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Nutrition

How does bovine milk-based fortification alter the oxidant–antioxidant profile of breast milk in preterm infants?

Ozge Aydemir[1] | **Yusuf Aydemir**[2] | **Ozge Surmeli Onay**[1]

[1]Division of Neonatology, Department of Pediatrics, Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, Turkey

[2]Division of Gastroenterology, Hepatology and Nutrition, Department of Pediatrics, Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, Turkey

Correspondence

Yusuf Aydemir, Division of Gastroenterology, Hepatology and Nutrition, Department of Pediatrics, Faculty of Medicine, Eskisehir Osmangazi University, Meselik, 26040 Eskisehir, Turkey.
Email: dryusufaydemir@yahoo.com

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Abstract

Objectives: Fortification of breast milk (BM) is recommended to enhance protein, vitamin, and mineral content, supporting improved growth in preterm infants. However, the impact of fortification on the oxidant–antioxidant balance in BM has not been previously studied. This study aims to evaluate the effects of fortification with a bovine milk-based fortifier on the total antioxidant capacity (TAC) and total oxidant status (TOS) in preterm BM.

Methods: In this prospective cohort study, transitional milk (TM) (6–10 days postpartum) and mature milk (MM) (>15 days postpartum) samples were collected from mothers of preterm infants receiving fortified BM. TAC and TOS were measured in BM samples before and after fortification. The oxidative stress index (OSI), defined as the TOS-to-TAC ratio, was used to assess oxidative stress levels.

Results: Seventy-five BM samples from 59 preterm infants, with a mean gestational age of 31.4 ± 2.8 weeks, were analyzed. TAC levels were consistent between TM and MM of the preterm infants. TOS levels and OSI were lower in TM compared to MM ($p = 0.019$ and $p = 0.033$, respectively). Fortification led to increased TAC and TOS in both TM ($p < 0.001$ each) and MM ($p < 0.001$ each). The OSI was higher in fortified TM ($p = 0.032$) compared to unfortified TM, while OSI remained unchanged in fortified MM ($p = 0.39$).

Conclusions: Preterm TM exhibits a more favorable oxidant–antioxidant profile compared to MM. Fortification elevates both TAC and TOS in preterm BM. In MM, the oxidant–antioxidant balance is maintained post-fortification; however, in TM, the increase in TOS exceeds that of TAC, resulting in a higher OSI.

KEYWORDS

bovine milk-based fortifier, oxidative stress index, total antioxidant capacity, total oxidant status

1 | INTRODUCTION

Reactive oxygen species (ROS) are generated as a by-product of cellular metabolism involving carbohydrates and lipids for energy production. These molecules are highly reactive and have the potential to cause damage to lipids, proteins, polysaccharides, and DNA. Under normal physiological conditions, antioxidants typically mitigate the detrimental effects of ROS. Oxidative stress (OS) is characterized by an imbalance between the levels of ROS and the protective mechanisms provided by antioxidants.[1]

The fast transition from intrauterine to relatively hyperoxic extrauterine environment exposes newborns to OS at birth. Preterm newborns are particularly vulnerable to OS because of their immature antioxidant defence mechanisms.[2] In preterm neonates, OS plays a role in the development of various pathological conditions and diseases, including respiratory distress syndrome (RDS), bronchopulmonary dysplasia (BPD), retinopathy of prematurity (ROP), necrotizing enterocolitis (NEC), periventricular leukomalacia (PVL), and intraventricular hemorrhage (IVH).[3,4]

[Correction added on 26 April 2025, after the first online publication: Headings have been updated.]

Breast milk (BM) serves not only as an optimal source of nutrients for growth and development but also provides several antioxidant constituents such as superoxide dismutase, glutathione peroxidase, catalase, vitamin E, vitamin C, and β-carotene which may protect newborns against OS at the early stage of life[5,6] Early in lactation, the BM of mothers delivering prematurely contains higher levels of protein and many bioactive molecules, which correspond to the increased requirements of preterm newborns.[7,8] However, these levels decrease in the first few weeks after delivery.[9] Fortification of preterm BM is recommended to provide additional protein, vitamins and minerals and improve growth in preterm infants. The ideal timing for the introduction of fortification is unknown and there is a wide variety in clinical practice.[10]

The effects of fortification on the oxidant–antioxidant profile of BM have not been studied to date. We aimed to evaluate the changes in oxidant–antioxidant balance of preterm BM after fortification with a commercially available bovine milk-based fortifier by measuring total antioxidant capacity (TAC) and total oxidant status (TOS).

What is Known

- The oxidant–antioxidant profile of preterms breast milk is different than term breast milk.
- It is known that the oxidant–antioxidant profile of breast milk is influenced by the mother's dietary habits and smoking, while the profile of stored milk is affected by storage conditions.

What is New

- During the transitional period of lactation, preterm breast milk shows a more favorable oxidant–antioxidant profile compared to the mature milk stage.
- After fortification, the oxidant–antioxidant balance remains stable in mature milk. However, in transitional milk, the increase in total oxidant status (TOS) surpasses the rise in total antioxidant capacity (TAC), leading to a higher oxidative stress index (OSI).

2 | METHODS

2.1 | Ethics statement

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Eskisehir Osmangazi University (Date:29.09.2020/No:45). Informed consent was obtained from the participants before recruitment.

2.1.1 | Study design

A single-center, prospective, and cohort study were conducted.

2.2 | Primary and secondary endpoints

The primary endpoint of the study was to demonstrate the changes in the TAC and TOS of preterm BM following fortification. The secondary endpoint was to assess the changes in the oxidant–antioxidant profile of BM through the transitional and mature milk stages of lactation and its relation to gestational age, perinatal factors and OS-related diseases of prematurity.

2.3 | Setting

The study was conducted in Level-III Neonatal Intensive Care Unit (NICU) of Eskisehir Osmangazi University Hospital, Eskisehir, Türkiye, between January 1, 2021 and December 31, 2021. Our hospital had baby-friendly hospital certificate. According to our NICU enteral nutrition protocol, the first choice was BM and the second choice was preterm infant formula in case of complete absence or inadequate amount of maternal BM. Minimal enteral nutrition was initiated on the first day of life at a volume of 10–20 mL/kg/day in infants with a birth weight of <1500 g and 20–40 mL/kg/day in infants with a birth weight of ≥1500 g. The enteral nutrition volume was increased by 10–15 mL/kg/day in infants with a birth weight of <1500 g and by 20–40 mL/kg/day in infants with a birth weight of ≥1500 g, as tolerated, until reaching a final volume of 150–180 mL/kg/day. All infants received enteral nutrition every 3 h (×8/day). Total parenteral nutrition was initiated according to nursery protocol in very low birth weight (<1500 g) infants and in those for whom enteral nutrition was not sufficient to achieve optimal energy supply. According to our unit's feeding protocol, the BM of infants with a birth weight of less than 1500 g or a birth weight between 1500 and 2000 g with inadequate weight gain is enriched with a BM fortifier to enhance mineral, vitamin, and protein content. Fortification started when the daily BM intake of infants monitored in our unit exceeds 80 mL/kg/day.

2.4 | Subjects

Inborn preterm infants admitted to our NICU and fed predominantly with fortified BM (more than 50% of the feeding volume) were included in the study. Mothers of infants with a birth weight of less than 1500 g were

invited to participate in the study on the first day of life, and mothers of infants with a birth weight between 1500 and 2000 g with inadequate weight gain were invited as soon as fortification was started. Inadequate weight gain was defined as either failure to regain birth weight by 10–14 days of age or gaining less than the expected 15–20 g/kg/day after the initial physiological weight loss period. Mothers without sufficient BM production for infant nutrition, mothers with history of smoking, substance or drug abuse, and infectious and inflammatory diseases were excluded from study.

2.5 | Demographics and clinical data

Maternal demographic characteristics including age, maternal morbidities (gestational hypertension, gestational diabetes mellitus, and chorioamnionitis), and antenatal steroid administration were noted on the data collection form. Natal data including gestational age, gender, birth weight, cesarean delivery, and 5-th min Apgar score were recorded. Postnatal clinical morbidities, including OS-related diseases of prematurity such BPD, ROP, NEC, PVL, and IVH were assessed. For defining patients with BPD, we used the criteria established by the NIH workshop.[11] Patients with any stage of ROP and those with severe ROP, classified as type 1 ROP according to the Early Treatment for Retinopathy of Prematurity (ETROP) study, were recorded.[12] Infants with Stage 2 and 3 diseases according to Modified Bell Staging are defined as NEC.[13] Infants with IVH ≥ Stage 2 according to Volpe criteria were recorded.[14]

2.6 | Oxidant–antioxidant profile analysis of BM

Mothers' BM was collected for TAC and TOS measurement before and after fortification during the transitional milk (TM) (6–10 days postpartum) and mature milk (MM) (>15 days postpartum) stages of lactation. For BM collection, one breast of the mother was pumped using an electric pump for at least 20 min, and only 2 mL of the pumped milk was taken. The remaining pumped milk was used for feeding the infant.

All samples were analyzed within 3 months of collection and kept at −80°C until analysis. After thawing, the samples were centrifuged for 15 min at a speed of 15,000 rpm and supernatants were taken for analysis. TAC and TOS were measured before and after addition of BM fortifier (Aptamil Eoprotin®) with colorimetric and automated methods described by Erel[15,16] (Rel Assay Diagnostics TAC and TOS assay kits, Mega Tip, Gaziantep, Türkiye) using Mindray BS 400 clinical chemistry analyser (Shenzhen Mindray Bio-Medical Electronics Co. Ltd., Shenzhen, China). Erel's TAC method is based on the bleaching of the characteristic color of a more stable 2.20-azino-bis (3-ethylbenz-thiazoline-6-sulfonicacid) radical cation by antioxidants. Relative antioxidant activities of individual antioxidants and their estimated contributions to TAC are free sulfhydryl groups of proteins (52.9%), uric acid (33.1%), vitamin C (4.7%), total bilirubin (2.4%), vitamin E (1.7%), and others (5.2%). Results were expressed in mmol of Trolox equivalent per liter. Erel's TOS method is based on the oxidation of ferrous ion to ferric ion in the presence of various oxidative species in acidic medium and the measurement of the ferric ion by xylenol orange. Main components of TOS are hydrogen peroxide and lipid hydroperoxide. The results were expressed in mmol H_2O_2/L. The TOS-to-TAC ratio was defined as the oxidative stress index (OSI), an indicator of the degree of OS.

2.7 | Data analysis

The sample size was calculated to be at least 36 patients, with 80% power to detect a mean change of 1.50 (SD 0.75) Trolox equivalent/L in TAC levels after the supplementation of BM in preterm infants. Sample size calculation was based on data reported in previous research articles by Deniz et al.[17] and Turhan et al.[18] regarding TAC levels in preterm BM. Statistical analyses were performed using SPSS version 21.0 (Armonk, NY: IBM Corp). The results are presented as number (*n*), frequency (%), mean with the respective standard deviation, and median with minimum and maximum values. For comparison of categorical variables between the groups, Chi-square test or Fisher's exact test was used. Continuous variables with normal distribution were compared using the Student's *t*-test, while those without normal distribution were compared using the Mann–Whitney *U*-test between two independent groups. For comparisons of dependent groups at different time points, two-way repeated measures analysis of variance or Wilcoxon's rank test was used. Correlation analyses were performed using Spearman's rank correlation test. Statistical significance was considered at $p < 0.05$.

3 | RESULTS

Seventy-five BM samples (40 TM and 35 MM) were collected from mothers of 59 preterm infants. During the study period, we excluded five mothers due to insufficient BM production, two mothers due to smoking habits, and three mothers due to infectious and inflammatory conditions (one with systemic lupus erythematosus, one with mastitis, and one with pneumonia). The mean gestational age of the infants was 31.4 ± 2.8 weeks, and the mean birth weight was

TABLE 1 Characteristics of the study population.

Features	Values
Maternal age*	32.4 ± 2.8
Gestational diabetes, *n* (%)	10 (16.9)
Gestational hypertension, *n* (%)	10 (16.9)
Chorioamnionitis, *n* (%)	0
Antenatal steroids given, *n* (%)	27 (45.8)
Gestational age,* week	31.4 ± 2.8
Birth weight,* g	1650 ± 627
Gender (F/M)	29/30
Cesarean delivery, *n* (%)	50 (84.7)
5-min Apgar score*	8 ± 1.4
Exclusively fed with BM, *n* (%)	29 (49.2)
Enteral feeding started,* day of life initiation of first feeding, (day of life)	2.74 ± 2.33
Respiratory distress syndrome, *n* (%)	24 (40.7)
Any intraventricular hemorrhage, *n* (%)	9 (15.3)
Intraventricular hemorrhage ≥ Stage 2, *n* (%)	3 (5)
Periventricular leukomalacia, *n* (%)	1 (1.7)
Necrotizing enterocolitis ≥ Stage 2, *n* (%)	4 (6.8)
Any retinopathy of prematurity, *n* (%)	9 (15.3)
Severe retinopathy of prematurity, *n* (%)	1 (1.7)
Bronchopulmonary dysplasia, *n* (%)	14 (23.7)

Abbreviation: BM, breast milk.
*Mean ± standard deviation.

1650 ± 627 g. Detailed data, including demographic and clinical characteristics of the mothers and infants, are provided in Table 1. TAC levels were similar in TM [1.00 (0.48–1.75) mmol Trolox equivalent/L] and MM [0.88 (0.50–1.43) mmol Trolox equivalent/L] ($p = 0.623$). In contrast, TOS levels were lower in TM [1.56 (0.14–4.97) μmol H_2O_2/L] compared to MM [1.74 (0.04–7.99) μmol H_2O_2/L] ($p = 0.019$). OSI was also lower in TM [0.128 (0.013–0.507) AU] compared with MM [0.192 (0.004–1.095) AU] ($p = 0.033$).

In both TM and MM of preterm infants, TAC, TOS, and OSI did not show significant linear correlations with gestational age. However, when comparing BM from extremely preterm infants (<28 weeks gestational age) to BM from infants born at >28 weeks gestational age, we observed notable differences. While TAC levels were similar between the groups, BM samples from extremely preterm infants exhibited considerably lower TOS levels in TM [0.48 (0.20–3.39) μmol H_2O_2/L vs. 1.72 (0.14–4.97) μmol H_2O_2/L, $p = 0.038$]. Similar differences were observed for OSI levels: TM-OSI [0.049 (0.019–0.257) AU vs. 0.144 (0.013–0.507) AU, $p = 0.018$]. In MM samples, TOS and OSI were also lower in extremely preterm infants, although these differences were not statistically significant. Table 2 presents TAC, TOS, and OSI values in TM and MM according to gestational age.

No association was found between TAC and TOS levels in BM and maternal age, antenatal factors (such as hypertension, pre-eclampsia, gestational diabetes, or antenatal steroid use), birth weight, gender, or mode of delivery.

After fortification with a human milk fortifier, TAC levels increased significantly in both TM and MM. The comparison of basal and fortified levels was as follows: TM [1.00 (0.48–1.75) mmol Trolox eq/L vs. 2.69 (1.34–3.52) mmol Trolox eq/L, $p < 0.001$] and MM [0.88 (0.50–1.43) mmol Trolox eq/L vs. 3.09 (1.44–3.64) mmol Trolox eq/L, $p < 0.001$] (Figure 1A). TOS also increased in both TM [1.56 (0.14–4.97) μmol H_2O_2/L vs. 5.17 (0.56–15.47) μmol H_2O_2/L, $p < 0.001$] and MM [1.74 (0.04–7.99) μmol H_2O_2/L vs. 7.09 (1.79–14.07) μmol H_2O_2/L, $p < 0.001$] (Figure 1B). The OSI was higher in fortified TM compared to unfortified TM [0.167 (0.061–1.154) AU vs. 0.115 (0.022–0.448) AU, $p = 0.032$], but did not change significantly in MM after fortification [0.234 (0.054–0.450) AU vs. 0.252 (0.074–0.779) AU, $p = 0.39$] (Figure 1C).

When we assessed TAC and TOS levels based on morbidity to better understand their association with OS-related preterm disorders, we found the following: Infants with BPD had lower TOS levels in both transitional milk (TM) [0.48 (0.20–3.61) μmol H_2O_2/L vs. 1.72 (0.14–4.97) μmol H_2O_2/L, $p = 0.022$] and mature milk (MM) [0.81 (0.22–4.06) μmol H_2O_2/L vs. 2.12 (0.04–7.99) μmol H_2O_2/L, $p = 0.023$], and lower OSI in TM [0.049 (0.019–0.215) AU vs. 0.149 (0.013–0.507) AU, $p = 0.006$] compared to infants without BPD. Among infants with NEC, TOS levels in MM were higher compared to those in infants without NEC [10.37 ± 0.84 μmol H_2O_2/L vs. 6.59 ± 0.514 μmol H_2O_2/L, $p = 0.011$]. After fortification, TAC, TOS, and OSI levels in both TM and MM were similar among infants with and without NEC and BPD. Infants with and without ROP had similar TAC and TOS levels in BM both before and after fortification. Table 3 demonstrates oxidant-antioxidant profile of TM and MM in relation to OS-related diseases of prematurity.

4 | DISCUSSION

The evidence supporting the use of BM for preterm infants is strong, offering substantial nutritional, anti-infective, and antioxidant health benefits in both the short and long term.[19] Providing BM to preterm infants has been shown to reduce the risk of NEC and contribute to sustained improvements in neurodevelopmental outcomes.[20,21] BM contains several antioxidant

TABLE 2 Transitional and mature breast milk oxidant-antioxidant profile according to gestational age.

Gestational age	TM-TAC	*p*-Value	TM-TOS	*p*-Value	TM-OSI	*p*-Value
≤28, week	1.08 (0.86–1.32)	0.225	0.48 (0.20–3.39)	0.038	0.049 (0.019–0.257)	
>28, week	0.97 (0.48–1.75)		1.72 (0.14–4.97)		0.144 (0.013–0.507)	**0.018**
	MM-TAC	***p*-Value**	**MM-TOS**	***p*-Value**	**MM-OSI**	***p*-Value**
≤28, week	0.86 (0.51–1.20)		0.94 (0.04–6.70)		0.121 (0.004–0.779)	
>28, week	0.91 (0.50–1.43)	0.294	2.02 (0.22–7.99)	0.118	0.217 (0.025–1.095)	0.088

Note: Values are given as medians with minimum and maximum values.

Abbreviations: MM-OSI, mature milk-oxidative stress index; MM-TAC, mature milk-total antioxidant capacity; MM-TOS, mature milk-total oxidant status; TM-OSI, transitional milk-oxidative stress index; TM-TAC, transitional milk-total antioxidant capacity; TM-TOS, transitional milk-total oxidant status.

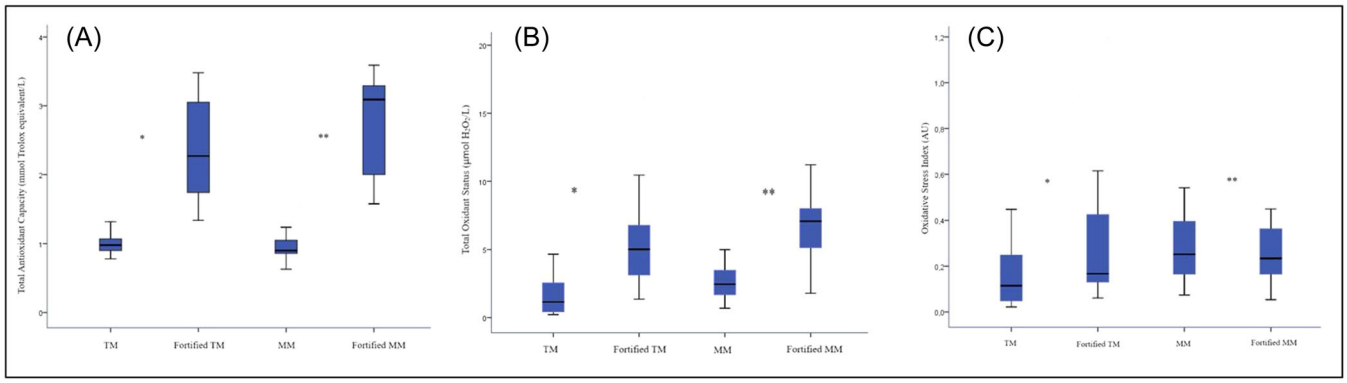

FIGURE 1 (A) Changes in total antioxidant capacity in transitional and mature preterm breast milk after fortification. (B) Changes in total oxidant status in transitional and mature preterm breast milk after fortification. (C) Changes oxidative stress index in transitional and mature preterm breast milk after fortification. In the boxplot graphics, central horizontal line is the median, box borders represent the interquartile range and whiskers represent the 5th and 95th percentiles. *$p < 0.001$ and **$p < 0.001$.

molecules and offers better antioxidant protection than formula feeding.[22] It is more effective at scavenging free radicals and exhibits less oxidation when subjected to physiological stress compared to formula.[23] The milk produced by mothers who give birth before their due date contains higher levels of protein and many bioactive molecules, which align with the increased needs of preterm neonates.[7,8] The antioxidant-oxidant properties of preterm BM and how they change throughout the stages of lactation have been areas of research interest. However, many of the studies face methodological limitations and have reported heterogeneous results. Only a small number of studies have evaluated oxidants alongside antioxidants and commented on their balance.[17,23–25] In a previous study, Deniz et al.[17] compared TAC and TOS of BM in term and preterm infants born before 34 weeks gestation. Consistent with previous literature,[26–28] they found that colostrum had the highest TAC levels, which decreased in the later stages of lactation in term BM. However, in preterm BM, TAC, which was significantly lower than in term BM during the colostrum stage, remained relatively constant throughout the different stages of lactation and was similar to term BM in the mature milk stage. TOS levels were lower in preterm BM compared to term BM across colostrum, transitional, and mature milk stages. Consequently, OSI was lower in preterm BM than in term BM, with statistical significance observed only during the transitional milk stage. In line with these findings, the present study observed lower TOS and OSI in TM, suggesting that TM has a better antioxidant-oxidant profile compared to MM in preterm infants. Additionally, in BM of extremely preterm infants TOS and OSI were lower in TM compared to older infants. This finding supports the idea that BM, especially during the transitional stage of lactation, offers a better antioxidant-oxidant balance for extremely preterm infants, who are more vulnerable to OS-related damage. BM that naturally provides protection seems to do the same for itself.

In early stages of lactation, preterm BM contains high levels of protein, fatty acids, and minerals. However, in later stages, preterm BM becomes similar in composition to term BM and may not provide sufficient amounts of protein, sodium, phosphate, or calcium to meet the specific needs of preterm infants.[9] The use of commercially produced fortifiers is recommended to augment BM for preterm infants, providing additional

TABLE 3 Oxidative stress-related diseases of prematurity and oxidant-antioxidant profile of transitional and mature breast milk.

	BPD			NEC ≥ Stage 2			Any ROP		
	+	-	*p*-Value	+	-	*p*-Value	+	-	*p*-Value
TM-TAC	0.98 (0.80–1.68)	1.00 (0.48–1.75)	0.816	1.05 (0.86–1.23)	1.00 (0.48–1.75)	1	0.98 (0.86–1.32)	1.00 (0.48-1.75)	0.754
TM-TOS	0.48 (0.20–3.61)	1.72 (0.14–4.97)	**0.022**	0.99 (0.42–1.57)	1.56 (0.14–4.97)	0.444	0.87 (0.22–3.39)	1.59 (0.14-4.66)	0.205
TM-OSI	0.049(0.019–0.21-5)	0.149 (0.013–0.507)	**0.006**	0.088 (0.049–0.128)	0.134 (0.013–0.507)	0.444	0.098 (0.022–0.257)	0.134 (0.013–0.507)	0.192
FTM-TAC	2.84 (1.54–3.4)	2.49 (1.34–3.53)	0.841	2.34 (1.75–2.94)	2.69 (1.34–3.53)	0.663	2.30 (1.54–2.98)	2.69 (1.34–3.52)	0.401
FTM-TOS	5.02 (0.56–9.04)	5.44 (1.93–15.47)	0.631	6,32 (2.98–9.66)	5.17 (0.56-15.47)	0.790	6.14 (1.36–9.66)	5.17 (0.56–15.47)	0.676
FTM-OSI	0.167 (0.035–0.425)	0.221 (0.061–1.154)	0.447	0.326 (0.101–0.552)	0.217 (0.035-1.15)	0.880	0.225 (0.07–0.552)	0.217 (0.035–1.154)	0.862
MM-TAC	0.86 (0.50–1.17)	0.90 (0.51–1.43)	0.174	0.90 (0.50–1.20)	0.88 (0.51-1.43)	0.981	0.86 (0.65–1.20)	0.89 (0.50–1.43)	0.479
MM-TOS	0.81 (0.22–4.06)	2.12 (0.04–7.99)	**0.023**	1.55 (0.22–2.46)	1.74 (0.04–7.99)	0.499	2.19 (0.53–6.70)	1.71 (0.04–7.99)	0.793
MM-OSI	0.127 (0.025–0.541)	0.198 (0.004–1.095)	0.151	0.218 (0.111–0.313)	0.192 (0.004–1.095)	0.822	0.183 (0.079–0.779)	0.194 (0.004–1.095)	0.984
FMM-TAC	3.11 (1.44–3.64)	3.07 (1.58–3.59)	0.529	3.07 (1.78–3.32)	3.1 (1.44–3.64)	0.940	3.05 (1.58–3.64)	3.12 (1.44–3.59)	0.362
FMM-TOS	7.51 (4.38–11.86)	7.09 (1.79–14.07)	0.483	10.79(8.01–11.86)	6.74 (1.79–14.07)	**0.011**	8.01 (4.55–11.86)	6.92 (1.79–14.07)	0.246
FMM-OSI	0.250 (0.044–0.441)	0.234 (0.054–0.451)	0.872	0.376 (0.044–0.450)	0.234 (0.054–0.451)	0.326	0.363 (0.136–0.450)	0.232 (0.044–0.451)	0.095

Note: Values are given as medians with minimum and maximum values.

Abbreviations: BPD, bronchopulmonary dysplasia; FMM-OSI, fortified mature milk-oxidative stress index; FMM-TAC, fortified mature milk-total antioxidant capacity; FMM-TOS, fortified mature milk-total oxidant status; FTM-OSI, fortified transitional milk-oxidative stress index; FTM-TAC, fortified transitional milk-total antioxidant capacity; FTM-TOS, fortified transitional milk-total oxidant status; MM-OSI, mature milk-oxidative stress index; MM-TAC, mature milk-total antioxidant capacity; MM-TOS, mature milk-total oxidant status; NEC, necrotizing enterocolitis; ROP, retinopathy of prematurity; TM-OSI, transitional milk-oxidative stress index; TM-TAC, transitional milk-total antioxidant capacity; TM-TOS, transitional milk-total oxidant status.

protein, calcium, phosphate, carbohydrates, as well as essential vitamins and minerals.[29] In theory, fortification could also be an option to increase the antioxidant capacity of preterm BM. Most multinutrient fortifiers contain antioxidant vitamins, which may improve the antioxidant capacity of BM. However, research on the effect of fortification on the antioxidant–oxidant properties of preterm BM is scarce, and an increase in oxidants could pose a potential risk. Friel et al.[30] investigated the effects of different feeding types on plasma and urinary oxidants in preterm infants. They compared infants fed with formula, BM, and BM supplemented with different proportions of human milk fortifier. They reported that urinary F2-isoprostanes were highest in infants who received more than 50% of their total BM as supplemented milk compared to infants who received formula or BM with a lesser proportion of fortification. The authors speculated that the inhibition of the antibacterial activity of BM by the addition of bovine protein-based HMF and improper storage conditions with subsequent bacterial growth may increase the body pool of oxidized lipids.

In the present study, we evaluated the antioxidant-oxidant balance in preterm BM after the addition of bovine milk-based HMF for the first time in the literature. We found that both TAC and TOS increased in transitional and mature BM following fortification. In preterm MM, the oxidant–antioxidant balance was preserved after fortification, as indicated by an unchanged OSI. However, in TM, TOS increased more than TAC after fortification, resulting in a higher OSI. This can partially be explained by the composition differences between transitional and mature BM. Dynamic changes occur in the lipid content of BM throughout lactation. It has been shown that most lipids decrease as the milk progresses from the colostral stage to the mature stage, with some reaching a peak at the transitional stage.[31] Lipid hydroperoxide is one of the major components of TOS measured by the method used in this study.[16] The higher lipid content of TM compared to MM makes it more prone to lipid peroxidation. This hypothesis is also supported by the study conducted by Friel et al.,[30] which reported higher urinary lipid peroxidation products in infants who were fed with BM with a higher proportion of fortification. This finding raises concerns about the ideal timing for starting fortification of preterm BM. The timing of HMF introduction for enterally fed preterm infants varies considerably, often due to a "lack of evidence and strong local tradition." The usual practice is to add fortifier to BM once a certain volume of enteral feeding is reached, which typically corresponds to the mature milk stage of lactation. The potential advantage of introducing HMF earlier is improved in-hospital growth of preterm infants. Observational studies comparing the early introduction of HMF (at enteral intakes of approximately 20–100 mL/kg/day) with later introduction (50–100 mL/kg/day) yield conflicting results, showing either improved growth or no discernible effect.[32–34] There is a concern that earlier HMF introduction might be associated with higher rates of feed intolerance, NEC, and an extended hospital stay. Despite some observational studies reporting increased gastric residuals and vomiting,[34] earlier fortification was not linked to NEC or sepsis[32,35] and did not result in an increased length of hospital stay.[33] In a meta-analysis comprising three randomized controlled trials involving 378 preterm infants, the timing of human milk fortifier (HMF) introduction was assessed. The evidence indicates that starting HMF earlier, at a volume of ≤40 mL/kg/day, compared to later, at ≥75 mL/kg/day, may have little to no impact on infant growth, feeding intolerance, sepsis, or NEC. However, these effects are highly uncertain due to imprecision and the risk of bias.[10] Additionally, none of these studies evaluated the effects of HMFs on the antioxidant-oxidant properties of BM or on the infants themselves.

In fact, our study was not well designed to investigate effects of BM's TAC, TOS, and OSI on OS-related morbidities in preterm infants. The number of subjects with these morbidities was too small to detect a statistically significant and reliable difference between groups. Due to the small number of infants with significant IVH and PVL, statistical analysis of BM TAC and TOS levels was not feasible. Additionally, TAC and TOS levels were not measured in infants themselves, acknowledging that BM is only one of the several other factors influencing the antioxidant and oxidant levels in the body. Nevertheless, our finding that TOS levels in MM were higher among infants with NEC compared to those without NEC is noteworthy, although it is difficult to interpret. OS contributes to several complications of prematurity, including NEC. Since NEC is a multifactorial disease, OS may play a role as a downstream component in its pathogenetic cascade. Maintaining the oxidant-antioxidant balance in BM could be an important strategy for NEC prevention in preterm infants, which might be supported by increasing antioxidant components in BM fortifiers.

BPD is another OS-related morbidity after preterm birth. Interestingly, we observed lower TOS both in TM and MM and lower OSI in TM, in infants with BPD compared to those without BPD. This finding aligns with our observation that infants born before 28 weeks of gestation have lower TOS and OSI in their BM, as these smallest infants constitute a large proportion of the BPD group in our study.

Our study has several limitations. First, it was constrained by a small sample size from a single center, which is insufficient to fully explore the relationship between OS-related morbidities of prematurity and the oxidant-antioxidant status of BM. A larger sample size is needed to establish a cause-and-effect relationship. The small sample size may also limit the

generalizability of our conclusions. While no infant in our study received donor BM, it is important to note that in many NICUs when maternal milk is insufficient, donor BM is often used. Donor milk is not always preterm BM and can come from women who delivered at term. Therefore, our concerns about the ideal timing for starting fortification of preterm BM may not apply to fortification practices for donor milk. Despite these limitations, the strength of our study lies in its originality; it addresses a topic that has not been previously investigated, and our findings provide valuable insights that could inform future research.

5 | CONCLUSION

In conclusion, during transitional period of lactation, BM has a better oxidant–antioxidant profile compared to the MM period in preterm infants. After fortification, the oxidant–antioxidant balance is preserved in MM. However, in TM, the increase in TOS was greater than the increase in TAC after fortification, resulting in a higher OSI. The safety of fortifying TM with respect to OS-related morbidities in preterm newborns requires further investigation. Additionally, further studies could focus on identifying the most effective strategy for increasing the TAC of fortified BM.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ORCID

Ozge Aydemir http://orcid.org/0000-0002-4106-0873
Yusuf Aydemir http://orcid.org/0000-0003-3318-2747
Ozge Surmeli Onay http://orcid.org/0000-0002-7658-3594

REFERENCES

1. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. *World Allergy Organ J*. 2012;5(1):9-19.
2. Torres-Cuevas I, Parra-Llorca A, Sánchez-Illana A, et al. Oxygen and oxidative stress in the perinatal period. *Redox Biol*. 2017;12:674-681.
3. Saugstad OD. The oxygen radical disease in neonatology. *Ind J Pediatr*. 1989;56(5):585-593.
4. Ozsurekci Y, Aykac K. Oxidative stress related diseases in newborns. *Oxid Med Cell Longevity*. 2016;2016:2768365.
5. Tsopmo A, Friel J. Human milk has Anti-Oxidant properties to protect premature infants. *Curr Pediatr Rev*. 2007;3:45-51.
6. L'Abbe MR, Friel JK, L'Abbe MR, Friel JK. Superoxide dismutase and glutathione peroxidase content of human milk from mothers of premature and full-term infants during the first 3 months of lactation. *J Pediatr Gastroenterol Nutr*. 2000;31(3):270-274.
7. Tudehope DI. Human milk and the nutritional needs of preterm infants. *J Pediatr*. 2013;162(3 suppl):S17-S25.
8. Boyce C, Watson M, Lazidis G, et al. Preterm human milk composition: a systematic literature review. *Br J Nutr*. 2016;116(6):1033-1045.
9. Zachariassen G, Fenger-Gron J, Hviid MV, Halken S. The content of macronutrients in milk from mothers of very preterm infants is highly variable. *Danish Med J*. 2013;60(6):4631.
10. Hilditch C, Keir A, Collins CT, Middleton P, Gomersall J. Early versus delayed introduction of human milk fortification in enterally fed preterm infants: a systematic review and meta-analysis. *J Paediatr Child Health*. 2022;58(1):30-38.
11. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. *Am J Respir Crit Care Med*. 2001;163(7):1723-1729.
12. Early Treatment For Retinopathy Of Prematurity Cooperative Group. Revised indications for the treatment of retinopathy of prematurity: results of the early treatment for retinopathy of prematurity randomized trial. *Arch Ophthalmol*. 2003;121(12):1684-1694.
13. Walsh MC, Kliegman RM. Necrotizing enterocolitis: treatment based on staging criteria. *Pediatr Clin North Am*. 1986;33(1):179-201.
14. Inder TE, Perlman JM, Volpe J. Preterm IVH/posthemorrhagic hydrocephalus. In: Volpe J, eds. *Volpe's Neurology of the Newborn*. Elsevier; 2018:637-698.
15. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin Biochem*. 2004;37(4):277-285. doi:10.1016/j.clinbiochem.2003.11.015
16. Erel O. A new automated colorimetric method for measuring total oxidant status. *Clin Biochem*. 2005;38(12):1103-1111.
17. Deniz A, Aydemir O, Cakil Saglik A, et al. Evaluation of total antioxidant capacity and total oxidant status of preterm and term breast milk during the course of lactation and within a nursing session. *Am J Perinatol*. 2021;38(3):258-264.
18. Turhan H, Atıcı T, Muslu T. Antioxidant capacity of breast milk of mothers who delivered prematurely is higher than that of mothers who delivered at term. *Int J Vitam Nutr Res*. 2011;81(6):368-371.
19. Schanler RJ. Outcomes of human milk-fed premature infants. *Semin Perinatol*. 2011;35(1):29-33.
20. Miller J, Tonkin E, Damarell RA, et al. A systematic review and meta-analysis of human milk feeding and morbidity in very low birth weight infants. *Nutrients*. 2018;10(6):707.
21. Isaacs EB, Fischl BR, Quinn BT, Chong WK, Gadian DG, Lucas A. Impact of breast milk on intelligence quotient, brain size, and white matter development. *Pediatr Res*. 2010;67(4):357-362.
22. Aycicek A, Erel O, Kocyigit A, Selek S, Demirkol MR. Breast milk provides better antioxidant power than does formula. *Nutrition*. 2006;22(6):616-619.
23. Friel JK, Martin SM, Langdon M, Herzberg GR, Buettner GR. Milk from mothers of both premature and full-term infants provides better antioxidant protection than does infant formula. *Pediatr Res*. 2002;51(5):612-618.
24. Sandal G, Uras N, Gokmen T, Oguz SS, Erdeve O, Dilmen U. Assessment of oxidant/antioxidant system in newborns and their breast milks. *J Matern-Fetal Neonatal Med*. 2013;26(5):540-543.
25. Hanna N. Effect of storage on breast milk antioxidant activity. *Arch Dis Childhood - Fetal Neonatal Ed*. 2004;89(6):F518-F520.

26. Sari FN, Akdag A, Dizdar EA, et al. Antioxidant capacity of fresh and stored breast milk: is -80°C optimal temperature for freeze storage? *J Matern-Fetal Neonatal Med*. 2012;25(6):777-782.
27. Xavier AM, Rai K, Hegde AM. Total antioxidant concentrations of breastmilk--an eye-opener to the negligent. *J Health Popul Nutr*. 2011;29(6):605-611.
28. Marinković V, Ranković-Janevski M, Spasić S, et al. Antioxidative activity of colostrum and human milk: effects of pasteurization and storage. *J Pediatr Gastroenterol Nutr*. 2016;62(6):901-906.
29. Brown JV, Lin L, Embleton ND, Harding JE, McGuire W. Multi-nutrient fortification of human milk for preterm infants. *Cochrane Database Syst Rev*. 2020;6(6):000343.
30. Friel JK, Diehl-Jones B, Cockell KA, et al. Evidence of oxidative stress in relation to feeding type during early life in premature infants. *Pediatr Res*. 2011;69(2):160-164.
31. Zhao M, Chen Z, Ye D, Yu R, Yang Q. Comprehensive lipidomic profiling of human milk from lactating women across varying lactation stages and gestational ages. *Food Chem*. 2025;463 (Pt 2):141242.
32. Tillman S, Brandon DH, Silva SG. Evaluation of human milk fortification from the time of the first feeding: effects on infants of less than 31 weeks gestational age. *J Perinatol*. 2012;32(7):525-531.
33. Maas C, Wiechers C, Bernhard W, Poets CF, Franz AR. Early feeding of fortified breast milk and in-hospital-growth in very premature infants: a retrospective cohort analysis. *BMC Pediatr*. 2013;13:178.
34. Moody GJ, Schanler RJ, Lau C, Shulman RJ. Feeding tolerance in premature infants fed fortified human milk. *J Pediatr Gastroenterol Nutr*. 2000;30(4):408-412.
35. Lapointe M, Barrington K, Savaria M, Janvier A. Preventing postnatal growth restriction in infants with birthweight less than 1300 g. *Acta Paediatr*. 2016;105(2):e54-e59.

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