Human versus bovine milk derived fortifiers in preterm infants - a systematic review and meta-analysis

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Online Supplementary Material

Supplemental Table 1: The characteristics of the included studies[1]

Study number	Study authors, year (References)	Participants	Intervention and Control	Primary Outcomes[2]	Secondary Outcomes[3]	Conclusions	Comments
1	Hagelberg et al 1990 (26)	preterm infats≤32 weeks or birth weight≤1500g (*n*=20)	mother's milk supplemented with human milk protein (HMP) (n=10) vs adapted cow's milk protein (CMP) (n=10) and introduced when the infant is on 150ml/Kg/day of milk feeds and continued for 3 weeks	weight gain (g/kg/day): 11.2±5.1 vs 13.1±4.58*¥ Gain in length(cm/week): 0.57±0.26 vs 0.71±0.42*¥ Gain in head circumference(cm/week): 0.78±0.18 vs 0.67±0.26*¥	calcium (mmol/l) at 3 weeks: 2.3(0.5-5) vs 2 (1-4)^ ¥ Phosphorus (mmol/l) at 3 weeks: 8.3 (3-13.2) vs 2.7 (0.6-7.4)^ ¥ Urea at 3 weeks (mmol/l): 3.2(1.9-7.4) vs 5.3 (1.4-12.8)^ ¥ Sepsis: 6/10 vs 3/10¥ NEC: 0/10 vs 0/10¥ Mortality during the study: 0/10 vs 1/10¥	No significant alterations in the aminoacid profiles of peripheral blood by the type of fortifier	The overall advantages of human milk protein needs to be studied further
2	Boehm et al 1993(27)	very low birth weight infants with birth weight <1500g (*n*=24)	human milk supplemented with human milk protein (n=11) vs bovine milk protein (n=13) for 14 days when the infant reached total feed volume of 150ml/Kg/day and continued for 14 days	alpha amino nitrogen (mmol/L): 1.63±0.23 vs 1.70±0.19*¥ Urea (mmol/L): 2.06±0.32 vs 1.94±0.28*¥ Prealbumin (mg/l): 94.2±16.8 vs 101.8±18.6*¥ Weight gain (g/day): 32.1 vs 31.3¥ Gain in length (cm/week): 1.19 vs 1.13¥	(EPO vs. placebo): NEC: 0/7 vs. 0/8¥; BPD: 5/7 vs. 8/8¥; ROP: 1/7 vs. 2/8¥; PDA: 4/7 vs. 3/8¥; IVH: 0/7 vs1/8¥; mortality: 0/7 vs. 1/9	Human milk enriched with a well balanced fortifier, even when based on non human cources can fulfil nutritional needs	Small sample size, short duration of treatment

3	Polberger et al 1999 (25)	preterm infants with birth weight 920g-1750g (*n*=32)	human milk fortified when the fed volume was 150ml/Kg/day with a bovine whey protein fortifier (n=16) vs ultrafiltered human milk protein fortifier (n=16) for 24 days.	gain in weight (g/Kg/day): 15.6±2.9 vs 14.7±3.2*¥ Gain in length 9cm/wk),mean±SD: 0.97±0.34 vs 1.02±0.23*¥ Gain in head circumference (cm/wk),mean±SD: 1.06±0.21 vs 1.02±0.23*¥	urea (mmol/l):1.3±0.7 vs 1.8±0.7*¥ Albumin (g/l): 31±4 vs 33±4*¥ Amino acid content F vs HMP: serine: 161±40 vs 192±95*# Proline: 220±53 vs 340±95*# Ornithins: 93±24 vs 141±53*#	Routine analysis of human milk protein and energy content to optimise the use of huma milk for feeding in preterm infants	Small sample size, short duration of treatment
4	Cristofalo et al (29)	preterm infants with birth weight 500g-1250g(*n*=53)	mother's milk supplemented with human milk protein (HMP) (n=29) vs adapted cow's milk protein (CMP) (n=24) and introduced when the infant is on 100ml/Kg/day of milk feeds and continued for 91 days	gain in weight (g/day): 15±5.8 vs 17±7.1*# Gain in length (cm/week): 0.84±0.21 vs 1.12±0.28*# Gain in head circumference (cm/week): 0.78±0.26 vs 0.88±0.18*¥	Mortality: 0/29 (0%) vs 2/24 (8%) NEC: 1/29 (3%) vs 5/24 (21%)¥ Surgical NEC: 0/29 (1%) vs 4/24 (17%)# Sepsis: 16/29 (55%) vs 19/24 (79%)¥	Recommends the use of exclusive human milk based diet for extremely preterm infants.	Small sample size
5	Sullivan 2010 et al (24)	preterm infants with birth weight 500 to 1250g (*n*=136)	mother's milk supplemented with human milk fortifier (HMF 100) (n=67) vs bovine milk based HMF (n=69) when the enteral intake was 100ml/Kg/day and continued for 91 days of age/discharge from hospital/attainment of 50% oral feedings	gain in weight (g/Kg/day): HM100 vs BOV: 14.2 (11.9, 15.8) vs vs15.1 (12.8,17) @ ¥ Gain in length (cm/wk): HM100 vs BOV: 0.86 (0.72,1.08) vs 0.94 (0.72,1.16) @ ¥ Gain in head circumference (cm/wk) HM100 vs BOV: 0.76 (0.62,0.85)vs 0.75 (0.62,0.86) @ ¥	NEC: 3/67 vs 11/69# NEC requiring surgery: 1/67 vs 7/69# Mortality: 1/67 vs 5/69#	The use of exclusive human milk based diet in extremely preterm infants is associated with reduced rates of NEC and surgical NEC	Advocates the use of newer technology to use exclusive human milk based diet.

6	O'Connor 2018(28)	preterm infants with birth weight <1250g (*n*=125)	human milk with added fortifier; human milk based fortifier (HMBF) (n=64) vs bovine milk based fortifier (BBF) (n=61) when 100ml/Kg/day feed was reached and continued until infants were 84 days of age/discharge/when they consumed ≥2 complete oral feeds daily over 3 days which ever was first	feeding interruption[n,(%)]:20/61 (32.8%) 17/64(26.6%) ,RD:-6.2 (-22.2,9.8) [¥] Weight(g) adjusted effect: 1124 (960,1065) vs 1303(1150,1456) [@¥] length: 7.3 (6.3,8.3) vs 8.1 (7.1,9.2) [@¥] head circumference(cm): 6.2 (5.5,6.8) vs 6.8 (6.1,7.4) [@¥]	mortality and morbidity index: 31/64 (48.4%) vs 30/61 (49.2%), RD: -0.7 (-18.3,16.8) [¥] Death: 3/64 (4.7%) vs 4/61 (6.6%), RD=-1.9 (-10.0,6.2) [¥] Late onset sepsis: 8/64 (12.5%) vs 14/61 (23%), RD=-10.5 (-23.8, 2.9) [¥] NEC all stages : 3/64 (4.7%) vs 6/61 (9.8%), RD=-5.2 (-14.2,3.9) [¥] NEC ≥stage 2: 3/64 (4.7%) vs 3/61 (4.9%),RD=-0.2 (-7.7,7.3) [¥] Severe ROP: 1/62 (1.6%) vs 6/59(10.2%),RD=-8.6 (-16.9,-0.02) [#] Severe brain injury: 111/64(17.2%) vs 8/61 (13.1%), RD=4.1(-8.5,16.6) BPD: 16/64(25%) vs 18/61 (29.5%),RD =-4.5(-20.1,11.1) [¥]	The use of human milk based fortifier did not improve feeding tolerance or reduce mortality and morbidity compared to bovine milk based fortifier.	Routine use of human milk based fortifier over bovine milk based fortifier not recommended

[1] Bov: bovine; BPD: bronchopulmonary dysplasia; CLD: Chronic lung disease; CI: Confidence interval; ELBW: Extremely low birth weight;HM: human milk; LOS: Late onset sepsis; NEC: Necrotizing enterocolitis; PDA: patent ductus artereosus; RD: risk difference; rhEPO: recombinant erythropoietin; ROP: Retinopathy of prematurity;

[2, 3] for these columns,* mean±SD; ^mean (range); [@] median (25th centile, 75th centile)) [#] *P*<0.01; [¥]*P*=NS (not significant)

Supplemental Table 2^: Sensitivity analysis

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Item	No: of studies	Sample size	RR (95% CI REM)	I^2 statistic (%)
Definite NEC				
low ROB on random sequence generation	2(O' Connor 2018, Sullivan 2010)	261	0·47 (0·14,1.54)	31
low ROB on allocation concealment	1(O' Connor 2018)	125	0·95 (0·20,4.54)	NA
low ROB on blinding	2 (O' Connor 2018, Cristofalo 2013)	178	0·46 (0·08,2.52)	44
HMF supplementation till discharge	3 (O' Connor 2018, Cristofalo 2013, Sullivan 2010)	314	**0·38 (0·15,0.95)**	9
Minimal or no industry bias	2 (O'Connor 2018, Hagelberg 1990)	145	0.95 (0.20,4.54)	NA
Surgical NEC				
low ROB on random sequence generation	1 (Sullivan 2010)	136	0.15 (0.02, 1.16)	NA
low ROB on allocation concealment	0	0	NA	NA
low ROB on blinding	1 (Cristofalo 2013)	53	0.09 (0.01, 1.64)	NA
HMF supplementation till discharge	2 (Cristofalo 2013, Sullivan 2010)	189	**0.13 (0.02, 0.67)**	0
Minimal or no industry bias	1 (Hagelberg 1990)	20	Not estimable	NA
mortality				
low ROB on random sequence generation	2 (O'Connor 2018, Sullivan 2010)	261	0.48 (0.14,1.59)	0
low ROB on allocation concealment	1 (O'Connor 2018)	125	0.71(0.17, 3.06)	NA
low ROB on blinding	2 (O'Connor 2018, Cristofalo 2013)	178	0.54 (0.15,2.00)	0
HMF supplementation till discharge	3 (O' Connor 2018, Cristofalo 2013, Sullivan 2010)	314	0.41 (0.14, 1.26)	0
Minimal or no industry bias	2 (O'Connor 2018, Hagelberg 1990)	145	0.62 (0.17, 2.32)	0

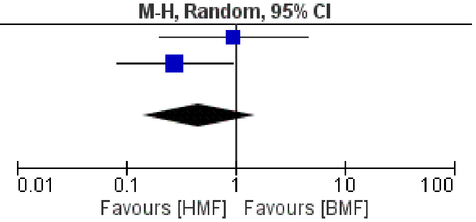
^ Definite NEC and surgical NEC are significantly decreased in HMF group vs BMF group whnen HMF is supplemented till discharge

Supplemental Figure 1: sensitivity analysis (Definite NEC)

1a) Low ROB on random sequence generation

	HMF		BMF			Risk Ratio	Risk Ratio
Study or Subgroup	**Events**	**Total**	**Events**	**Total**	**Weight**	**M-H, Random, 95% CI**	**M-H, Random, 95% CI**
O'Connor 2018	3	64	3	61	42.0%	0.95 [0.20, 4.54]	
Sullivan 2010	3	67	11	69	58.0%	0.28 [0.08, 0.96]	
Total (95% CI)		**131**		**130**	**100.0%**	**0.47 [0.14, 1.54]**	
Total events	6		14				

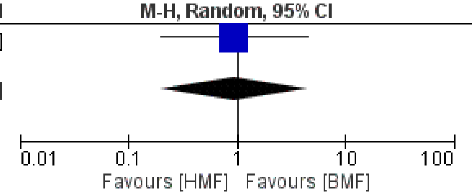
Heterogeneity: Tau² = 0.24; Chi² = 1.46, df = 1 (P = 0.23); I² = 31%
Test for overall effect: Z = 1.25 (P = 0.21)



1b) Low ROB on allocation concealment

	HMF		BMF			Risk Ratio	Risk Ratio
Study or Subgroup	**Events**	**Total**	**Events**	**Total**	**Weight**	**M-H, Random, 95% CI**	**M-H, Random, 95% CI**
O'Connor 2018	3	64	3	61	100.0%	0.95 [0.20, 4.54]	
Total (95% CI)		**64**		**61**	**100.0%**	**0.95 [0.20, 4.54]**	
Total events	3		3				

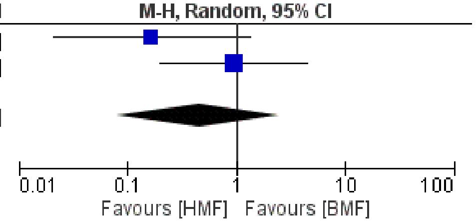
Heterogeneity: Not applicable
Test for overall effect: Z = 0.06 (P = 0.95)



1c) Low ROB on blinding

	HMF		BMF			Risk Ratio	Risk Ratio
Study or Subgroup	**Events**	**Total**	**Events**	**Total**	**Weight**	**M-H, Random, 95% CI**	**M-H, Random, 95% CI**
Cristofalo 2013	1	29	5	24	42.2%	0.17 [0.02, 1.32]	
O'Connor 2018	3	64	3	61	57.8%	0.95 [0.20, 4.54]	
Total (95% CI)		**93**		**85**	**100.0%**	**0.46 [0.08, 2.52]**	
Total events	4		8				

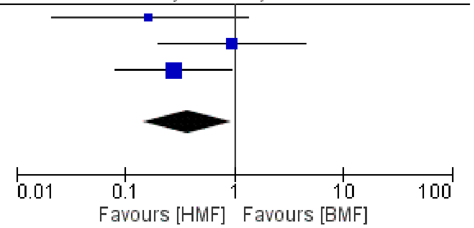
Heterogeneity: Tau² = 0.69; Chi² = 1.78, df = 1 (P = 0.18); I² = 44%
Test for overall effect: Z = 0.90 (P = 0.37)



1d) HMF supplementation till discharge

Study or Subgroup	HMF Events	HMF Total	BMF Events	BMF Total	Weight	Risk Ratio M-H, Random, 95% CI
Cristofalo 2013	1	29	5	24	18.9%	0.17 [0.02, 1.32]
O'Connor 2018	3	64	3	61	32.1%	0.95 [0.20, 4.54]
Sullivan 2010	3	67	11	69	49.0%	0.28 [0.08, 0.96]
Total (95% CI)		**160**		**154**	**100.0%**	**0.38 [0.15, 0.95]**
Total events	7		19			

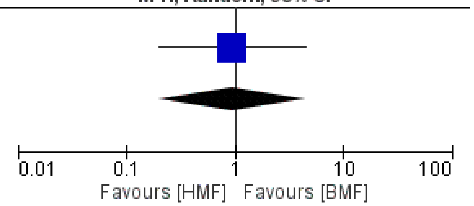
Heterogeneity: Tau² = 0.06; Chi² = 2.19, df = 2 (P = 0.33); I² = 9%
Test for overall effect: Z = 2.06 (P = 0.04)



1e) Minimal or no industry bias

Study or Subgroup	HMF Events	HMF Total	BMF Events	BMF Total	Weight	Risk Ratio M-H, Random, 95% CI
Hagelberg 1990	0	10	0	10		Not estimable
O'Connor 2018	3	64	3	61	100.0%	0.95 [0.20, 4.54]
Total (95% CI)		**74**		**71**	**100.0%**	**0.95 [0.20, 4.54]**
Total events	3		3			

Heterogeneity: Not applicable
Test for overall effect: Z = 0.06 (P = 0.95)

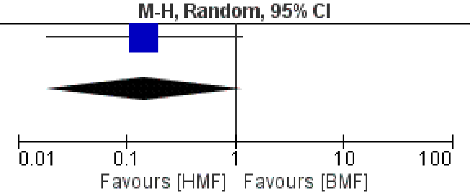


Supplemental Figure 2: sensitivity analysis (Surgical NEC)

2a) Low ROB on random sequence generation

Study or Subgroup	HMF Events	HMF Total	BMF Events	BMF Total	Weight	Risk Ratio M-H, Random, 95% CI	Risk Ratio M-H, Random, 95% CI
Sullivan 2010	1	67	7	69	100.0%	0.15 [0.02, 1.16]	
Total (95% CI)		**67**		**69**	**100.0%**	**0.15 [0.02, 1.16]**	
Total events	1		7				

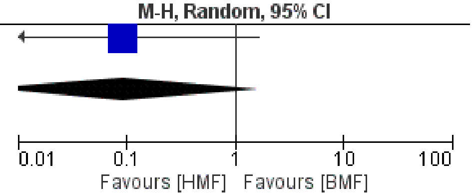
Heterogeneity: Not applicable
Test for overall effect: Z = 1.82 (P = 0.07)



2b) Low ROB on blinding

Study or Subgroup	HMF Events	HMF Total	BMF Events	BMF Total	Weight	Risk Ratio M-H, Random, 95% CI	Risk Ratio M-H, Random, 95% CI
Cristofalo 2013	0	29	4	24	100.0%	0.09 [0.01, 1.64]	
Total (95% CI)		**29**		**24**	**100.0%**	**0.09 [0.01, 1.64]**	
Total events	0		4				

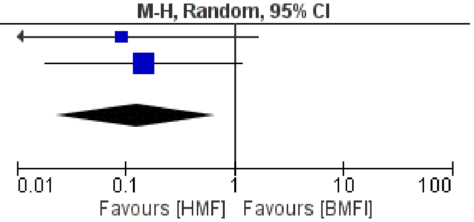
Heterogeneity: Not applicable
Test for overall effect: Z = 1.62 (P = 0.10)



2c) HMF supplementation till discharge

Study or Subgroup	HMF Events	HMF Total	BMF Events	BMF Total	Weight	Risk Ratio M-H, Random, 95% CI	Risk Ratio M-H, Random, 95% CI
Cristofalo 2013	0	29	4	24	34.1%	0.09 [0.01, 1.64]	
Sullivan 2010	1	67	7	69	65.9%	0.15 [0.02, 1.16]	
Total (95% CI)		**96**		**93**	**100.0%**	**0.13 [0.02, 0.67]**	
Total events	1		11				

Heterogeneity: Tau² = 0.00; Chi² = 0.07, df = 1 (P = 0.80); I² = 0%
Test for overall effect: Z = 2.42 (P = 0.02)

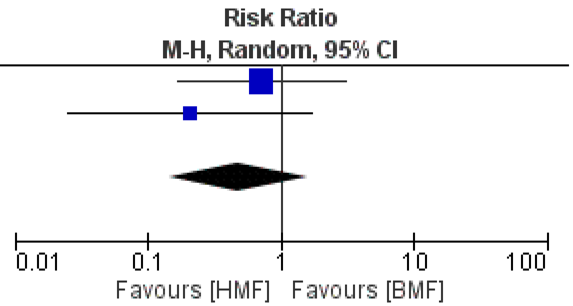


Supplemental Figure 3: sensitivity analysis (mortality)

3a) Low ROB on random sequence generation

Study or Subgroup	HMF Events	HMF Total	BMF Events	BMF Total	Weight	Risk Ratio M-H, Random, 95% CI
O'Connor 2018	3	64	4	61	68.0%	0.71 [0.17, 3.06]
Sullivan 2010	1	67	5	69	32.0%	0.21 [0.02, 1.72]
Total (95% CI)		**131**		**130**	**100.0%**	**0.48 [0.14, 1.59]**
Total events	4		9			

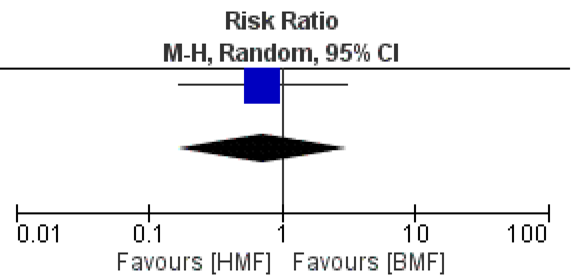
Heterogeneity: Tau² = 0.00; Chi² = 0.92, df = 1 (P = 0.34); I² = 0%
Test for overall effect: Z = 1.20 (P = 0.23)



3b) Low ROB on allocation concealment

Study or Subgroup	HMF Events	HMF Total	BMF Events	BMF Total	Weight	Risk Ratio M-H, Random, 95% CI
O'Connor 2018	3	64	4	61	100.0%	0.71 [0.17, 3.06]
Total (95% CI)		**64**		**61**	**100.0%**	**0.71 [0.17, 3.06]**
Total events	3		4			

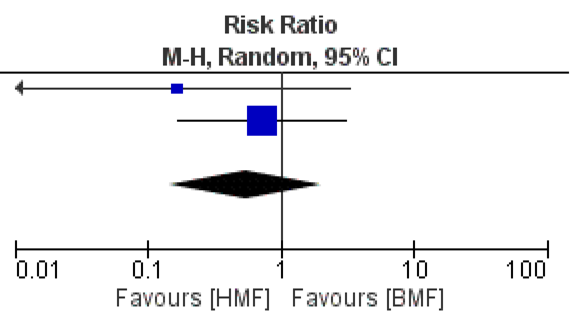
Heterogeneity: Not applicable
Test for overall effect: Z = 0.45 (P = 0.65)



3c) Low ROB on blinding

Study or Subgroup	HMF Events	HMF Total	BMF Events	BMF Total	Weight	Risk Ratio M-H, Random, 95% CI
Cristofalo 2013	0	29	2	24	19.2%	0.17 [0.01, 3.31]
O'Connor 2018	3	64	4	61	80.8%	0.71 [0.17, 3.06]
Total (95% CI)		**93**		**85**	**100.0%**	**0.54 [0.15, 2.00]**
Total events	3		6			

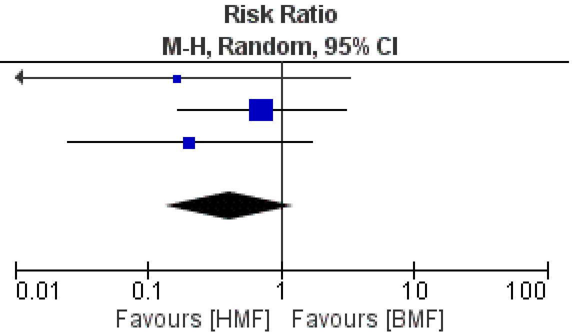
Heterogeneity: Tau² = 0.00; Chi² = 0.75, df = 1 (P = 0.39); I² = 0%
Test for overall effect: Z = 0.92 (P = 0.36)



3d) HMF supplementation till discharge

Study or Subgroup	HMF Events	HMF Total	BMF Events	BMF Total	Weight	Risk Ratio M-H, Random, 95% CI
Cristofalo 2013	0	29	2	24	13.9%	0.17 [0.01, 3.31]
O'Connor 2018	3	64	4	61	58.6%	0.71 [0.17, 3.06]
Sullivan 2010	1	67	5	69	27.6%	0.21 [0.02, 1.72]
Total (95% CI)		**160**		**154**	**100.0%**	**0.41 [0.14, 1.26]**
Total events	4		11			

Heterogeneity: Tau² = 0.00; Chi² = 1.35, df = 2 (P = 0.51); I² = 0%
Test for overall effect: Z = 1.55 (P = 0.12)



3e) Minimal or no industry bias

Study or Subgroup	HMF Events	HMF Total	BMF Events	BMF Total	Weight	Risk Ratio M-H, Random, 95% CI
Hagelberg 1990	0	10	1	10	18.2%	0.33 [0.02, 7.32]
O'Connor 2018	3	64	4	61	81.8%	0.71 [0.17, 3.06]
Total (95% CI)		**74**		**71**	**100.0%**	**0.62 [0.17, 2.32]**
Total events	3		5			

Heterogeneity: Tau² = 0.00; Chi² = 0.19, df = 1 (P = 0.66); I² = 0%
Test for overall effect: Z = 0.71 (P = 0.48)