Review

Donor human milk protects against bronchopulmonary dysplasia: a systematic review and meta-analysis

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Abstract: Bronchopulmonary dysplasia (BPD) is the most common complication after preterm birth. Pasteurized donor human milk (DHM) has increasingly become the standard of care for very preterm infants over the use of preterm formula (PF) if mother's own milk (MOM) is unavailable. Studies have reported beneficial effects of DHM on BPD. We conducted a systematic review and meta-analysis of randomized controlled trials (RCTs) and observational studies on the effects of DHM on BPD and other respiratory outcomes. Eighteen studies met the inclusion criteria. Meta-analysis of RCT's could not demonstrate that supplementation of MOM with DHM reduced BPD when compared to PF (3 studies, risk ratio [RR] 0.89, 95% confidence interval [CI] 0.60-1.32). However, meta-analysis of observational studies showed that DHM supplementation reduced BPD (8 studies, RR 0.78, 95% CI 0.67-0.90). An exclusive human milk diet reduced the risk of BPD, compared to a diet with PF and/or bovine milk-based fortifier (3 studies, RR 0.80, 95% CI 0.68-0.95). Feeding raw MOM, compared to feeding pasteurized MOM, protected against BPD (2 studies, RR 0.77, 95% CI 0.62-0.96). In conclusion, our data suggest that DHM protects against BPD in very preterm infants, but pasteurization of human milk reduces the benefit.

Keywords: donor human milk; bronchopulmonary dysplasia; breast milk; preterm formula; pasteurization

1. Introduction

The nutritional and immunological benefits of providing human milk to very preterm (gestational age [GA] <32 weeks) or very low birth weight (VLBW, i.e., birth weight < 1500 g) infants have been increasingly recognized [1-5]. Official bodies such as the American Academy of Pediatrics [6] or the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition [7] recommend mother's own milk (MOM) as the first choice in VLBW infant feeding and advocate for making strong efforts to promote lactation. When MOM is not available, pasteurized donor human milk (DHM) is the preferred choice [6,7]. When MOM and DHM are not available, preterm formula (PF) should be used [7].

Although evidence exists regarding the protective effects of MOM in reducing the risk of necrotizing enterocolitis (NEC), late-onset sepsis (LOS), and retinopathy of prematurity (ROP) in VLBW infants, questions remain regarding whether DHM provides the same benefits [5,8-14]. Numerous MOM components which could contribute to protect against adverse outcomes of prematurity are reduced or absent in DHM which is usually pasteurized [14-16].

Bronchopulmonary dysplasia (BPD) is the most common complication of extreme preterm birth. Infants who develop BPD manifest aberrant or arrested pulmonary development and can experience lifelong alterations in cardiopulmonary function [17,18]. Optimal nutritional support is considered a cornerstone in the treatment/prevention of BPD [17,19]. Several studies have reported protective effects of DHM in the development of BPD [9,20]. However, a systematic review of the evidence in the literature has not been performed to date. Therefore, we aimed to conduct a systematic review and meta-analysis of the

interventional and observational studies reporting data on the effects of pasteurized DHM on BPD as well as other indicators of pulmonary outcome in preterm infants.

2. Materials and Methods

A protocol was developed prospectively that detailed the specific objectives, criteria for study selection, the approach to assessing study quality, clinical outcomes, and statistical methodology. The study was carried out and reported according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) [21]. The PRISMA checklist for this report can be found in supplementary material (Figure S1).

2.1. Data Sources and Search Strategies

A comprehensive literature search was conducted using PubMed/MEDLINE and EMBASE, from their inception to December 1, 2017. The search strategy for PubMed used the following terms, including Mesh terms: (breast milk OR infant feeding OR donor milk OR pasteurized human milk OR preterm formula) AND (preterm infant OR very low birth weight infant) AND (outcome OR bronchopulmonary dysplasia OR BPD). A similar strategy was used in the other databases. No language limit was applied. Translation was performed where necessary. Randomized controlled trials (RCTs) and observational studies were included in the review. Narrative reviews, systematic reviews, case reports, letters, editorials, and commentaries were excluded but read to identify potential additional studies. Additional strategies to identify studies included use of "related articles" feature on PubMed, and use of "cited by" tool in Web of Science and Google Scholar.

2.2. Eligibility Criteria and Study Selection

Studies were included if they were RCTs, cohort studies or case-control studies, involving the use of DHM in very preterm (GA <32 weeks) or VLBW (BW <1500g) infants, included a study and control group divided according to feeding policy, and reported results on BPD, days of mechanical ventilation (MV) or days on oxygen (O_2). BPD was defined as oxygen dependency at 28 days (BPD28) or as oxygen dependency at 36 weeks post-menstrual age (BPD36). To identify relevant studies, two reviewers (EV-M, EV) independently screened the results of the searches and applied inclusion criteria using a structured form. Discrepancies were identified and resolved through discussion or in consultation with the other researchers.

2.3. Data Extraction and Assessment of Risk of Bias

Two investigators (EV-M, EV) extracted the data by using a data collection form designed for this review. The following information was collected: study type, number of patients, number and name of centers, study period, inclusion/exclusion criteria, patient characteristics (GA, BW), feeding intervention or observation (MOM, DHM, and/or PF, type of fortifier, duration of intervention/observation), and outcome (incidence of BPD, days on mechanical ventilation, days on oxygen). Two other investigators (MP, GC) independently validated the accuracy of the extracted data.

Two reviewers (EV-M, EV) independently assessed risk of bias in each study, using two predetermined tools. Risk of bias in RCTs was assessed by using the Cochrane "Risk of Bias Assessment Tool" [22]. For each domain (random number generation, allocation concealment, blinding of intervention and outcome assessors, completeness of follow-up, selectivity of reporting, and other potential sources of bias) the risk was assessed as low, high, or unclear. Risk of bias in observational studies was assessed using the Newcastle-Ottawa scale for quality assessment of cohort and case-control studies [23]. This scale uses a rating system (range: 0-9) that gives points for selection (0-4), comparability (0-2) and outcome/exposure (0-3). Discrepancies during the data extraction and assessment of risk of bias process were resolved by discussion and consensus among all reviewers.

2.4. Statistical Analysis

Studies were combined and analyzed using comprehensive meta-analysis V 3.0 software (Biostat Inc., Englewood, NJ, USA). For dichotomous outcomes, the Mantel-Haenszel (MH) risk ratio (RR) with 95%

confidence interval (CI) was calculated from the data provided in the studies. For continuous outcomes, the mean difference (MD) with 95% CI was calculated. When studies reported continuous variables as median and range or interquartile range, we asked authors for mean and standard deviation (SD). If they did not provide the requested data, we estimated the mean and SD using the method of Wan et al. [24].

Due to anticipated heterogeneity, summary statistics were calculated with a random-effects model. This model accounts for variability between studies as well as within studies. Subgroup analyses were conducted according to the mixed-effects model [25]. In this model, a random-effects model is used to combine studies within each subgroup and a fixed-effect model is used to combine subgroups and yield the overall effect. The study-to-study variance (tau-squared) is not assumed to be the same for all subgroups. This value is computed within subgroups and not pooled across subgroups. Statistical heterogeneity was assessed by Cochran's Q statistic and by the I^2 statistic, which is derived from Q and describes the proportion of total variation that is due to heterogeneity beyond chance [26]. We used the Egger's regression test and funnel plots to assess publication bias. A probability value of less than 0.05 (0.10 for heterogeneity) was considered statistically significant.

3. Results

Based on the titles and abstracts of 1081 citations, we identified 139 potentially relevant studies (Figure 1), 18 of which met the inclusion criteria [9,20,27-42]. The main characteristics of the included studies are shown in Table 1. Seven studies [9,29,30,37,39,40,42] were RCTs and 11 studies [20,27,28,31-34,36,38,41,43] were observational. Definitions of feeding type varied across studies. None of the studies had BPD as their primary outcome. To pool data, we classified feeding type comparisons into 3 categories: 1) DHM vs. PF; 2) MOM vs. DHM; 3) raw MOM vs. treated MOM (pasteurized or frozen).
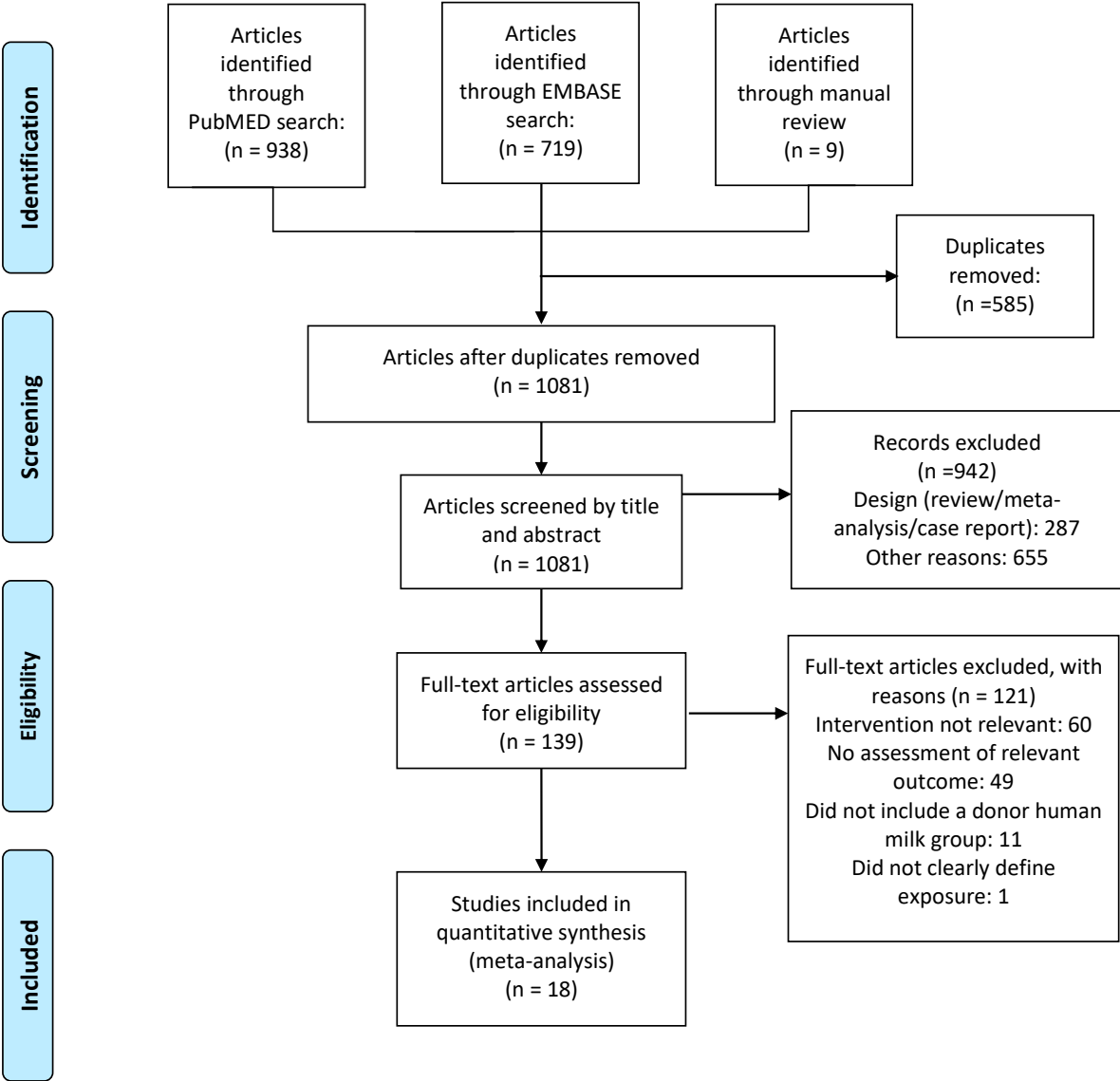
Figure 1. Flow diagram of literature search process.

Table 1. Synoptic table of characteristics of included randomized controlled trials.

First author, year	n of infants (centers)	Inclusion criteria	Primary outcome(s)	Respiratory outcome(s)	Studied intervention	Duration of intervention	Fortification	Comments
Corpeleijn, 2016 [29]	373 (6)	BW <1500g, MOM insufficiently available	Composite incidence of NEC, serious infection (sepsis or meningitis), or all-cause mortality between 72h and 60 days of life	BPD28	- MOM + DHM - MOM + PF	10 days or hospital transfer or death	Bovine fortifier added to MOM and DHM	Median MOM intakes were higher in the DHM group, 89.1% in the DHM group vs. 84.5% in the PF group
Cossey, 2013 [30]	303 (1)	GA <32 weeks, BW <1500g	Incidence of proven late-onset sepsis	BPD36, days on MV	- Raw MOM + PF - Pasteurized MOM + PF	8 weeks or discharge	Not specified	MV defined as respiratory support
Cristofalo, 2013 [42]	53 (7)	BW 500-1250g, no intention to provide MOM, parenteral nutrition within 48 hours, enteral feeding within 21 days	Duration of parenteral nutrition	Days on MV, days on O_2	- Exclusive DHM - Exclusive PF	91 days of age or discharge, or attainment of 50% oral feedings.	DHM fortifier added to DHM	
O'Connor, 2016 [40]	363 (4)	BW <1500g, enteral feeding within 7 days	Bayley-III score at 18 months	BPD36	- MOM + DHM - MOM + PF	90 days or discharge	Bovine fortifier added to MOM and DHM	MOM + DHM group: MOM = 58% of intake. MOM + PF group: MOM = 63% of intake.

First author, year	n of infants (centers)	Inclusion criteria	Primary outcome(s)	Respiratory outcome(s)	Studied intervention	Duration of intervention	Fortification	Comments
Omarsdottir, 2015 [37]	140 (2)	GA <28 weeks, intention to provide MOM, intention to breastfeed	CMV transmission to infants from breast milk, rate of symptomatic CMV infection	BPD36	- Frozen MOM + DHM - Fresh MOM + frozen MOM + DHM	Until 32 weeks PMA	Not specified	Frozen MOM: stored for ≥3 days at -20°C Fresh MOM: fed immediately or after refrigeration at +4°C
Schanler, 2005 [9]	243 (1)	GA ≤29 weeks	Incidence of late-onset sepsis and/or NEC	BPD36, days on MV	- MOM + DHM - MOM + PF	90 days or discharge	Bovine fortifier added to MOM and DHM	
Sullivan, 2010 [39]	207 (12)	BW 500-1250g, intention to provide MOM	NEC	BPD36, days on MV, days on O₂	- MOM + DHM + DHM fortifier - MOM + PF + bovine fortifier	91 days or ≥50% oral feedings or discharge	Donor DHM-based fortifier in DHM group, bovine fortifier in PF group.	

BPD28: Bronchopulmonary dysplasia, defined as oxygen dependency at day 28 of life; BPD36: Bronchopulmonary dysplasia, defined as oxygen dependency at 36 weeks corrected gestational age; MOM: Mother's own milk; PF: Preterm formula; DHM: Donor human milk; MV: mechanical ventilation; GA: gestational age; BW: birth weight; PMA: post-menstrual age; CMV: Cytomegalovirus; NEC: necrotizing enterocolitis.

Table 2. Synoptic table of characteristics of included observational studies.

First author, year	Study design	n of infants (centers)	Inclusion criteria	Primary outcome(s)	Respiratory outcome(s)	Groups	Duration of intervention	Fortification	Comments
Assad 2015 [27]	Retrospective cohort	293 (1)	GA <29 and/or BW ≤1500g	Feeding intolerance, time to full feeds, length of stay	BPD36	- MOM + DHM + DHM-based fortifier; - MOM + bovine fortifier - MOM + bovine fortifier + PF - Exclusive PF	Until discharge	DHM fortifier in exclusive human diet, bovine fortifier in bovine groups	
Colacci 2017 [28]	Retrospective cohort	85 (1)	BW <1000g, GA<37 weeks	Neurodevelopmental impairment (Bayley-III score)	BPD36	- MOM + DHM + DHM-based fortifier - MOM + bovine fortifier + PF	At least 4 weeks, until weight ≥1500g, or 34 weeks PMA	DHM-based fortifier in exclusive human milk group, bovine fortifier in other group	
Dicky 2017 [31]	Retrospective cohort	926 (63)	GA <32 weeks, alive at 7d of life	In-hospital mortality, short-term morbidity, weight gain	BPD36 Days on MV, Days on oxygen	- Raw MOM + PF/DHM - Pasteurized MOM + PF/DHM	Until 33 weeks corrected age or until discharge	Bovine fortifier added to both groups	The supplement (PF, DHM, infant formula) to raw or pasteurized MOM varied per center
Ginovart 2016 [32]	Retrospective cohort	186 (1)	BW <1500g	Retinopathy of prematurity	BPD28, BPD36, days on MV, days on O_2	- MOM + DHM - MOM + PF	4 weeks	Bovine fortifier added to MOM and to DHM	Additional data provided by authors, and from later (2017) report of Ginovart et al. [44]

First author, year	Study design	n of infants (centers)	Inclusion criteria	Primary outcome(s)	Respiratory outcome(s)	Groups	Duration of intervention	Fortification	Comments
Giuliani 2012 [33]	Retrospective cohort	92 (1)	GA >23 weeks, BW <1500g.	Growth, and short-term clinical outcomes	BPD28	- Mainly MOM (>80%) - Mainly DHM (>80%)	32 weeks corrected GA	Bovine fortifier added to MOM	
Hair 2016 [34]	Retrospective cohort	1587 (4)	BW <1250g.	NEC, mortality	BPD36, days on MV	- MOM + DHM + DHM-based fortifier; - MOM + bovine fortifier + PF;	32-34 corrected GA, or 60 days of life, depending on center	DHM-based fortifier in DHM group, Bovine fortifier in bovine group	
Kreissl 2017 [43]	Prospective cohort	283 (1)	GA <32 weeks, BW <1500g	Time to full enteral feeding	BPD36	- MOM + DHM - MOM + PF	Until term or discharge. DHM group received DHM until MOM was available or until reaching 140 mL/kg/d, then switched to term formula.	Bovine fortifier added to MOM and DHM when infant reached intake of 100 mL/kg/d	DHM provided by other preterm mothers
Lee 2016 [36]	Retrospective cohort	46 (1)	BW <1500g	Morbidity, duration parenteral nutrition, length of hospital stay	BPD36, days on MV, days on O_2	- MOM + DHM - MOM + PF	Not specified	Not specified	Days on MV: defined as invasive ventilation. Days on O_2 defined as non-invasive ventilation.

First author, year	Study design	n of infants (centers)	Inclusion criteria	Primary outcome(s)	Respiratory outcome(s)	Groups	Duration of intervention	Fortification	Comments
Madore 2017 [41]	Retrospective case-control	81 (1)	BW <1000g	Growth, neurodevelopment	BPD36	- Exclusive MOM - DHM >50% - PF >50%	First month of life	Bovine fortifier added to MOM and to DHM	
Sisk 2017 [38]	Retrospective cohort	563 (1)	GA ≤32w and BW ≤1500g	NEC stage ≥2	BPD36	- MOM ≥50% - DHM ≥50% - PF ≥50%	Within 2 hours of birth until 34 weeks PMA	Bovine fortifier added to MOM and to DHM	
Spiegler 2016 [20]	Prospective cohort	1433 (48)	GA 22-31 6/7, BW <1500g,	BPD	BPD36, days on MV, days on O_2	- MOM + DHM - MOM + DHM + PF - Exclusive PF	Until discharge	Bovine fortifier added to MOM and to DHM	

BPD28: Bronchopulmonary dysplasia defined as oxygen dependency after day 28 of life. BPD36: Bronchopulmonary dysplasia, defined as oxygen dependency at 36 weeks corrected gestational age; MOM: Mother's own milk. PF: Preterm formula; DHM: Donor human milk; GA: gestational age; BW: birth weight. PMA: post-menstrual age. NEC: necrotizing enterocolitis

3.1. Quality assessment and publication bias

The six RCTs included in the review scored low risk of bias for random sequence generation, allocation concealment, incomplete outcome data, selective reporting and other bias (Table A1). Three studies [9,30,39] scored high or unknown risk of bias on blinding (Table A1). The 12 observational studies included scored between 6 and 9 points on the Newcastle-Ottawa scale, out of a possible 9 points (Table A2). The median score of included studies was 7 points.

We found no evidence of publication bias in any of the analyses that we performed, either through visual inspection of funnel plots or Egger's regression intercept. However, the limited amount of studies per outcome makes these tests inconclusive.

3.2. Randomized controlled trials: donor human milk vs. preterm formula

Five RCTs [9,29,39,40,42] randomized infants to receive DHM or PF when MOM was insufficiently available. Thus, they compared infants receiving MOM supplemented with DHM vs. infants receiving MOM supplemented with PF. Three of these studies [9,39,40] reported on the rate of BPD36. As shown in Figure 2, meta-analysis could not detect a statistically significant effect of DHM on BPD36 (RR 0.89, 95% CI 0.60 to 1.32, p = 0.562). The RCT of Sullivan et al. used a DHM-based fortifier in the DHM group, and a bovine milk-based fortifier in the PF group. The studies of Schanler et al. [9] and O'Connor et al. [40] used a bovine milk-based fortifier in both groups. Exclusion of the study of Sullivan et al. [39] for using a different type of fortifier did not significantly affect the results of the meta-analysis on BPD36 (RR 0.85, 95% CI 0.39 to 1.85, p = 0.676). One study [29] reported on BPD28 and could not find any significant effect (RR 1.06, 95% CI 0.75 to 1.51, p = 0.724).

Study name	BPD36-Yes / Total		Statistics for BPD36					Relative Weight (%)
	DHM	PF	MH risk ratio	Lower limit	Upper limit	*p*-Value		
Schanler 2005	12 / 81	25 / 92	0.55	0.29	1.01	0.055		24
Sullivan 2010	48 / 138	27 / 69	0.89	0.61	1.29	0.535		38
O'Connor 2016	44 / 175	37 / 179	1.22	0.83	1.79	0.318		38
OVERALL			**0.89**	**0.60**	**1.32**	**0.562**		

Heterogeneity: Q = 4.8; I^2 = 58.2%; p = 0.092

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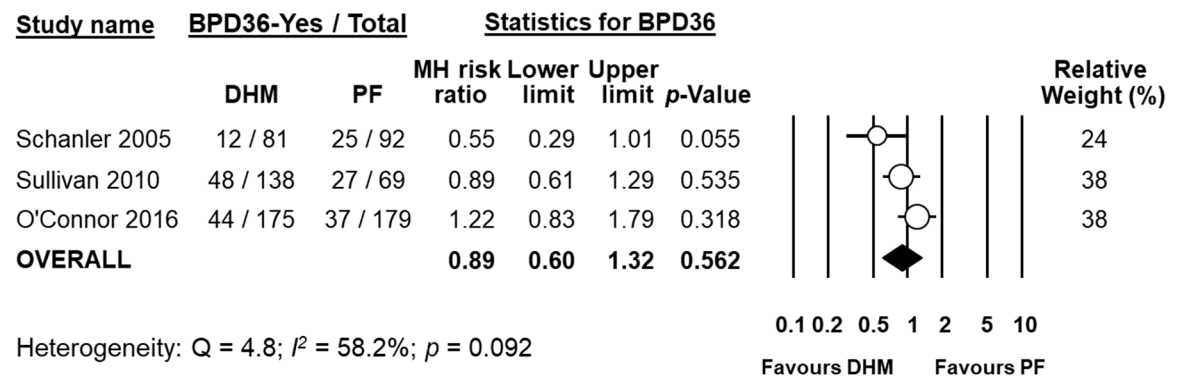
Favours DHM   Favours PF

Figure 2. Meta-analysis of randomized controlled trials assessing the effects of supplementation of MOM with DHM, compared with supplementation with PF, on risk of BPD36. DHM: donor human milk; PF: preterm formula; BPD36: bronchopulmonary dysplasia defined as oxygen dependency at 36 weeks post-menstrual age; MH: Mantel-Haenszel.

Three studies [9,39,42] reported data on days on mechanical ventilation, and meta-analysis showed a significant reduction in this outcome for infants receiving DHM (MD -5.73 days, 95% CI -10.68 to -0.77, p = 0.023, Figure 3). Two studies [39,42] reported data on days on oxygen, and meta-analysis could not find a significant reduction in this outcome for infants receiving DHM (MD -9.11 days, 95% CI -24.82 to 6.60, p = 0.256).
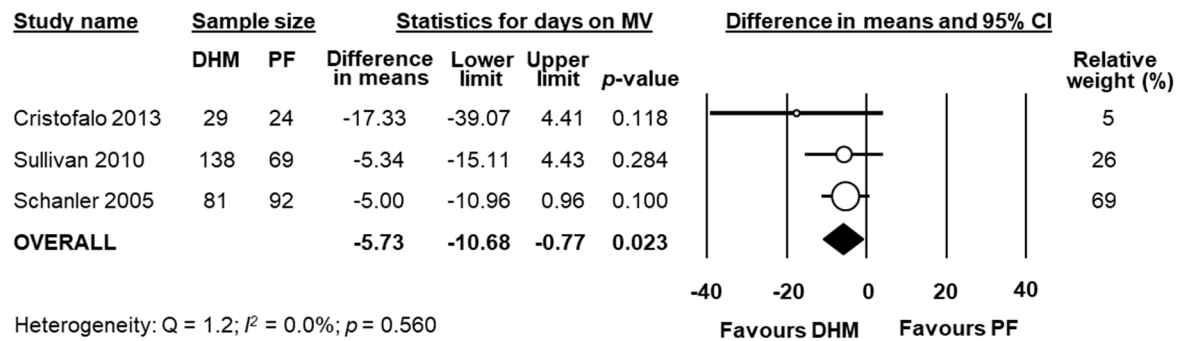
Study name	Sample size		Statistics for days on MV				Difference in means and 95% CI	Relative weight (%)
	DHM	PF	Difference in means	Lower limit	Upper limit	*p*-value		
Cristofalo 2013	29	24	-17.33	-39.07	4.41	0.118		5
Sullivan 2010	138	69	-5.34	-15.11	4.43	0.284		26
Schanler 2005	81	92	-5.00	-10.96	0.96	0.100		69
OVERALL			-5.73	-10.68	-0.77	0.023		

Heterogeneity: Q = 1.2; I^2 = 0.0%; *p* = 0.560

Figure 3. Meta-analysis of randomized controlled trials assessing the effects of supplementation of MOM with DHM, compared with supplementation with PF, on mean days on MV. DHM: donor human milk; PF: preterm formula; MV: mechanical ventilation.

3.3. Observational studies: donor human milk vs. preterm formula

Eight observational studies compared infants receiving MOM supplemented with DHM to infants receiving MOM supplemented with PF [20,27,28,32,34-36,38] of which all reported data on BPD36. Three of these studies [27,28,34] used a DHM-based fortifier in the DHM-group, and a bovine milk-based fortifier in the PF group. When these 3 studies were pooled, meta-analysis showed a significant protective effect of DHM on BPD36 (RR 0.80, 95% CI 0.68 to 0.95, *p* = 0.009). This protective effect of DHM on BPD36 was also observed when the 5 studies [20,32,35,36,38] that did not use DHM-based fortifier were pooled (RR 0.71, 95% CI 0.53 to 0.95, *p* = 0.022) and when the 8 studies were combined in a mixed-effects meta-analysis (RR 0.78, 95% CI 0.67 to 0.89, *p* = 0.0005, Figure 2). The study of Ginovart et al. [32] also reported data on BPD28, and found no significant difference in this outcome between the group receiving DHM and the group receiving PF (RR 1.18, 95% CI 0.68 to 2.05, *p* = 0.560).

Study name	BPD36-Yes/ Total		Statistics for BPD36				MH risk ratio and 95% CI	Relative weight (%)
	DHM	PF	MH risk ratio	Lower limit	Upper limit	*p*-value		
Spiegler 2016	25 / 223	50 / 239	0.54	0.34	0.84	0.006		45
Kreissl 2017	17 / 133	24 / 150	0.80	0.45	1.42	0.445		26
Sisk 2017	15 / 139	14 / 113	0.87	0.44	1.73	0.693		19
Lee 2016	3 / 18	5 / 28	0.93	0.25	3.44	0.917		5
Ginnovart 2016	7 / 114	3 / 72	1.47	0.39	5.52	0.565		5
Studies with bovine fortifier			0.71	0.53	0.95	0.022		
Assad 2016	13 / 87	54 / 206	0.57	0.33	0.99	0.046		8
Colacci 2016	19 / 39	31 / 46	0.72	0.49	1.06	0.094		16
Hair 2016	391 / 819	432 / 768	0.85	0.77	0.93	0.001		75
Studies with human fortifier			0.80	0.68	0.95	0.009		
OVERALL			0.78	0.67	0.90	0.001		

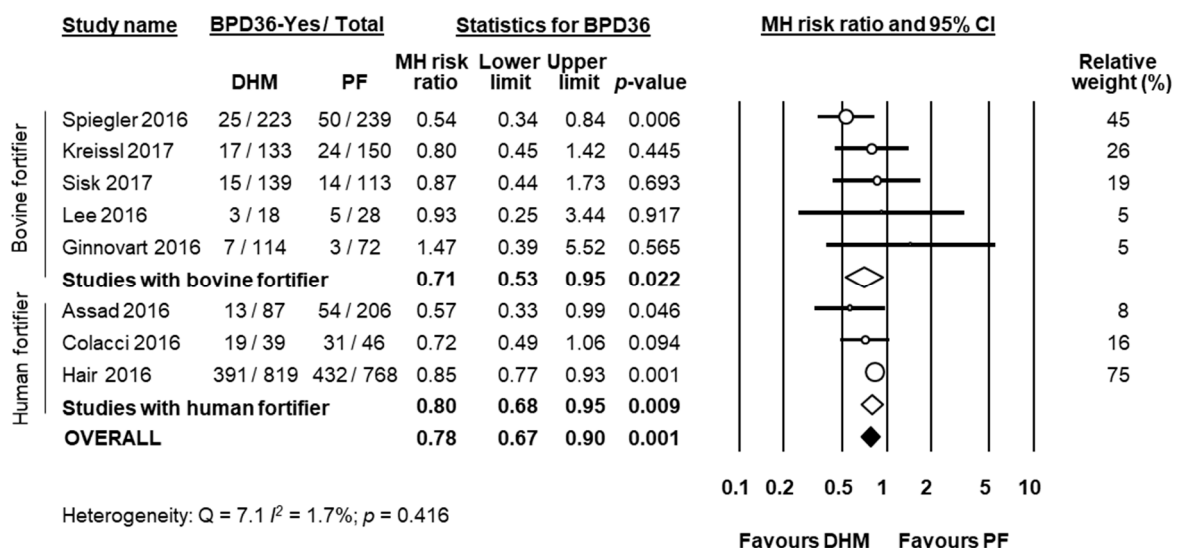Heterogeneity: Q = 7.1 I^2 = 1.7%; *p* = 0.416

Figure 4. Meta-analysis of observational studies assessing the effects of supplementation of MOM with DHM, compared with supplementation with PF, on risk of BPD36. MOM: mother's own milk; DHM: donor human milk; PF: preterm formula; BPD36: bronchopulmonary dysplasia defined as oxygen dependency at 36 weeks post-menstrual age; MH: Mantel-Haenszel.

Four studies [20,32,34,36] reported data on mean days on mechanical ventilation, and meta-analysis showed a significant reduction in difference in means in the DHM-group (MD 2.14 days, 95% CI -4.08 to -0.21, *p* = 0.030, Figure 5). Three studies [20,32,36] reported data mean on days on oxygen, and meta-analysis could not find a significant difference in mean days on oxygen in the DHM group (MD -2.78 days, 95% CI -6.32 to 0.76, *p* = 0.123, Figure 6).
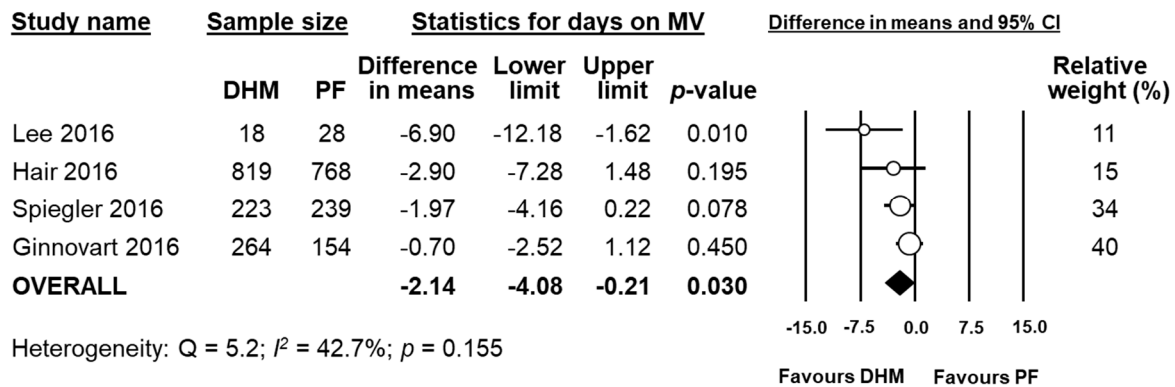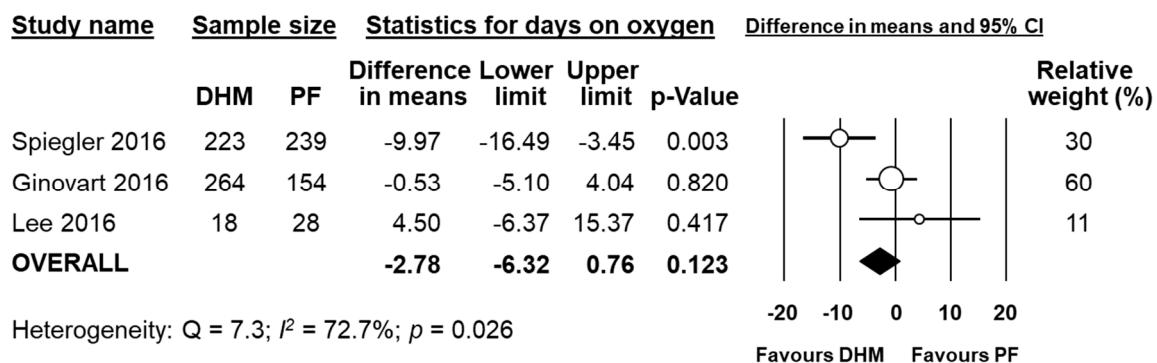
Study name	Sample size DHM	Sample size PF	Difference in means	Lower limit	Upper limit	p-value	Difference in means and 95% CI	Relative weight (%)
Lee 2016	18	28	-6.90	-12.18	-1.62	0.010		11
Hair 2016	819	768	-2.90	-7.28	1.48	0.195		15
Spiegler 2016	223	239	-1.97	-4.16	0.22	0.078		34
Ginnovart 2016	264	154	-0.70	-2.52	1.12	0.450		40
OVERALL			**-2.14**	**-4.08**	**-0.21**	**0.030**		

Heterogeneity: Q = 5.2; I^2 = 42.7%; *p* = 0.155

Favours DHM   Favours PF

Figure 5. Meta-analysis of observational studies assessing the effects of supplementation of MOM with DHM, compared with supplementation with PF, on mean days on mechanical ventilation. MOM: mother's own milk; DHM: donor human milk; PF: preterm formula.

Study name	Sample size DHM	Sample size PF	Difference in means	Lower limit	Upper limit	p-Value	Difference in means and 95% CI	Relative weight (%)
Spiegler 2016	223	239	-9.97	-16.49	-3.45	0.003		30
Ginovart 2016	264	154	-0.53	-5.10	4.04	0.820		60
Lee 2016	18	28	4.50	-6.37	15.37	0.417		11
OVERALL			**-2.78**	**-6.32**	**0.76**	**0.123**		

Heterogeneity: Q = 7.3; I^2 = 72.7%; *p* = 0.026

Favours DHM   Favours PF

Figure 6. Meta-analysis of observational studies assessing the effects of supplementation of MOM with DHM, compared with supplementation with PF, on mean days on oxygen. DHM: donor human milk; PF: preterm formula.

3.4. Mother's own milk vs. donor human milk

Five observational studies [9,27,33,38,41] compared infants who received mainly MOM vs. infants who received mainly DHM. There were significant differences in group design. Guiliani et al. [33] compared infants receiving >80% MOM (supplemented with <20% DHM) vs. infants receiving <20% MOM (supplemented with >80% DHM). Sisk et al. [38] and Madore et al. [41] compared infants receiving ≥50% MOM to infants receiving ≥50% DHM. The RCT of Schanler et al.[9] randomized infants to receive either DHM or PF as supplementation when insufficient MOM was available, but also provided data on infants who only received MOM. We treated it as observational for this comparison, and compared infants who received exclusive MOM to infants who received MOM supplemented with DHM.

Four of the five studies reported on BPD36, and meta-analysis could not find significant difference in BPD36 risk in the MOM-group compared to the DHM-group (RR 1.24, 95% CI 0.87 to 1.77, *p* = 0.231, Figure

7). The study of Giuliani et al. [33] reported on BPD28 and did not find any significant effect of MOM on this outcome (RR 0.91, 95% CI 0.43 to 1.93, *p* = 0.804).
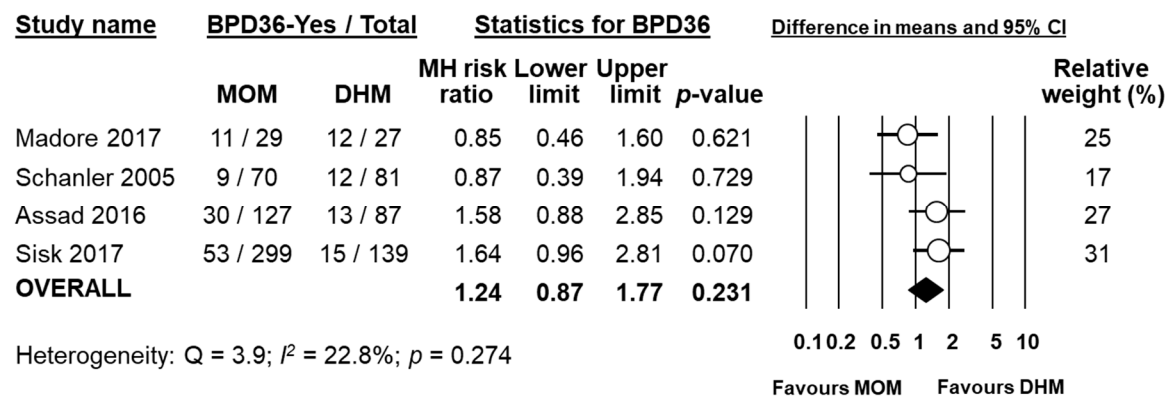
Study name	BPD36-Yes / Total		Statistics for BPD36				Difference in means and 95% CI	Relative weight (%)
	MOM	DHM	MH risk ratio	Lower limit	Upper limit	*p*-value		
Madore 2017	11 / 29	12 / 27	0.85	0.46	1.60	0.621		25
Schanler 2005	9 / 70	12 / 81	0.87	0.39	1.94	0.729		17
Assad 2016	30 / 127	13 / 87	1.58	0.88	2.85	0.129		27
Sisk 2017	53 / 299	15 / 139	1.64	0.96	2.81	0.070		31
OVERALL			1.24	0.87	1.77	0.231		

0.1 0.2 0.5 1 2 5 10

Favours MOM   Favours DHM

Heterogeneity: Q = 3.9; I^2 = 22.8%; *p* = 0.274

Figure 7. Meta-analysis of observational studies assessing the effects of receiving mainly MOM vs. receiving mainly DHM, on risk of BPD36. MOM: mother's own milk; DHM: donor human milk; BPD36: bronchopulmonary dysplasia defined as oxygen dependency at 36 weeks post-menstrual age; MH: Mantel-Haenszel.

3.5. Raw mother's own milk vs. treated mother's own milk (pasteurized or frozen)

Two studies compared raw or fresh MOM to pasteurized MOM [30,31]. Cossey et al. [30] carried out a RCT where infants were randomized to receive either raw or pasteurized MOM. Dicky et al. [31] carried out a multi-center observational trial comparing infants of centers where MOM was pasteurized and centers where it was not pasteurized. Individually, either study did not find a significant reduction in BPD36 risk in the raw MOM group when using unadjusted data (Figure 8), although Dicky et al. found a significant reduction on BPD36 risk in the raw MOM group when they adjusted their data for confounders (adjusted OR 0.40, 95% CI 0.27 to 0.67, *p* < 0.001). Since the study of Dicky et al. had a quasi-randomized design, we combined it with the RCT of Cossey et al. in a random effects meta-analysis. This analysis showed a significant reduction of BPD36 risk in the raw MOM group (RR 0.77, 95% CI 0.62 to 0.96, *p* = 0.018, Figure 8).

Study name	BPD36-Yes / Total		Statistics for BPD36				MH risk ratio and 95% CI	Relative weight (%)
	Raw MOM	Pasteurized MOM	MH risk ratio	Lower limit	Upper limit	p-Value		
Cossey 2013	24 / 151	35 / 152	0.69	0.43	1.10	0.121		21
Dicky 2017	132 / 636	76 / 290	0.79	0.62	1.01	0.063		78
OVERALL			0.77	0.62	0.96	0.018		

0.1 0.2 0.5 1 2 5 10

Favours DHM   Favours PF

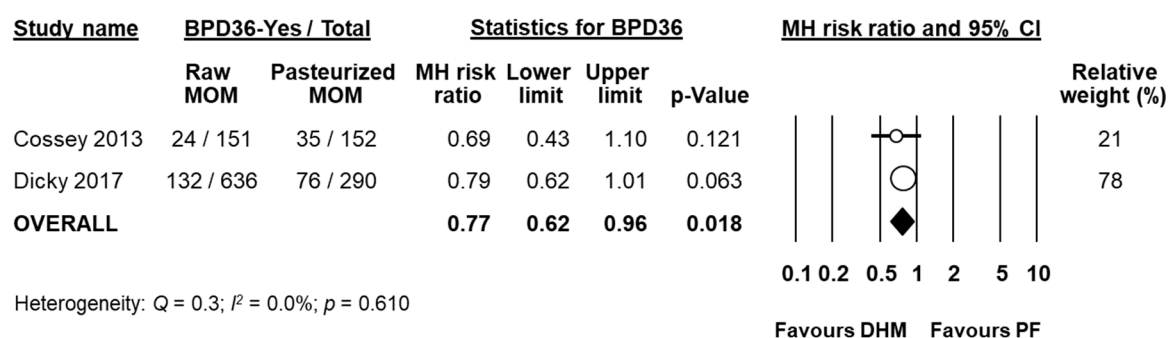Heterogeneity: Q = 0.3; I^2 = 0.0%; *p* = 0.610

Figure 8. Meta-analysis of studies assessing the effects of receiving raw MOM vs. receiving pasteurized MOM, on risk of BPD36. MOM: mother's own milk; DHM: donor human milk; BPD36: bronchopulmonary dysplasia defined as oxygen dependency at 36 weeks post-menstrual age; MH: Mantel-Haenszel.

One RCT [37] studied the effect of freezing MOM vs. providing it fresh to infants. Infants were randomized to receive only freeze-thawed MOM (frozen at -20˚C for at least 3 days), or to receive fresh MOM or MOM refrigerated at 4˚C, supplemented with frozen MOM when no fresh MOM was available. The mothers of all included infants had intention to breastfeed. Supplementation in both groups was with pasteurized DHM

when no MOM was available. The authors found an increase in BPD36 risk in the group receiving fresh MOM, although it was not significant (RR 1.47, 95% CI 0.98 to 2.21, *p* = 0.062).

4. Discussion

The present study is the first systematic analysis of evidence to date regarding the possible benefits of DHM on BPD. Meta-analysis of RCTs (3 studies) could not demonstrate that supplementation of MOM with DHM had a significant effect on BPD36 risk, when compared to supplementation with PF. Meta-analysis of RCTs did find that supplementation with DHM significantly reduced the mean days on mechanical ventilation. Moreover, meta-analysis of observational studies (8 studies) showed a protective effect of DHM supplementation on BPD36 and on days on mechanical ventilation, but not on days on oxygen. Additionally, an exclusive human milk diet (i.e., MOM and/or DHM and DHM-derived fortifier) significantly reduced the risk of BPD36, when compared to a diet that included PF and/or bovine milk-based fortifier. We also found that feeding infants raw MOM, compared to feeding them pasteurized MOM, protected against BPD36. In conclusion, our data suggest that DHM could protect against BPD in very preterm/VLBW infants. However, the process of pasteurization appears to reduce the beneficial properties of human milk on BPD development.

Our study has several limitations. First, the number of included studies is small. Second, no RCT was primarily designed and powered to detect the effects of DHM on BPD. Third, most RCTs comparing the effects of DHM and PF have included infants receiving some or primarily MOM in both groups because of the inability to assign feeding type ethically [45]. The proportion of MOM and supplementation with either DHM or PF varied across studies. Fourth, there was substantial heterogeneity among the studies in population, timing of initiation and duration of the intervention. An additional limitation, inherent to any meta-analysis on BPD, is the heterogeneity of the definition of the condition [46-49]. Finally, many studies were observational, which potentially reduced the reliability of the results. Despite all these limitations, our meta-analysis shows an additional benefit of using DHM instead of PF in VLBW infants.

Out of 18 studies we included in our analysis, 12 were published in 2016 or 2017. The use of DHM in the NICU is a topic of much discussion, and the recency of most studies we included reflects this. As more centers start using DHM in clinical practice, and as more RCTs and observational studies are reported, the results of our meta-analyses could change or be confirmed further.

There are several hypothetical mechanisms by which human milk may exert a protective effect against BPD: (i) by improving the nutritional status and growth of the infants; (ii) by reducing postnatal inflammatory processes such as NEC and LOS; (iii) by modulating the immune functions; and (iv) through the antioxidant properties of human milk. However, the term human milk feeding is frequently used to encompass both MOM and DHM, implying that the multiple beneficial outcomes attributed to MOM can be generalized to DHM [13,14]. This assumption may not be correct because of the important differences between DHM and MOM. As discussed below, part of these differences may be attributed to pasteurization, but there are additional factors that could play a role in the development of outcomes such as BPD. DHM is typically donated by women who have delivered a term infant and this milk has different levels of nutrients and protective components, including cytokines, growth factors, and lactoferrin than the milk provided by a mother for her own preterm infant [13,14]. Moreover, it has been suggested that the beneficial effects of MOM may be unique to the specific mother-infant dyad, thereby providing maximum protection to the mother's own infant [13,14].

The concern about transmission of infectious agents through breast milk led to the obligatory requirement for pasteurization of DHM. Holder pasteurization (62.5 °C for 30 min) of DHM is performed in

order to inactivate the microbial agents that may be present [50]. However, pasteurization also destroys or significantly decreases many of the protective elements in human milk, including, among others, lysozymes, secretory immunoglobulin A, growth factors, lactoferrin, antioxidants, and commensal bacteria [13,14,50-52]. Although numerous studies have characterized the effects of holder pasteurization on the biological properties of DHM (see [50] for review), the impact of pasteurization on clinical outcomes has been scarcely investigated. Since DHM should always be pasteurized, it is ethically impossible to study the clinical impact of unpasteurized DHM. However, there are studies comparing pasteurized to unpasteurized MOM. The main purpose of this pasteurization of MOM is the prevention of cytomegalovirus (CMV) infection [53].

We included two studies in our systematic review that reported the clinical outcomes of VLBW infants receiving unpasteurized or pasteurized MOM. The study of Cossey et al. [30] was a single center RCT, whereas the study of Dicky et al. [31] compared infants from 33 French NICUs that used pasteurized MOM to infants from 30 French NICUs that did not pasteurize MOM. Due to the quasi- randomized design of the study of Dicky et al, we combined it with the study of Cossey et al. in a meta-analysis, which showed that infants fed pasteurized MOM had a significantly higher risk of BPD than infants fed raw MOM (see Figure 8). Therefore, our data suggest that the possible protective effect of human milk on BPD decreases with pasteurization.

As mentioned above, holder pasteurization not only inactivates all common pathogens in human milk but also affects the commensal microflora [50]. The microbiome of breast milk has been associated with many of the benefits of raw MOM [54], namely with respect to the prevention of NEC [55,56]. The beneficial effects on the prevention of NEC could not be repeated with pasteurized DHM [29], which may be due to the loss of the microbiome [56,57]. The microbiome of breast milk can be "regrown" in pasteurized DHM with the inoculation of unprocessed MOM [58] which awaits clinical testing. BPD has also been associated with changes in the microbiome of the airways [59,60] and may even be associated with the gut microbiome [61]. Unfortunately, we have no complete understanding about the origin, dynamics and biological function of the respiratory microbiome [62]. Nevertheless, the effect of MOM's microbiome on the gut microbiome and possibly the respiratory microbiome may explain our finding on the loss of beneficial effects after pasteurization of human milk.

In some NICUs, MOM is routinely frozen to reduce the risk of CMV transmission [53]. In our systematic review we included one RCT [37] which studied the effect of freezing MOM compared to providing fresh MOM. They found a close to significant increased risk of BPD in the group receiving fresh MOM (RR 1.47, 95% CI 0.98 to 2.21, *p* = 0.062). It is worth noting that this study was not powered to detect differences in BPD. Further studies are needed to elucidate the effect of MOM-freezing on neonatal outcomes.

Both MOM and DHM must be fortified to provide sufficient support for growth and development in the postnatal period of the VLBW infant [63-66]. At present, most NICUs use bovine milk-derived fortifiers. However, the recent availability of a DHM-derived fortifier has led to the introduction of the concept of "exclusive human milk diet" [27,28,34,39]. This diet consists of MOM, DHM if MOM is not adequately available, and fortification with DHM-derived fortifier. Our data suggest that this exclusive human milk diet may reduce the risk of developing BPD. However, this result should be interpreted with care. Most studies evaluating the use of an exclusive human milk diet [28,34,39] do not isolate the individual effects of the DHM-derived fortifier, because the group receiving bovine products is a mixture of infants fed DHM plus bovine milk-derived fortifier and infants fed PF. Therefore, it is not possible to determine whether the lower risk of BPD was due to the benefit of DHM-derived fortifier or to the benefit of avoiding PF. Only the study of Assad et al. [27] provided data on infants not exposed to PF and could not find a significant change in BPD risk when using DHM-derived fortifier, compared to bovine-derived fortifier.

Maintaining a sufficient (unpasteurized) MOM supply is challenging for mothers of VLBW infants, necessitating supplementation with either DHM or PF. Our results suggest the superiority of DHM over PF in reducing BPD, but we should not forget that MOM is more effective in the reduction of multiple morbidities and is less expensive to acquire than DHM. Therefore, NICU care providers should prioritize interventions to support initiation and maintenance of lactation and make efforts to identify and solve lactation problems in mothers of VLBW infants.

Appendix A

Table A1. Assessment of risk of bias of included randomized controlled trials.

Study	Random Sequence Generation	Allocation Concealment	Blinding of Participants and Personnel	Blinding of Outcome Assessment	Incomplete Outcome Data	Selective Reporting	Other Bias
Corpeleijn 2016 [29]	LR	LR	LR	LR	LR	LR	LR
Cossey 2013 [30]	LR	LR	HR	UR	LR	LR	LR
Cristofalo 2013 [42]	LR	LR	LR	LR	LR	LR	LR
O'Connor 2016 [40]	LR	LR	LR	LR	LR	LR	LR
Omarsdottir 2015 [37]	UR	UR	HR	UR	LR	LR	
Schanler 2005 [9]	LR	LR	HR	HR	LR	LR	LR
Sullivan 2010 [39]	LR	LR	HR	LR	LR	LR	LR

Table A2. Assessment of methodological quality of included observational studies, using the Newcastle-Ottawa Scale.

Study name	Study design	Selection (0-4 points)	Comparability (0-2)	Outcome (0-3)	Total (0-9)
Assad 2015[27]	Retrospective cohort	4	0	3	7
Colacci 2017 [28]	Retrospective cohort	4	0	3	7
Dicky 2017 [31]	Retrospective cohort	4	2	3	9
Ginovart 2016	Retrospective cohort	4	0	3	7
Giuliani 2012 [33]	Retrospective case-control	4	0	3	7
Hair 2016 [34]	Retrospective cohort	4	0	3	7
Kreissl [43]	Prospective cohort	4	0	3	7
Lee [36]	Retrospective cohort	4	0	2	6
Madore 2017 [41]	Retrospective case-control	4	2	3	9
Sisk 2017 [38]	Retrospective cohort	4	0	3	7
Spiegler [20]	Prospective cohort	4	2	3	9

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