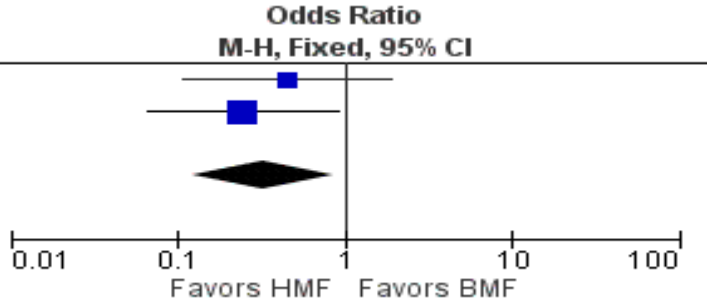


Figure *3*

Comparison: Human milk fortifier versus Bovine milk fortifier, Outcome: Incidence of necrotizing enterocolitis in observational studies

Study or Subgroup	HMF Events	HMF Total	BMF Events	BMF Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Box 2018	3	20	1	64	0.3%	11.12 [1.09, 113.78]
Bushati2021	0	15	2	49	0.9%	0.61 [0.03, 13.47]
Colacci2017	4	39	5	46	3.0%	0.94 [0.23, 3.76]
Delaney Manthe2019	7	104	7	101	4.9%	0.97 [0.33, 2.87]
Hair 2016	56	819	128	768	90.9%	0.37 [0.26, 0.51]
Total (95% CI)		**997**		**1028**	**100.0%**	**0.45 [0.33, 0.60]**
Total events	70		143			

Heterogeneity: Chi² = 11.78, df = 4 (P = 0.02); I² = 66%
Test for overall effect: Z = 5.27 (P < 0.00001)

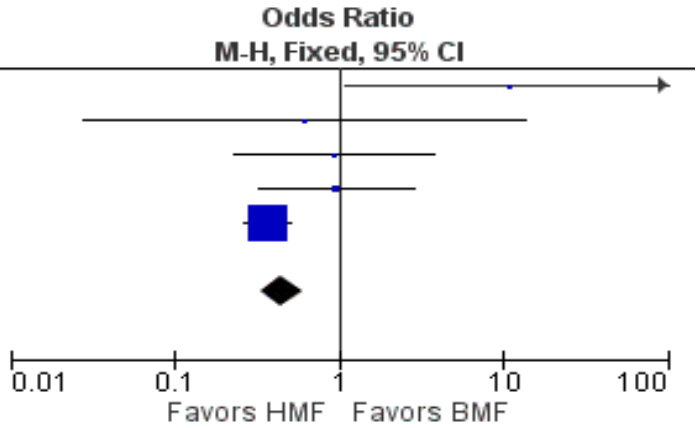


Figure *4*

Comparison: Human milk fortifier versus Bovine milk fortifier, Outcome: feeding intolerance, RCT

Study or Subgroup	HMF Events	HMF Total	BMF Events	BMF Total	Weight	Odds Ratio M-H, Fixed, 95% CI
O'Connor 2018	17	64	20	61	100.0%	0.74 [0.34, 1.60]
Total (95% CI)		**64**		**61**	**100.0%**	**0.74 [0.34, 1.60]**
Total events	17		20			

Heterogeneity: Not applicable
Test for overall effect: Z = 0.76 (P = 0.45)

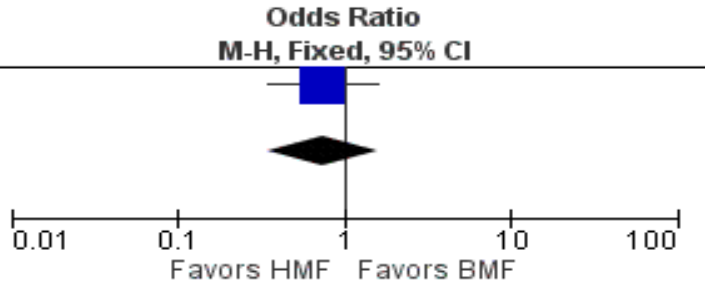


Figure *5*

Comparison: Human milk fortifier versus Bovine milk fortifier, Outcome: feeding intolerance, observational study

Study or Subgroup	HMF Mean	HMF SD	HMF Total	BMF Mean	BMF SD	BMF Total	Weight	Mean Difference IV, Fixed, 95% CI
Bushati2021	2	3	15	5	4	49	100.0%	-3.00 [-4.89, -1.11]
Total (95% CI)			**15**			**49**	**100.0%**	**-3.00 [-4.89, -1.11]**

Heterogeneity: Not applicable
Test for overall effect: Z = 3.12 (P = 0.002)

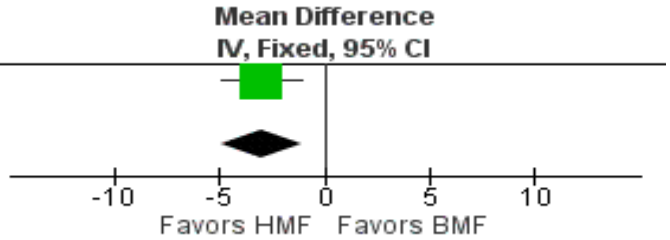


Figure *6*

Comparison: Human milk fortifier versus Bovine milk fortifier, Outcome: neurodevelopment at 6 months, observational study

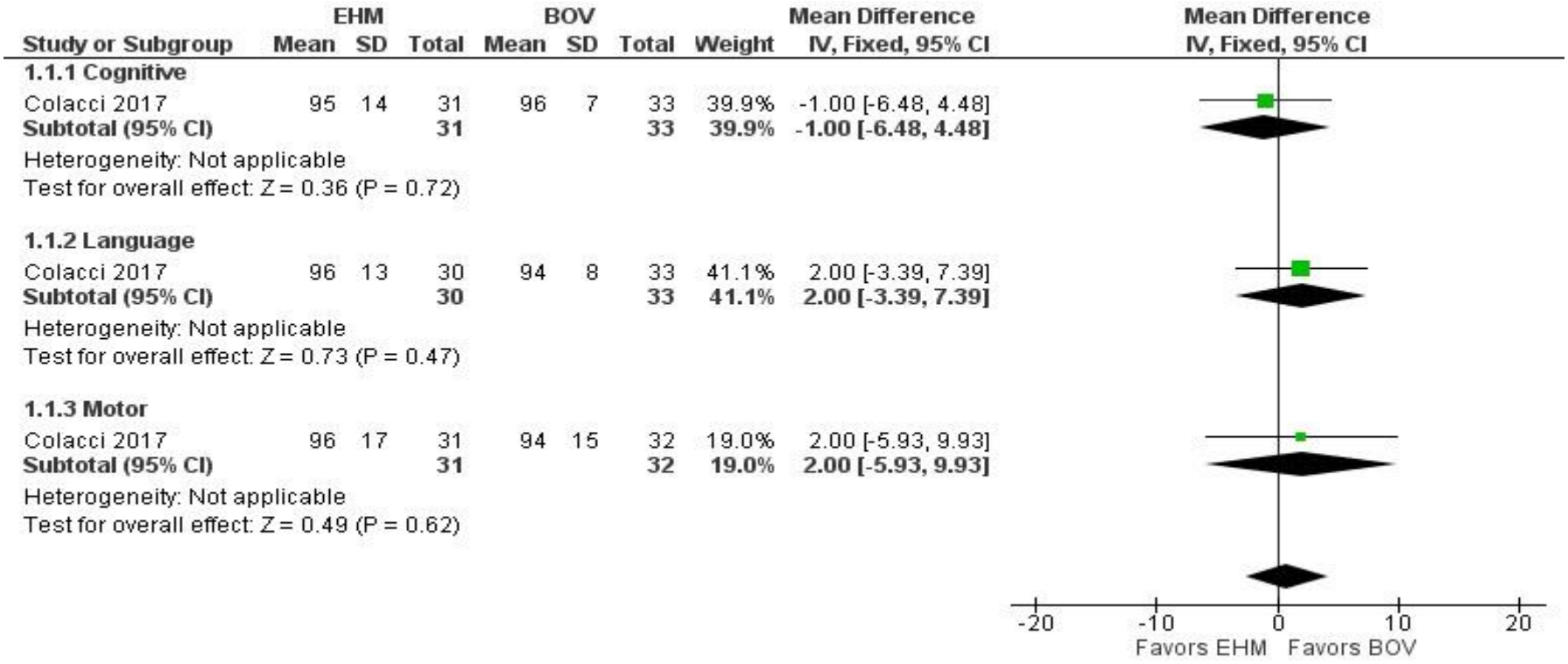


Figure *7*

Comparison: Human milk fortifier versus Bovine milk fortifier, Outcome: neurodevelopment at 12 months, observational study

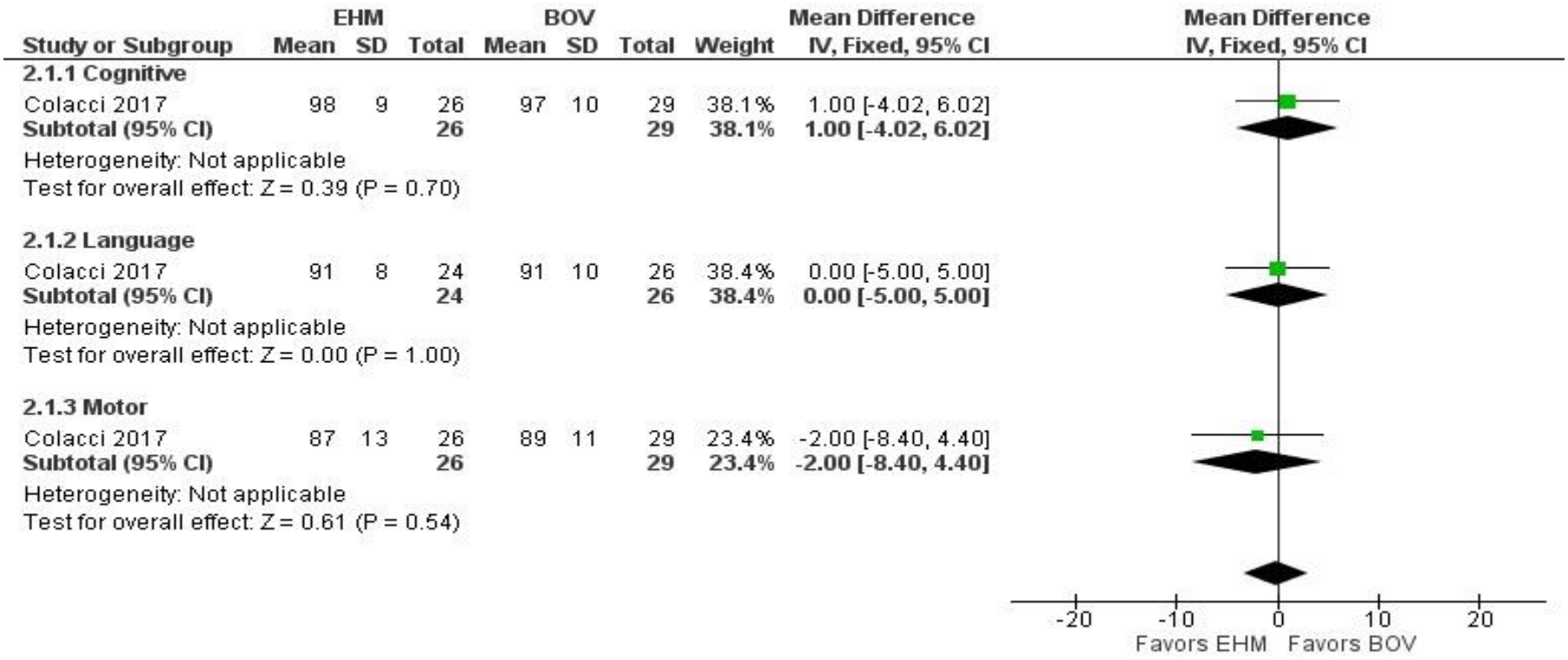


Figure *8*

Comparison: Human milk fortifier versus Bovine milk fortifier, Outcome: neurodevelopment at 18 months, observational study

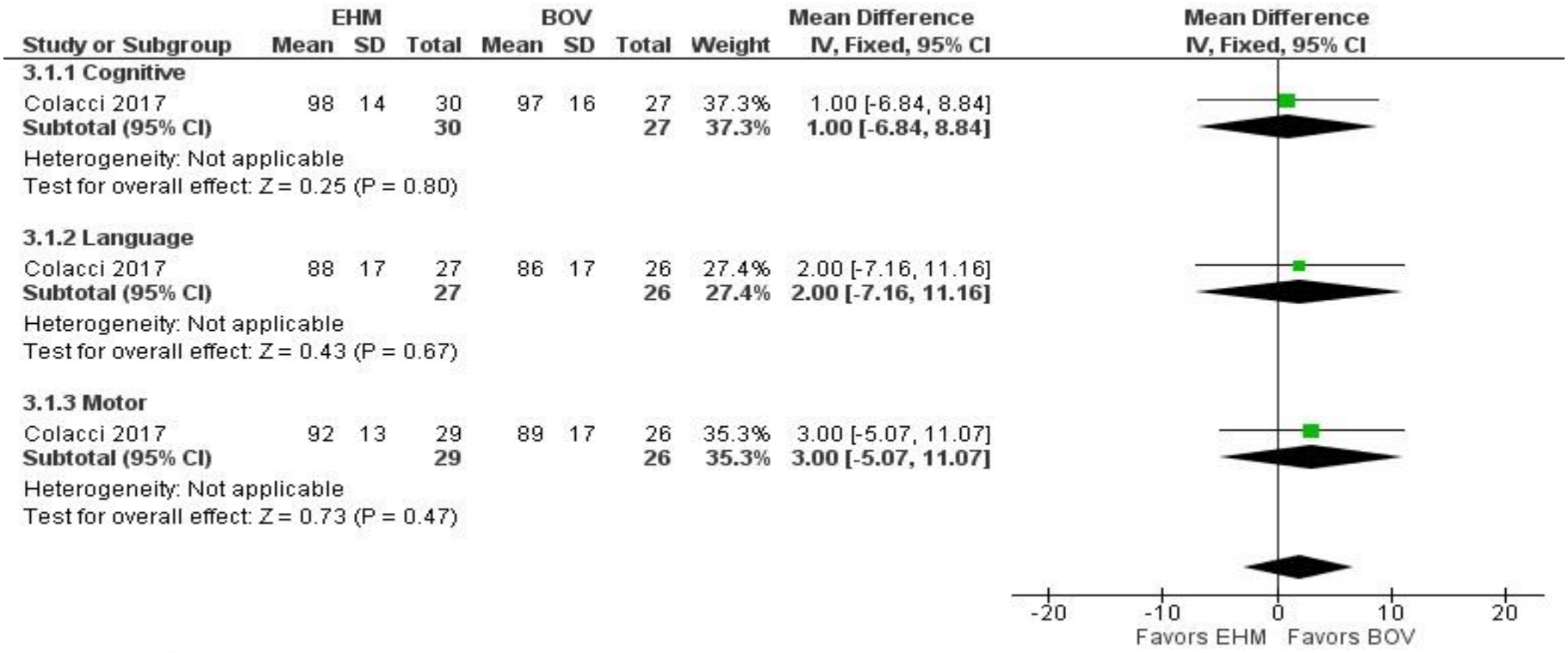


Figure *9*

Comparison: Human milk fortifier versus Bovine milk fortifier, Outcome: Incidence of bronchopulmonary dysplasia, RCTs

Study or Subgroup	HMF Events	HMF Total	BMF Events	BMF Total	Weight	Odds Ratio M-H, Fixed, 95% CI
O'Connor 2018	16	64	18	61	43.6%	0.80 [0.36, 1.75]
Sullivan2010	22	67	27	69	56.4%	0.76 [0.38, 1.54]
Total (95% CI)		**131**		**130**	**100.0%**	**0.78 [0.46, 1.31]**
Total events	38		45			

Heterogeneity: Chi² = 0.01, df = 1 (P = 0.93); I² = 0%

Test for overall effect: Z = 0.95 (P = 0.34)

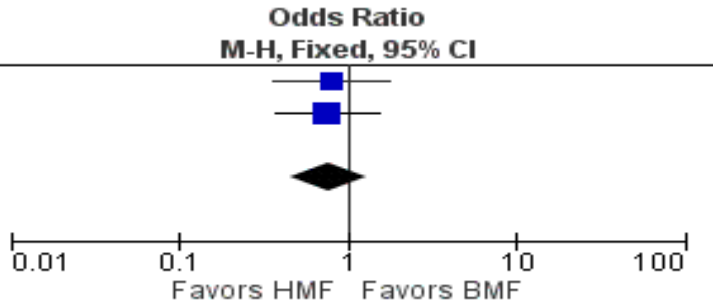


Figure *10*

Comparison: Human milk fortifier versus Bovine milk fortifier, Outcome: Incidence of bronchopulmonary dysplasia, observational studies

Study or Subgroup	HMF Events	HMF Total	BMF Events	BMF Total	Weight	Odds Ratio M-H, Random, 95% CI
Assad2016	13	87	30	127	6.1%	0.57 [0.28, 1.16]
Colacci2017	19	39	31	46	4.0%	0.46 [0.19, 1.11]
Delaney Manthe2019	50	104	65	101	10.0%	0.51 [0.29, 0.90]
Hair 2016	390	819	432	768	79.9%	0.71 [0.58, 0.86]
Total (95% CI)		**1049**		**1042**	**100.0%**	**0.66 [0.56, 0.79]**
Total events	472		558			

Heterogeneity: Tau² = 0.00; Chi² = 2.06, df = 3 (P = 0.56); I² = 0%

Test for overall effect: Z = 4.54 (P < 0.00001)

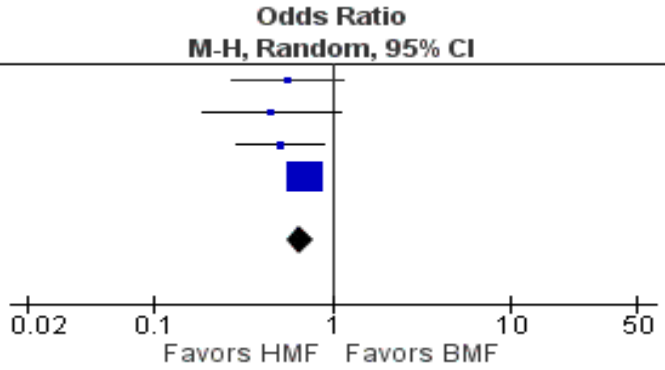


Figure *11*

Comparison: Human milk fortifier versus Bovine milk fortifier, Outcome: Incidence of retinopathy of prematurity, RCT

Study or Subgroup	HMF Events	HMF Total	BMF Events	BMF Total	Weight	Odds Ratio M-H, Fixed, 95% CI
O'Connor 2018	1	62	6	59	100.0%	0.14 [0.02, 1.24]
Total (95% CI)		**62**		**59**	**100.0%**	**0.14 [0.02, 1.24]**
Total events	1		6			

Heterogeneity: Not applicable
Test for overall effect: Z = 1.76 (P = 0.08)

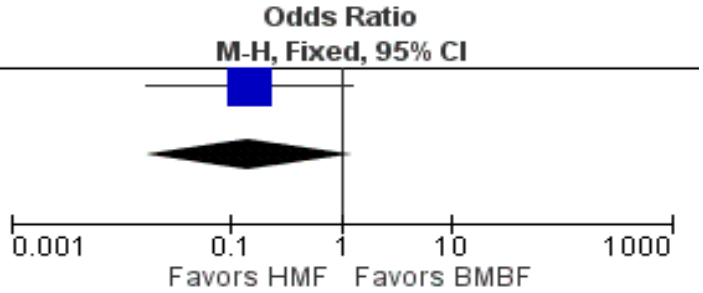
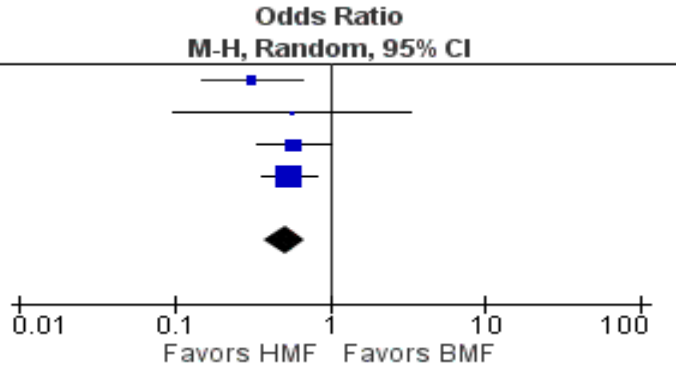


Figure *12*

Comparison: Human milk fortifier versus Bovine milk fortifier, Outcome: Incidence of retinopathy of prematurity, observational studies

Study or Subgroup	HMF Events	HMF Total	BMF Events	BMF Total	Weight	Odds Ratio M-H, Random, 95% CI
Assad2016	11	87	40	127	15.7%	0.31 [0.15, 0.66]
Colacci2017	2	39	4	46	2.8%	0.57 [0.10, 3.28]
Delaney Manthe2019	50	104	62	101	27.6%	0.58 [0.33, 1.01]
Hair 2016	42	819	69	768	53.9%	0.55 [0.37, 0.81]
Total (95% CI)		**1049**		**1042**	**100.0%**	**0.51 [0.38, 0.68]**
Total events	105		175			

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 2.02$, df = 3 (P = 0.57); $I^2 = 0\%$
Test for overall effect: Z = 4.51 (P < 0.00001)



Characteristics of Intervention Studies

Assad et.al., 2016

Methods	Retrospective Cohort
Participants	**Participants:** Preterm infants < 28 weeks and /or very low birth weight < 1500 grams enrolled from March 2009 until March 2014 **Setting:** Children's Hospital at Sinai, Baltimore, MD USA **Number enrolled into study:** *N* = 293 • **Group 1,** Entirely human milk (EHM): *n* = 87 • **Group 2,** Bovine-based fortifier, and maternal milk: *n* = 127 • **Group 3,** Combination of maternal milk, bovine-based fortifier and maternal milk: *n* = 49 • **Group 4,** Formula: *n* = 30 **Gender, males (as defined by researchers):** • **Group 1:** *n* = 60% • **Group 2:** *n* = 50% • **Group 3:** *n* = 59% • **Group 4:** *n* = 9% **Race / ethnicity or nationality (as defined by researchers):** • **Group 1:** Caucasian *n* = 24 (27%), African American *n* = 53 (61%), Other *n* = 10 (11%) • **Group 2:** Caucasian *n* = 36 (28%), African American *n* = 85 (67%), Other *n* = 6 (5%) • **Group 3:** Caucasian *n* = 14 (28%), African American *n* = 32 (68%), Other *n* = 3 (6%) • **Group 4:** Caucasian *n* = 9 (30%), African American *n* =21 (70%), Other *n* = 0 (0%) **Age, average gestational age in weeks (standard deviation)** • **Group 1,** *n* = 27.7 (± 2.7) • **Group 2,** *n* = 28.3 (± 2.8) • **Group 3,** *n* = 27.6 (± 2.8) • **Group 4,** *n* = 29.8 (± 2.5) **Inclusion Criteria:** • All infants < 28weeks gestational age (GA) and/or 1500 grams **Exclusion Criteria:** • Infants that died before initiation of feeds • Infants transferred to an outside hospital before a GA of 34 weeks. **Covariates Identified:** • None

Interventions	**Both:** 1. No changes to clinical practice, feeding strategies, respiratory management or attending physician staffing 2. Similar feeding schedules including slow advances (10 to 20ml/kg -1 per day and subsequent fortification with either BMF or HMF or DMDF after reaching ~120 - 150ml/kg -1 per day (full enteral feeds) 3. Feeding initiated within the first 8 hours of life • **Group 1:** Infants born from March 2012 to 2014 • **Group 2,3,4:** Infants born March 2009 to 2012
Outcomes	**Primary outcome(s):** • Examine effect of EHM diet on length of stay, *incidence of feeding intolerance (feeds being interrupted and held for 24 hours or longer due to emesis, abdominal distension, bloody stools, or suspicion of NEC) and time to full feeds **Secondary outcome(s):** • Examine effect of EHM diet on the incidence of necrotizing enterocolitis (NEC), and cost effectiveness. **Safety outcome(s):** • None noted
Results	**Results:** • Feeding intolerance was worse in all groups compared to Group 1 ($p < .001$) • 94% of infants did not have feeds held compared with 66%, 37%, and 66% for groups 2, 3, and 4 respectively. • Incidence of NEC was decreased in EHM diet group infants that were the same GA of other infants in the study (1.1 versus 10%, $p < .001$) **Limitations:** None noted

Box et al., 2018

Methods	Retrospective Cohort (abstract)
Participants	**Participants:** Preterm Infants (<1250) **Setting:** NICU **Number enrolled into study:** *N* = 84 • **Group 1,** Human Milk Fortifier (HMF): *n* = 20 • **Group 2,** Cow's Milk Fortifier (CMF): *n* = 64 **Gender, males (as defined by researchers):** • Not reported **Race / ethnicity or nationality (as defined by researchers):** • Not reported **Gestational Age:** • Not reported **Inclusion Criteria:** • Preterm infants <1250 gm **Exclusion Criteria:** • Not reported **Covariates Identified:** • Not reported
Interventions	**Study was a pre-post** • **Group 1:** Mother's breast milk was fortified with Prolacta (HMF group) 6 months after Jan 2014 • **Group 2:** Mother's breast milk was fortified with cow's milk fortifier (CMF group) 6 months before Jan 2014
Outcomes	**Primary outcome(s):** • *Extra uterine growth restriction measured in 2 ways - Weight below the 10th percentile at discharge and Mean of difference between birth weight percentile and discharge percentile. **Secondary outcome(s):** • *Necrotizing enterocolitis (NEC) • Electrolyte imbalance • Length of hospital stays • *Retinopathy of prematurity (ROP) • *Bronchopulmonary dysplasia (BPD) • Intra ventricular hemorrhage (IVH)

	• Mortality *Outcomes of interest to the CMH CPG /CAT development team
Results	**Results:** • Growth restriction was higher in the HMF group in comparison to CMF group, 65% versus 57%, respectively. • Mean of difference between birth weight percentile and discharge percentile significantly higher in HMF group versus CMF group, 29.25±28.93 versus 16.87± 16.5, p-value = .02. • NEC was significantly higher with 1.5% in CMF group compared to 15% in HMF group, p-value = .01 • No difference in electrolyte imbalance • No difference in length of hospital stays • No difference in ROP • No difference in BPD • No difference in IVH • No difference in mortality **Limitations:** • The study was an abstract with a significant number of potential confounding variables unreported. • Number of subjects were not clear

Bushati et. al., 2021

Methods	Cohort
Participants	**Participants:** ELBW infants with birth weight of ≤ 1,000 gm **Setting:** Fifty-two bed level III NICU located in a large urban teaching hospital **Number enrolled into study:** *N* = 64 • **Group 1,** Historical – ELBW infants born between January 1, 2018 to May 26, 2019 and received MOM or PDHM with BOV fortifier: *n* = 49 • **Group 2,** EHMD- ELBW infants born between May 27, 2019 to December 31, 2019 and received MOM or PDHM with HM fortifier: *n* = 15 **Gender, males (as defined by researchers):** • **Group 1:** *n* = 21(42.9%) • **Group 2:** *n* = 10(66.7%) **Race / ethnicity or nationality (as defined by researchers):** • not provided **Gestational age, mean in weeks ± SD** • **Group 1**: 26 ± 2 • **Group 2**: 28 ± 3 **Inclusion Criteria:** • ELBW (BW ≤ 1,000 g) born between January 1, 2018 to May 26, 2019 and from May 27, 2019 to December 31, 2019 **Exclusion Criteria:** • Infants excluded if they passed away in first 7 days of life • Infants excluded if they had major congenital anomalies **Covariates Identified:** • none identified
Interventions	**Group 1:** Received human milk (HM), either mother's own milk (MOM) or pasteurized donor human milk (PDHM) with an added bovine milk fortifier (BOV-f) until infants reached 34 weeks postmenstrual age **Group 2:** Received an exclusive human milk diet (MOM or PDHM) with human milk fortifier (HM-f) until infants reached 34 weeks postmenstrual age

Outcomes	**Primary outcome(s):** • Decrease number of days with no oral intake (decrease feeding intolerance) • Grow velocity **Secondary outcome(s):** • Decrease in number of spontaneous intestinal perforation (SIP) • Decrease in NEC • Decrease in hospital length of stay
Results	**Results:** • EHMD group tolerated feeds better (40% vs. 90%) needing days of no oral intake after feeds • No statistical significance in growth when comparing z-scores between the two groups • Two cases of NEC (stage ≥ 2) in historical cohort with one case prior to receiving BOV-f vs. 2 cases of NEC (stage 1) in the EHMD cohort while receiving HM-f with no reported SIP • Lower weight at discharge in the EHMD group vs. the historical group looking at z-scores however, weight gain velocity of EHMD cohort similar to the historical cohort **Limitations:** • Small sample size • Use of clinical discretion to determine no oral intake had potential for bias • Unknown volume of MOM or PHDM consumed by the infant

Colacci et. al., 2017

Methods	Cohort
Participants	**Setting:** Single-center, Level-III NICU in Chicago, IL **Number enrolled into study:** • **Group 1,** Infants admitted from July 2011 to June 2012 fed breast milk with BF and/or bovine milk-based fortifiers and formula: *n* = 46 • **Group 2,** Infants admitted from July 2012 to June 2013 fed EHM: *n* = 39 **Gender, males:** Not disclosed **Race/ethnicity or nationality:** Not disclosed **Gestational Age (GA), weeks (mean + SD):** • **Group 1:** 26 ± 1.9 • **Group 2:** 26 ± 1.9 **Inclusion Criteria:** • Infants born between July 2011 to June 2013 • GA < 37 weeks • Birth weight < 1,000 g **Exclusion Criteria:** • Death before discharge • Major congenital anomaly • Genetic abnormality **Covariates Identified:** • Maternal education, median household income, Cesarean delivery, Chorioamnionitis, administration of surfactant, SNAPPE-II, intraventricular hemorrhage, necrotizing enterocolitis, retinopathy of prematurity, postmenstrual age **Sample size:** With 36 in each group would allow detection of a score difference of 10, assuming a standard deviation of 15 (based on the test's reference value) in the cognitive domain at any follow-up visit with a power = 0.8 and an α = 0.05.
Interventions	**Both:** All other nutrition practices were unchanged between the two cohorts • Parenteral protein and dextrose solutions were started immediately after birth and intravenous lipids were initiated within 48 hours of birth. • Enteral feedings were to start within 48 hours as trophic feeding (20 mL/kg/day for up to 3 days) if the clinical team deemed appropriate. If tolerated, feedings increased by 20 mL/kg/day. • Full feedings were achieved when enteral nutrition provided 100 kcal/kg/day and parenteral nutrition had been discontinued. • Feedings remained fortified until infants took all feedings by mouth. • Unfortified mother's own milk and transitional formula were the primary forms of nutrition prescribed at discharge.

	• **Group 1:**
	  ○ Feeding initiation: Mother's own milk and/ or premature infant formula feeding fortification: Bovine milk-based fortifier for human milk feedings at 100 mL/kg/d
	  ○ Feeding transition: Fortified human milk and/or premature formula used until full oral feedings
	• **Group 2:**
	  ○ Feeding initiation: Mother's own milk and/or donor milk
	  ○ Feeding fortification: Human milk-based fortifier at 100 mL/kg/d
	  ▪ Feeding transition after achieving 1. Human milk-based fortifier use for at least 4 weeks 2. Current weight 1,500 g or 34 weeks postmenstrual age (whichever occurs first), then bovine milk-based products introduced with either bovine milk-based fortifier if mother's milk available or premature infant formula:--Day 1: two feedings per day contain bovine product --Day 2: three feedings per day contain bovine product--Day 3: four feedings per day contain bovine product --Day 4: five feedings per day contain bovine product--Day 5: All feedings contain bovine product and continue until full oral feedings

Outcomes	**Primary outcome(s):** • *Bayley-III Scales of Infant and Toddler Development, Third Edition in which cognition, language, and motor domains were measured at 6-, 12-, and 18-months corrected age **Secondary outcome(s):** • *NEC • *ROP • *BPD **Safety outcome(s):** • none reported ***Outcomes of interest to the CMH CPG /CAT development team
Results	**Results:** • Seventy-one (84%) infants attended at least one follow up visit • Bayley-III scores in cognition, language, and motor did not different between the two groups • Due to infants lost to follow up, the sample size calculated to compare the two groups was not met **Limitations:** • Fourteen infants (9 from BOV group and 4 from EHM) were lost to follow-up

Delaney Manthe et.al., 2019

Methods	Cohort
Participants	**Setting:** Single-center NICU in Charlottesville, Virginia **Number enrolled into study:** *N* = 205 • **Group 1,** Infants admitted 20 months preceding the introduction of exclusive human milk diet (EHMD) given cow's milk-based human milk fortifier (CMB-HMF): *n* = 101 • **Group 2,** Infants admitted after the introduction of EHMD only: *n* = 104 **Gender, males:** • **Group 1**: 50.5% • **Group 2:** 47.1% **Race / ethnicity or nationality:** • **Group 1:** Caucasian: 64.4%/African American: 28.7%/Hispanic: 4.0%/Other: 3.0% • **Group 2:** Caucasian: 61.5%/African American: 24.0%/Hispanic: 10.6%/Other: 3.8% **Gestational Age (GA), weeks (mean + SD):** • **Group 1:** 26 ± 2.5 • **Group 2:** 26 ± 2.1 **Inclusion Criteria:** • Infants with a birth weight 1250 g or less • Infants tolerating mother's own milk (MOM) or pasteurized human milk (PHM) feeds at 80 mL/kg/d, continued until the infant reached a corrected gestational age (CGA) of 34 weeks **Exclusion Criteria:** • Infant weighed more than 1250 g at birth • Infant was within 1 week of reaching a CGA of 34 weeks • Admitted 48 hours after birth • Received fewer than 2 full doses HMB-HMF feeds • Died within 48 hours of admission • Infant had congenital/ severe gastrointestinal disease preventing attaining goal feeding volumes with human milk-based fortifier (HMB-HMF) human milk **Covariates Identified:** • Not disclosed **Sample size:** Based on evaluating the number of infante admitted during the 2012-2015 calendar years, it was determined that 80 infants per year would prove to be representative of the population

Interventions	• **Group 1:** Infants received MOM and/or PHM fortified with CMB-HMF (dose and detailed administration were not disclosed). • **Group 2:** The HMB-HMF products were thawed for 12 to 17 minutes in the same warmers used to thaw bottles of PHM, or alternatively, were thawed in the patient bedside refrigerator, allowing 1 hour per 10 mL of HMB-HMF. Nurses added 70-mL MOM or PHM to the 30-mL container of Prolact+ H2MF +6. If ordered, a 10-mL container of ProlactCR was thawed in the same way and the ordered volume mixed with the fortified milk.
Outcomes	**Primary outcome(s):** • *Decrease the incidence of NEC • Decrease the number of days of parenteral nutrition • Maintaining Adequate growth **Secondary outcome(s):** • *ROP • *BPD *Outcomes of interest to the CMH CPG /CAT development team
Results	**Results:** • NEC (surgical), declined from 4 cases to 1 case • ROP declined in the EHMD group from 62% to 48.5% • BPD cases were less in the EHMD group compared to the CMB-HMF group (48.5% vs. 65% respectively) **Limitations:** • Limited sample size • Study design impacted power of data; unable to determine statistical difference • Unable to table growth velocity: median was reported instead of mean

Hair et.al., 2016

Methods	**Retrospective Cohort**
Participants	**Participants:** Infants with a birth weight <1250 gm **Setting:** Four NICU in Texas, Illinois, Florida, and California **Number enrolled into study**: *N* = 1587 • **Group 1**, Human Based Diet (HUM): *n* = 819 • **Group 2**, Bovine Based Diet (BOV): *n* = 768 **Gender, males (as defined by researchers):** • **Group 1**: *n* = 50.2% • **Group 2**: *n* = 49.5% **Race / ethnicity or nationality (as defined by researchers):** • Not reported **Gestational Age, mean in weeks:** • **Group 1:** 26.5 ± 2.5 • **Group 2:** 26.4 ± 2.3 **Inclusion Criteria:** • Infants with birth weight <1250 gm **Exclusion Criteria:** • Major congenital anomalies • Diet within the first 12 hours of admission • Transferred in from an outside hospital after 1 week of age **Covariates Identified:** • Necrotizing enterocolitis (NEC) • Surgical NEC • Late-onset sepsis • Intraventricular hemorrhage (IVH) • Retinopathy of prematurity (ROP) • Patent ductus arteriosus (PDA) • Bronchopulmonary dysplasia (BPD) • Antenatal steroids • Study site
Interventions	**Texas** • **Group 1:** ○ Infants received mother's own milk, donor human milk (HM), and a donor HM-derived fortifier (HUM)

	○ Enteral feeds started with trophic feeds of 20mL/kg/day for 3 days ○ Feeds were advanced by 20mL/kg/day as tolerated to 140–160mL/kg/ day. ○ Pasteurized donor HM-derived fortifier, Prolact+H2MF (Prolacta Bioscience, Industry, CA) was added once feeds reached 60mL/kg/day for an additional 4 Cal/oz. ○ At 100mL/ kg/day, fortification was increased to provide an additional 6 Cal/oz. If weight gain was <15 g/kg/day, fortification was increased to 8 Cal/oz and then 10Cal/oz. ○ Infants received a HUM diet until 34weeks post menstrual age (PMA) • **Group 2:** ○ Infants advanced with similar protocol, but patient received BOV fortifier ○ Fortification did not occur until 100ml/kg/day **California** • **Group 1:** ○ The HUM diet was used for all very low birth weight (VLBW) infants (Birth weight <1,000 g) ○ Infants were fed mother's own milk or donor HM using a NICU Standardized Feeding Protocol ○ Feeds were fortified with Prolact+H2MF to an additional 4 Cal/oz at 100 mL/kg/day volume of feeds ○ Advancement to an additional 6 Cal/oz occurred at 150 mL/kg/day. ○ If infants needed more concentrated feeds, fortification with the donor HM-derived fortifier was increased to provide an additional 8 Cal/oz. ○ This regimen was continued until 60 days of age, and then infants were transitioned to either mother's own milk with a bovine fortifier or preterm formula if mother's own milk was not available. • **Group 2:** ○ Not reported **Illinois** • **Group 1:** ○ Infants received mother's own milk and a donor HM-derived fortifier (Prolact+ H2MF). ○ If mother's own milk was not available, then donor HM was provided for infants weighing <1,500 gm ○ Trophic feeds were started at 10–20 mL/kg/day for 3 days ○ Feeds were advanced by 20 mL/kg/day to reach a total volume goal of 150–160 mL/kg/day. ○ For infants <1,000 gm Prolact+H2MF was added once feeds reached 100–120 mL/kg/day for an additional 4 Cal/oz. ○ Fortification was advanced to 6 Cal/oz if weight gain was <15 gm/kg/day, and further advanced to 8 Cal/oz if there was continued low weight gain ○ Infants were transitioned off this diet at 1,500 gm or 34 weeks PMA • **Group 2:** ○ Similar to HUM protocol but received bovine fortifier **Florida** • **Group 1**: ○ Infants received mother's own milk and/or donor milk if <750 g BW and <26 weeks gestational age ○ Enteral feeds were started with trophic feeds of 10–20 mL/kg/day for 3–5 days. ○ Feeds were advanced by 10–30 mL/kg/day to reach a total volume goal of 150–160 mL/kg/day

	○ HM fortifier, Prolact+H2MF was added once feeds reached 100–120 mL/kg/day for an additional 4 Cal/oz ○ If weight gain was determined to be sub-optimal, an additional 2–4 Cal/oz of fortification was added to the feeds for a total of 6–8 kcal/oz. ○ Infants were transitioned off the diet at approximately 32 weeks PMA • **Group 2:** ○ Bovine fortifier was introduced at approximately 120–150 mL/kg/day
Outcomes	**Primary outcome(s):** • *NEC and mortality **Secondary outcome(s):** • Late onset sepsis • *ROP • *BPD • PDA • IVH • Ventilator days • PMA at discharge *Outcomes of interest to the CMH CPG /CAT development team
Results	**Results:** • The HUM group had significantly lower incidence of: • NEC 16.7% versus 6.9%, p < .00001 • Mortality 17.2% versus 13.6%, p = .04 • Late-onset sepsis 30.3% versus 19.0%, p < .00001 • ROP 9% versus 5.2%, p = .003 and • BPD 56.3% versus 47.7%, p = .0015 **Limitations:** • Difference in birth weight and birth head circumference between study groups • Retrospective study lends to several biases such as misclassification biases, particularly in relation to the diagnosis of NEC • It is possible that over the period this study occurred, there were changes in practice aside from type of milk and fortifier • Mother's milk and pasteurized donor HM likely increased during the study period and potentially contributed to the improved outcomes.

O'Connor et.al., 2018

Methods	**Randomized Control Trail**
Participants	**Participants:** Neonatal Intensive Care Unit (NICU) Infants <1250 gm **Setting:** Two tertiary NICU in Toronto, Canada **Randomized into study:** *N* = 127 • **Group 1,** Human Milk-Based Fortifier (HMBF): *n* = 64 • **Group 2,** Bovine Milk-Based Fortifier (BMBF): *n* = 63 **Completed Study:** *N* = 125 • **Group 1:** *n* = 64 • **Group 2:** *n* = 61 **Gender, males (as defined by researchers):** • **Group 1:** *n* = 25 (39.1%) • **Group 2:** *n* = 29 (46.0%) **Race / ethnicity or nationality (as defined by researchers):** • Mother's ethnicity reported but not infants **Gestational Age, mean in weeks:** • **Group 1:** 27.9 ± 2.7 • **Group 2:** 27.5 ± 2.3 **Inclusion Criteria:** • Birth weight <1250 gm • Parents consented to supplemental donor milk **Exclusion Criteria:** • Receipt of formula or BMBF prior to randomizations • Enteral feeding had not commenced within 14 days of birth • Chromosomal or congenital anomaly affecting growth was identified prior to enrollment • Participation in another study affecting nutritional management, • Reasonable likelihood of transfer to an NICU where human research ethics approval had not been secured. **Power Analysis:** • Sample size of 62 infants per group was estimated to be sufficient to observe a reduction in feeding interruptions from the local baseline of ~30% (for exclusively mother's milk–fed VLBW infants with BMBF) to 10% (with HMBF), with 80% power and an α-level of 0.05
Interventions	**Both:** • Study day 1 was defined as the day nutrient fortification of enteral feeds started • The feeding intervention continued until infants were 84 days of age, discharge, or when they consumed ≥2 complete oral feeds daily over 3 day, whichever came first

	• Mother's milk was provided first. Pasteurized donor human milk provided as a supplement
	• Once a feeding order reached ≥100 mL/kg per day, nutrient fortification of milk commenced
	• The goal was to discontinue PN at 120 mL/kg per day, with full enteral feeding defined as 160 mL/kg per day
	• Enteral feeds were further concentrated if, after full enteral feeds were achieved, weekly weight gain was <15 gm/kg per day
	• At the end of the feeding intervention, infants were weaned from the study feeds over 3 day
	  ○ **Group 1:** In the HMBF arm (intervention), Prolact + 4, Prolact + 6 and Prolact + 8 (Prolacta Bioscience) were mixed with human milk according to the manufacturer's instructions, in the ratios 1:4, 3:7, and 2:3, respectively. Fortification began at 0.81 kcal/mL (24 kcal/oz), and when the infant had reached 140 mL/kg per day it was increased to 0.88 kcal/mL (26 kcal/oz)
	  ○ **Group 2:** Human milk was nutrient enriched with Similac Human Milk Fortifier Powder (Abbott Nutrition) to 0.72 kcal/mL (22 kcal/oz) and 0.78 kcal/mL (24 kcal/oz), and with the addition of powdered formula (Similac Neosure, Abbott Nutrition) to further concentrate feeds to 0.88 kcal/mL (26 kcal/oz). Fortification began at 0.72 kcal/mL (2 packages/100 mL human milk), and when the infant reached 140 mL/kg per day it was increased to 0.78 kcal/mL (4 packages/100 mL human milk). To mimic the average mother's milk protein concentration of 1.2 g/dL, an intact-protein powder modular (Beneprotein, Nestle) was routinely added to donor milk (0.4 g/100 mL) after nutrient fortification had reached 0.78 kcal/mL.

Outcomes	**Primary outcome(s):** • *Percentage of infants with an interruption in enteral feedings, unrelated to a clinical procedure that lasted for >12 hours or a >50% reduction in volume over the same time frame. **Secondary outcome(s):** • Dichotomous mortality and morbidity index • Fecal calprotectin • *Growth • NEC Stage >II • Late onset sepsis • Chronic lung disease • *Severe retinopathy of prematurity *Outcomes of interest to the CMH CPG or CAT development team
Notes	**Limitations:** • Sample size not large enough to detect significant difference in NEC • The study does not address if differences would have been observed if fortification would have been started at 40ml/kg/d.

Risk of Bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Infants were randomly assigned to either the HMBF or BMBF group through an online third-party service. Randomization occurred in blocks of 4, stratified by birth weight (<1000, 1000–1249 g) and recruitment center.

Allocation concealment (selection bias)	Low risk	Allocation of treatment was blinded
Blinding of participants and personnel (performance bias)	Low risk	Parents and staff were blinded
Blinding of outcome assessment (detection bias)	Low risk	staff abstracting data from the medical records, remained blinded to the feeding assignments
Incomplete outcome data (attrition bias)	Low risk	Per-protocol analysis was used, but sensitivity analysis showed it would not have effected the outcomes.
Selective reporting (reporting bias)	Low risk	All outcomes reported
Other bias	Unclear risk	

Sullivan et. al., 2010

Methods	**Randomized Control Trail**

Participants	**Participants:** Very low birth weight infants (500-1250g)
	Setting: Twelve NICUs- 11 in the United States and 1 in Austria
	Randomized into study by type of HMF they received and type of milk they were given if no mother's own milk was available: *N* = 207
	• **Group 1, HM100**: *n* = 67
	• **Group 2, HM40**: *n* = 71
	• **Group 3, BOV**: *n* = 69
	
	Completed Study: *N* = 176
	• **Group 1**: *n* = 56
	• **Group 2**: *n* = 61
	• **Group 3**: *n* = 59
	
	Gender, males (as defined by researchers):
	• **Group 1**: *n* = 32 (48%)
	• **Group 2**: *n* = 25 (35%)
	• **Group 3:** *n* = 36 (52%)
	
	Race / ethnicity or nationality (as defined by researchers):
	• **Group 1**: *n* = 20 (30%)
	• **Group 2**: *n* = 17 (24%)
	• **Group 3**: *n* = 10 (14%)
	
	Gestational age, mean in weeks ± SD
	• **Group 1**: 27.2 ± 2.2
	• **Group 2**: 27.1 ± 2.3
	• **Group 3**: 27.3 ± 2.0
	
	Inclusion Criteria:
	• Birth weight of 500 – 1250 g
	• Intention to receive mother's milk
	• Ability to adhere to a feeding protocol based on:
	  ○ the use of mother's own milk
	  ○ initiation of enteral feeding before 21 days after birth
	  ○ initiation of PN within 48 hours of birth
	
	Exclusion Criteria:
	• Major congenital malformations

	• Likelihood of transfer to a non-study institution during the study period **Power Analysis:** Sample size calculation was based on the primary outcome of duration of PN (parenteral nutrition), a surrogate of feeding intolerance, and neonatal morbidity. • For a 40% reduction in PN days, a sample size of 62 infants per group was needed for a 2-sided alpha error of 2.5% and power of 90% • An estimated proportion of protocol non-adherence of 5% indicated a final sample size of 69 infants per group
Interventions	**Both:** • Received mother's own milk • Provided trophic feedings with initiation 1 to 4 days after birth and continued at 10 to 20 mL/kg/d as tolerated up to 5 days • Daily body weight and weekly recumbent length and head circumference were recorded. ○ Group 1: pasteurized donor human milk-based human milk fortifier (HMF) + donor human milk (if mother's own milk was unavailable). HMF was added in when milk intake reached 100 mL/kg/day ○ Group 2: pasteurized donor human milk-based human milk fortifier (HMF) + donor human milk (if mother's own milk was unavailable). HMF was added in when milk intake reached 40 mL/dg/day ○ Group 3: bovine milk based HMF and preterm formula if no mother's own milk was available. Bovine milk-based fortifier was added in when milk intake reached 100 mL/kg/da
Outcomes	**Primary outcome(s):** • Improved health benefits including reduction in duration of parenteral nutrition, late-onset sepsis, and *NEC **Secondary outcome(s)** • *ROP • *BPD **Safety outcome(s):** • None reported *Outcomes of interest to the CMH CPG or CAT development team
Notes	**Results:** • Progression of first enteral feed to achievement of full enteral feed was similar across groups 1, 2, and 3. • No significant differences found for duration of parenteral nutrition or incidence of late-onset sepsis across groups. • Notable (significant) differences noted in occurrence of NEC with fewer cases in the HM100(6%) and HM40(8.5%) groups compared to the BOV (20%) group. • A multivariate logistic regression that controlled for possible contributing variables to impact NEC occurrence (5-minute APGAR score, quantity of MOM received, gestational age, receipt of prenatal and postnatal steroids, black race, BPD) found an odds ratio for NEC with an exclusive human milk diet to be 0.23 (95% CI = 0.08, 0.66), P = .007 *The study was not powered for secondary outcomes.
Risk of Bias	

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	High risk	Randomization based on weight and if they were appropriate or small for gestational age.
Allocation concealment (selection bias)	Low risk	Randomization performed in blocks of 4 based on weight and size for gestational age- investigators were unaware of block size
Blinding of participants and personnel (performance bias)	High risk	Caregivers not blinded due to need to properly handle mother's own milk but would not impact outcome; investigators would be able to see the physical differences between human milk and formula used/provided.
Blinding of outcome assessment (detection bias)	Unclear risk	No description of who prepared the milk/fortifier - insufficient information to permit judgement of low or high risk
Incomplete outcome data (attrition bias)	Low risk	Any randomized infant remained in their group for final analysis
Selective reporting (reporting bias)	Low risk	All priority outcomes were reported
Other bias	Unclear risk	Insufficient information to determine whether an important risk of bias exists

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References marked with an asterisk indicate studies included the meta-analysis. Citations are marked with an asterisk.

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