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Impact of perinatal factors on breast milk composition and volume in preterm infants

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Maternal age, type of delivery, and newborn sex can influence the volume and composition of human milk (HM) expressed by mothers of premature infants. Initiating and maintaining breastfeeding in these mothers is a significant challenge, and records of milk volume expressed during the first few days are crucial for sustaining adequate production. To assess HM production in mothers of preterm infants during the first 15 days of life, examining the relationship between production, type of delivery, and maternal age. Additionally, we aim to analyze the macronutrient composition of HM based on various factors, such as the infant's sex, type of delivery, and maternal age. This is a prospective longitudinal study conducted over 2 years, evaluating HM production and macronutrient composition in 45 mothers of 52 premature infants born at ≤ 32 weeks of gestational age and/or weighing ≤ 1500 g, admitted to the NICU of the University Clinical Hospital of Santiago de Compostela, a Level III Q3+ facility. The study focused on the first 15 days of life, recording the volume of milk expressed and collecting milk samples on days 3, 7, and 15 for nutritional analysis of macronutrients. The study examined the influence of maternal age, type of delivery, and newborn sex on these factors. Vaginal deliveries and maternal age < 35 years were associated with a trend toward greater volumes of milk expressed compared to cesarean deliveries and maternal age ≥ 35 years. If the amount of HM expressed on day 4 was less than 140 mL/day, it significantly predicted a total expression of < 500 mL/day by day 15. We found that 64.3% of our sample expressed less than 140 mL/day on day 4, and 73.8% of these mothers did not reach 500 mL/day by day 15. Regarding macronutrients, HM contained more fat following vaginal delivery compared to cesarean delivery, with significant differences observed on day 3 of life. No differences were observed based on maternal age or newborn sex. In conclusion, HM production on day 4 is a good predictor of production by day 15; maternal age and cesarean delivery seem to negatively influence HM production; the composition of HM shows higher fat content in the colostrum of mothers who had vaginal deliveries.

Keywords Premature newborn, Breastfeeding, Volume, Macronutrients, Maternal age, Type of delivery, Sex

Abbreviations

HM	Human milk
DHA	Docosahexaenoic acid
LC-PUFA	Long-chain polyunsaturated fatty acids
GA	Gestational age
IC	Informed consent
DHMB	Donor Human Milk Bank
WHO	World Health Organization
SENeo	Spanish Society of Neonatology

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NEC	Necrotizing enterocolitis
BMI	Body mass index

Background
The composition of human milk (HM) for premature infants differs from that of full-term newborns. It offers greater protective effects, higher levels of anti-inflammatory and immunological factors, more proteins, lipids, and minerals (such as sodium, calcium, and phosphorus), less lactose, and a higher energy content compared to full-term newborns (58–70 kcal/dL vs. 48–64 kcal/dL)[1]. Premature infants absorb a very high percentage of the lipids in HM (90%), providing them with significant energy and long-chain polyunsaturated fatty acids (LC-PUFA), such as docosahexaenoic (DHA) and arachidonic acid (AA), which are crucial for the composition of cell membranes and the retinal and neurological development of the premature infant[1].

HM changes its composition over the course of days, within the same day, and even during a single feeding. It is a living tissue and represents the best means of transitioning and adapting from intrauterine to extrauterine life, containing microorganisms from the mother that are highly beneficial for the progressive establishment of the newborn's gut microbiota, thereby strengthening their immune system with bacteria like Bifidobacterium or Lactobacillus from the very first moment colostrum is administered to the oral mucosa, reducing the time to achieve full enteral feeding and lowering the risk of necrotizing enterocolitis, among other benefits[2,3]. However, the HM of premature infants needs to be fortified with calcium, phosphorus, protein, and calorie supplements to meet the high nutritional demands and achieve adequate anthropometric parameters[4].

Other factors such as maternal age, the sex of the newborn, or the type of delivery may influence both the amount of HM extracted and the composition of its macronutrients. For example, it has been observed that HM from mothers with male infants has a higher carbohydrate content than that from mothers with female infants[5].

Initiating and maintaining breastfeeding in premature infants is more challenging due to the separation of the dyad, stress, and personal factors that may influence this process. All of these factors make HM a challenge for mothers. However, being able to spend as much time as desired next to their child, prolonged and early Kangaroo Mother Care, as well as promoting early and frequent HM extraction next to the incubator or during Kangaroo Care, can partially reverse this situation[6].

The normal volume of HM required to ensure adequate production in mothers remains unknown. However, it has been demonstrated that an HM volume exceeding 500 mL/day at 2 weeks of life, strongly predicts successful feeding with the mother's own milk at hospital discharge in mothers of very low birth weight newborns[7]. Other studies have shown that the volume of HM expressed on the fourth postpartum day can indicate whether breastfeeding will be successful, with production of less than 140 mL/day on day 4 being associated with a 95-fold higher risk of low milk production at 6 weeks postpartum[8].

The objective of our study is to assess HM production in mothers of preterm infants during the first 15 days of life, examining the relationship between production, type of delivery, and maternal age. Additionally, we aim to analyze the macronutrient composition of HM based on various factors, such as the infant's sex, type of delivery, and maternal age.

Materials and methods
This was a 2-year longitudinal prospective study evaluating HM production and macronutrient composition in 45 mothers of 52 premature infants ≤ 32 weeks of gestational age (GA) and/or ≤ 1500 g of weight admitted to the NICU of the University Clinical Hospital of Santiago de Compostela, Level III Q3+, according to the neonatal care levels of the Standards Committee of the Spanish Society of Neonatology[9]. Ethical approval was obtained from the Galicia Santiago-Lugo Research Ethics Committee under registration number 2019/229, version 3.0.

The study period covers the first 15 days of life, during which the volume of milk extracted daily was recorded, and milk samples were taken on days 3, 7, and 15 of life for nutritional analysis (fats, lactose and proteins).

Inclusion criteria

- All lactating mothers of premature infants ≤ 32 weeks of GA and/or ≤ 1500 g of weight.

Exclusion criteria

- Mothers who did not agree to participate in the study and did not sign the informed consent.
- Mothers who intended to breastfeed but abandoned it in the first 3 days.

Clinical variables studied

- *Maternal variables*: Maternal age (considered advanced if ≥ 35 years and young if < 35 years); type of delivery (vaginal/cesarean); nutritional analysis on days 3, 7, and 15 of 5 mL of fresh HM and the amount of HM extracted on days 3, 4, 7, and 15.
- *Newborn variables*: Sex (male/female); gestational age; twin status; birth weight and weight on days 3, 7, and 15.

Macronutrient analysis
5 mL of HM was collected in the morning, between 8 and 9 am, using a breast pump, as manual extraction promotes a higher fat content than extraction using a mechanical pump[10]. The analysis was performed using a human milk analyzer with FTIR technology (MilkoSkan Mars FOSS Denmark)[11], with prior calibration. The

HM samples were fresh, extracted at the moment, and were agitated to homogenize them. They were then heated in a water bath (37 °C) before analysis.

Statistical analysis

We used the IBM SPSS Statistics version 20.0 software. The normal distribution of quantitative variables was studied using the Shapiro–Wilk test. All studied variables met criteria for non-parametric distribution except for weight, so non-parametric tests were used throughout the study for homogeneity reasons.

From a descriptive perspective, quantitative variables are presented using the median and interquartile range. For the statistical comparison of quantitative variables, the non-parametric Mann–Whitney U test was used. The statistical comparison of qualitative variables was performed using the chi-square test.

A p-value ≤ 0.05 was considered statistically significant.

Results

Forty-five mothers of fifty-two premature infants ≤ 32 weeks of gestational age (GA) and/or ≤ 1500 g of birth weight were included in the study. All mothers were of Caucasian ethnicity, from the northwestern region of Spain, and followed a varied diet without restrictions.

Figure 1 presents an overview of participants and samples flow chart in our study,

Table 1 presents the descriptive variables of the studied sample. The median maternal age was 35 years, the median gestational age was 31 weeks, and the median birth weight was 1375 g. The majority of deliveries occurred via cesarean section.

Figure 2 depicts the median human milk production at 3, 4, 7, and 15 days of the study. A gradual increase is observed over the study period, without exceeding 500 mL at day 15.

The influence of the type of delivery on HM production was studied on days 3, 7, and 15 using the Mann–Whitney U test, and although no significant differences were found, there was a trend towards higher HM production in vaginal deliveries on days 7 and 15 (Table 2).

The influence of maternal age on HM production was studied on days 3, 7, and 15 using the Mann–Whitney U test. No significant variations were found between these variables. However, we observed a trend indicating that older mothers (≥ 35 years) produce a lower volume of HM compared to younger mothers across the three time points studied (Table 2).

The results of the HM macronutrient composition (median) from our sample are shown in Fig. 3. The protein content barely varies over the 15 days; however, fats and carbohydrates progressively increase.

The nutritional analysis of HM based on the newborn's sex does not reveal significant differences (Table 3).

When relating macronutrients and the type of delivery, significant differences were found in the fat content at 3 days, showing that infants born vaginally had a higher fat content in colostrum (*p* 0.03) compared to those

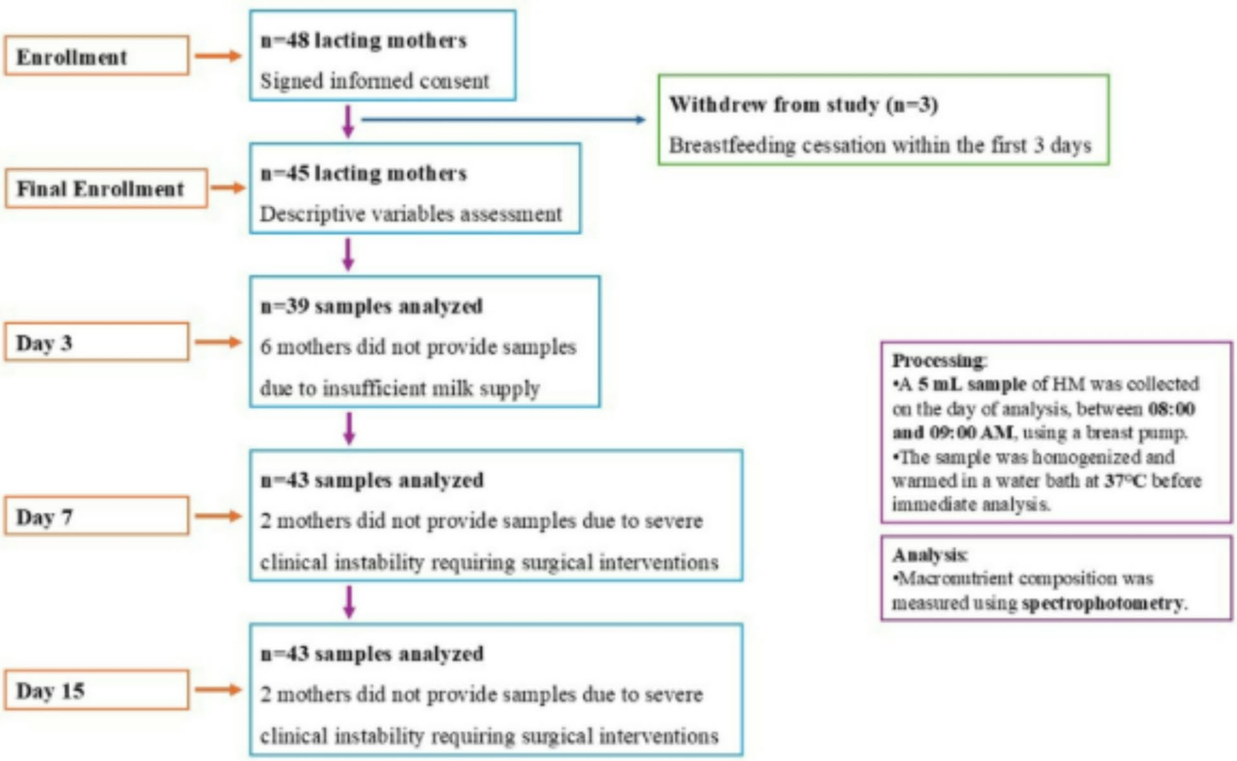

Fig. 1. Participants and samples flow chart.

Variable	Median (IQR)
Maternal age (years)	35 (31.5–38)
Gestational Age (GA) (weeks)	31 (29–32)
Twin status (n%)	8 (15.4%)
Sex (n%)	Male: 28 (56.8%) Female: 24 (46.2%)
Type of delivery (n%)	Vaginal: 17 (33%) Cesarean: 35 (67%)
Birth weight (grams)	1375 (1012–1667)
Newborn weight (grams) (day 3)	1240 (927–1667)
Newborn weight (grams) (day 7)	1280 (990–1515)
Newborn weight (grams) (day 15)	1520 (1125–1755)

Table 1. Sample Descriptives. *n* number; % percentage, *M* males, *F* females, *IQR* interquartile range.

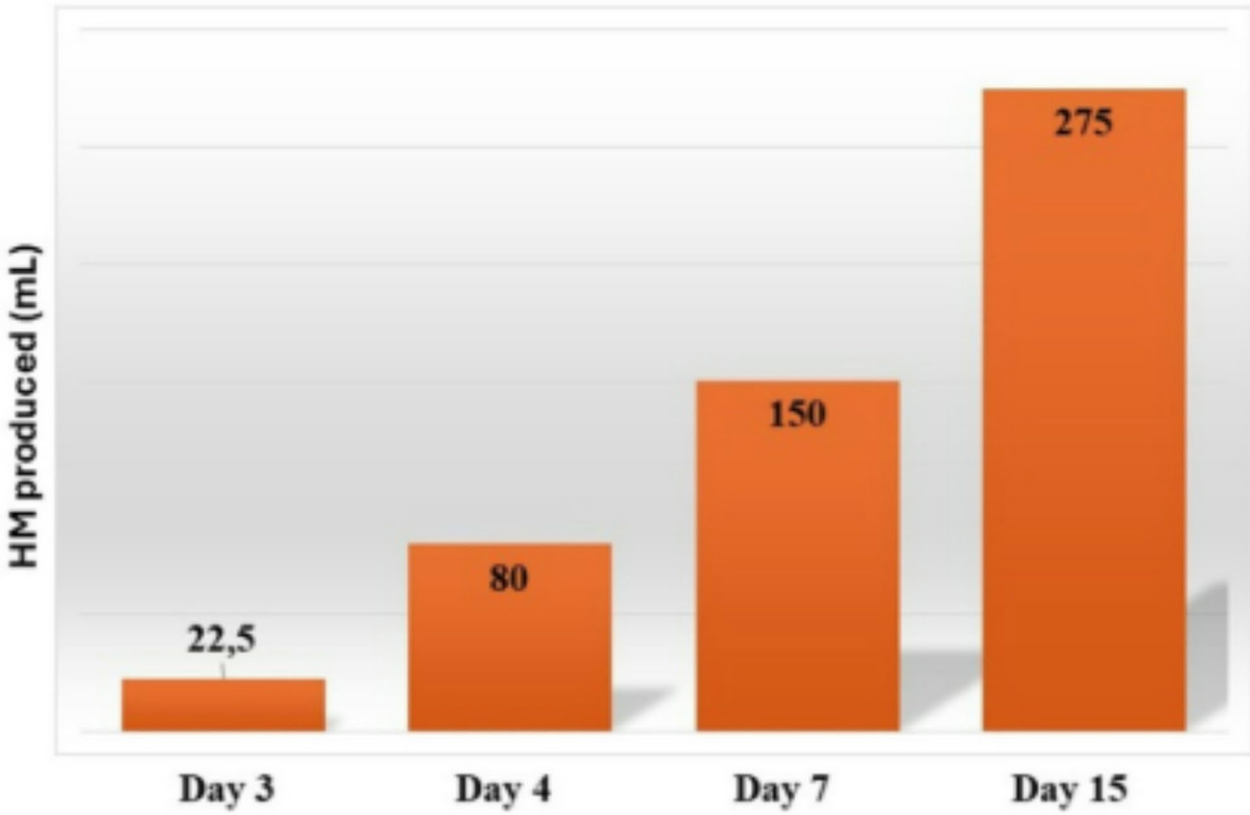

Fig. 2. Evolution of HM Produced (median) in mL on days 3, 4, 7, and 15.

Vaginal delivery (n = 17)	Cesarean (n = 35)	*p*
HM day 3: 20 mL (10–60)	HM day 3: 25 mL (5–80)	$p = 0.91$
HM day 7: 307.5 mL (47.5–367.5)	HM day 7: 145 mL (45–300)	$p = 0.66$
HM day 15: 440 mL (110–600)	HM day 15: 250 mL (131–450)	$p = 0.77$
< 35 years (n = 20)	**≥ 35 years (n = 32)**	***p***
HM day 3: 32.5 mL (75–97.5)	HM day 3: 11 mL (5–44)	$p = 0.82$
HM day 7: 205 mL (55–395)	HM day 7: 125 mL (36–327.5)	$p = 0.66$
HM day 15: 370 mL (220–705)	HM day 15: 197.5 mL (105–450)	$p = 0.84$

Table 2. HM Production (mL) in Relation to the Type of Delivery and Maternal Age. Mann–Whitney U test.

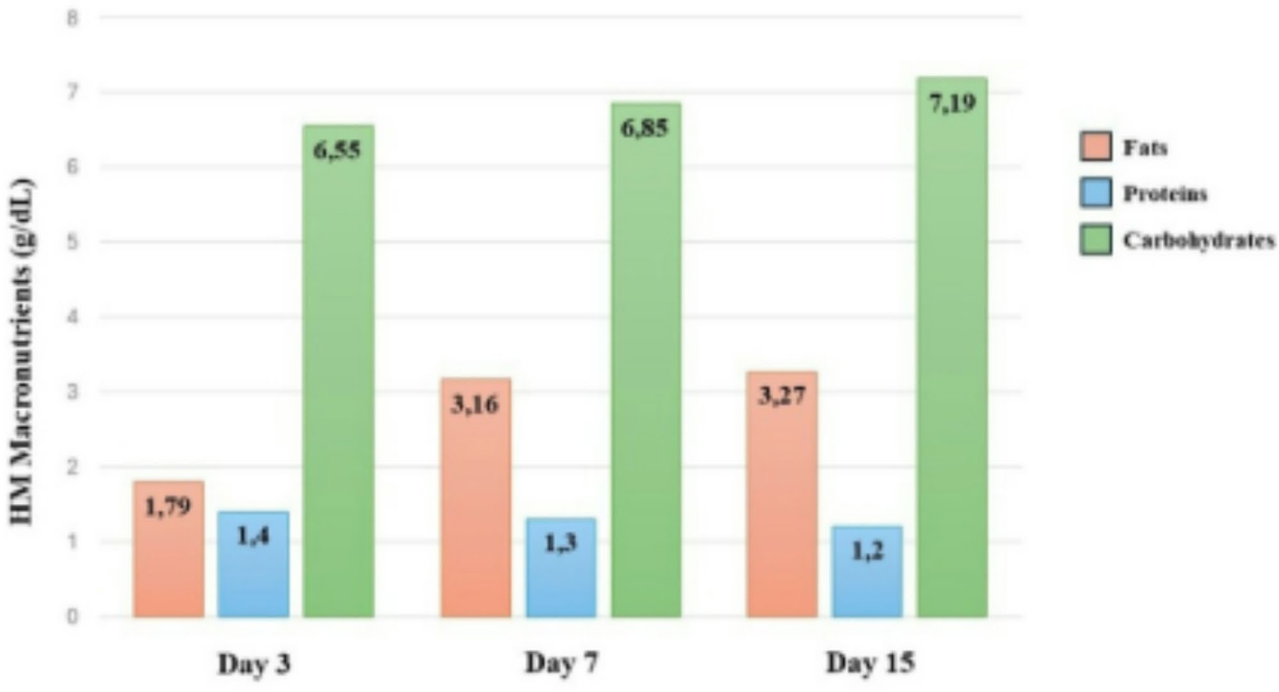

Fig. 3. HM Macronutrients (g/dL) on days 3, 7, and 15 of life.

	Male (n = 28)	Female (n = 24)	*p*
3 days			
Fats (g/dL)	1.78 (1.63–2.27)	1.79 (1.25–3.02)	$p = 1$
Proteins (g/dL)	1.30 (1.30–1.40)	1.40 (1.30–1.45)	$p = 0.34$
Carbohydrates (g/dL)	6.63 (6.31–6.67)	6.55 (5.67–6.86)	$p = 0.75$
7 days			
Fats (g/dL)	3.25 (2.77–3.79)	2.97 (2.59–3.83)	$p = 0.67$
Proteins (g/dL)	1.30 (1.30–1.40)	1.30 (1.30–1.40)	$p = 0.56$
Carbohydrates (g/dL)	6.95 (6.65–7.22)	6.79 (5.82–7.02)	$p = 0.29$
15 days			
Fats (g/dL)	3.31 (2.92–4.22)	3.37 (2.82–3.81)	$p = 0.76$
Proteins (g/dL)	1.30 (1.25–1.30)	1.30 (1.20–1.30)	$p = 0.11$
Carbohydrates (g/dL)	7.20 (6.83–7.42)	7.24 (7.04–7.39)	$p = 0.66$

Table 3. HM Composition Based on the Sex of the Newborn.

born via cesarean (Table 4). The trend at 7 and 15 days is similar to that observed in colostrum, though not statistically significant.

No significant differences were found in the composition of HM based on maternal age at any of the three time points studied. (Table 5).

We also analyzed the relationship between HM production on day 4 and day 15, observing that a production of less than 140 mL on day 4 is a clear predictor of extracting less than 500 mL on day 15, with statistical significance (*p* 0.003). Additionally, there is a greater tendency to produce more than 140 mL on day 4 and have more than 500 mL on day 15. (Table 6).

Discussion

Achieving adequate human milk production in mothers of premature infants is a complex task. In a previous study, we demonstrated that promoting early Kangaroo Mother Care reduces stress in mothers and stimulates increased milk production[12].

The median HM volume in our sample is 80 mL on day 4, which is considerably lower than the 140 mL/day required to ensure adequate production 6 weeks postpartum, as reported in the literature[8]. Therefore, it is highly likely that a significant proportion of our mothers will have issues with lactogenesis. This is an important fact

	Vaginal (n = 17)	Cesarean (n = 35)	*p*
3 days			
Fats (g/dL)	2.12 (1.75–2.815)	1.62 (1.20–1.99)	$p = 0.03$
Proteins (g/dL)	1.30 (1.30–1.40)	1.40 (1.30–1.40)	$p = 0.27$
Carbohydrates (g/dL)	6.63 (6.495–6.81)	6.46 (5.84–6.67)	$p = 0.08$
7 days			
Fats (g/dL)	3.25 (2.87–4.04)	3.16 (2.65–3.62)	$p = 0.27$
Proteins (g/dL)	1.30 (1.30–1.40)	1.30 (1.30–1.40)	$p = 0.80$
Carbohydrates (g/dL)	6.95 (6.65–7.22)	6.85 (6.01–7.03)	$p = 0.11$
15 days			
Fats (g/dL)	3.45 (3.035–3.89)	3.21 (2.82–4.03)	$p = 0.25$
Proteins (g/dL)	1.30 (1.30–1.30)	1.30 (1.20–1.30)	$p = 0.49$
Carbohydrates (g/dL)	7.37 (7.09–7.475)	7.19 (6.93–7.30)	p = 0.10

Table 4. HM Composition Based on the Type of Delivery. Mann–Whitney U test.

	Young (< 35 years) (n = 20)	Advanced (≥ 35 years) (n = 32)	*p*
3 days			
Fats (g/dL)	1.99 (1.74–2.69)	1.64 (1.32–2.16)	$p = 0.13$
Proteins (g/dL)	1.30 (1.30–1.50)	1.35 (1.30–1.40)	$p = 0.92$
Carbohydrates (g/dL)	6.46 (6.04–6.67)	6.57 (6.11–6.75)	$p = 0.59$
7 days			
Fats (g/dL)	3.16 (2.75–3.96)	3.11 (2.49–3.61)	$p = 0.32$
Proteins (g/dL)	1.30 (1.30–1.40)	1.30 (1.30–1.40)	$p = 0.88$
Carbohydrates (g/dL)	6.85 (6.32–7.15)	6.87 (6.26–7.05)	$p = 0.75$
15 days			
Fats (g/dL)	3.50 (2.86–4.40)	3.15 (2.95–3.37)	$p = 0.49$
Proteins (g/dL)	1.30 (1.20–1.30)	1.30 (1.20–1.30)	$p = 0.5$
Carbohydrates (g/dL)	7.20 (6.95–7.40)	7.17 (6.96–7.40)	$p = 0.82$

Table 5. HM Composition Based on Maternal Age (Young/Advanced Age). Mann–Whitney U test.

	15 days		
	< 500 mL (%)	**≥ 500 mL (%)**	
4 days			
< 140 mL	57.1	7.2	64.3
≥ 140 mL	16.7	19	35.7
	73.8	26.2	100

Table 6. Distribution of mothers according to HM production on days 4 and 15. Chi-square test, $p = 0.003$.

to consider because early intervention in these mothers' milk production could help achieve adequate volumes, ensuring optimal production at discharge.

In our study, the median HM in the sample of mothers at 15 days is 275 mL/day, which is below the 500 mL/day volume marked in the literature as a quality indicator for successful lactogenesis at discharge[7].

Our study largely coincided with the SARS-CoV-2 pandemic, during which mothers spent less time in the hospital due to periods of full or partial lockdowns, experiencing uncertainty and doubts about the risks that HM could pose to their infants. This factor may have contributed to the low production of HM in mothers of premature infants. Additionally, having a Donor Human Milk Bank (DHMB) in the same hospital helped reassure mothers since their infants' nutrition was covered. This negative impact of the pandemic on the reduction of HM production and volume has also been observed in other studies[13], despite the fact that the Initiative for the Humanization of Childbirth and Lactation Care (IHAN), the World Health Organization (WHO), UNICEF, the Spanish Society of Neonatology (SENeo), and other scientific societies have always recommended HM, even if the mother is COVID-positive.

In our study, only 11 of the 45 mothers had a HM volume greater than 500 mL/day in the second week. Therefore, only 26% of our mothers will have adequate lactogenesis with sufficient HM production at discharge, while the remaining 74% will have problems ensuring exclusive HM at discharge, according to Hoban's study[7]. In other reviewed articles, even lower percentages have been found, where only 6.3% of mothers reach the volume of 500 mL/day at 2 weeks[14].

Moreover, 64.3% of our sample of mothers extracted less than 140 mL/day on day 4 postpartum, and 73.8% of them did not reach the volume of 500 mL/day on day 15. We statistically demonstrated in our study that HM production below 140 mL/day on day 4 postpartum predicts a volume lower than 500 mL/day on day 15.

This finding highlights the importance of recording the volumes of milk extracted by mothers in Neonatology Services from the start of breastfeeding. It is crucial to inform mothers about HM and emphasize the importance of early colostrum extraction, which contributes to higher HM production and provides healthy microbial colonization when administered to the oral mucosa of the premature infant early and in a scheduled manner. This reduces the incidence of necrotizing enterocolitis (NEC), bronchopulmonary dysplasia, late-onset sepsis, or ventilator-associated pneumonia[15–18].

Initiating early colostrum extraction within the first hour after birth will increase HM volume by shortening the time of lactogenesis II[19,20]. This is especially important in mothers of premature infants since the volume extracted by these mothers is lower compared to when birth occurs at term[21].

In our study, we observed that the type of delivery does not influence the HM volume expressed. However, we identified a non-significant trend suggesting that vaginal delivery resulted in a higher volume of HM extracted compared to cesarean. The literature has demonstrated that cesarean delivery results in a lower volume of milk on day 4, related to the delay in the first HM extractions. Cesarean section can delay the onset of lactation due to postoperative routines that disrupt early bonding, with these first hours being critical for the initiation of lactogenesis and for prolonging its duration[22,23]. It has been observed in the literature that if the cesarean is elective, HM rates will be lower compared to emergency cesarean when the labor process has already begun, and a metabolic and endocrine cascade typical of labor has developed[24].

We also found that maternal age does not significantly influence milk production. However, we observed a non-significant trend where younger mothers (< 35 years) extracted more HM (32.5 mL at 3 days, 205 mL at 7 days, and 370 mL at 15 days) compared to older mothers (11 mL at 3 days, 125 mL at 7 days, and 197.5 mL at 15 days). A possible explanation for not finding a significant difference could be the cutoff point used to define advanced maternal age. We set this at 35 years based on the obstetric standards of our center, but these differences might become more pronounced with a larger sample size and a more refined maternal age stratification in the study. The reviewed literature also does not report significant statistical differences in milk production based on maternal age[25].

Regarding macronutrients, we compared our results with a recent meta-analysis conducted in Spain[26], where it was analyzed the HM macronutrient composition from mothers of preterm infants. Our data are comparable with those reported in this meta-analysis. We obtained protein values of 1.3 g/dL and 1.2 g/dL at 7 and 15 days, respectively, and Borrás Novel reported 1.5 g/dL and 1.3 g/dL, respectively. Regarding fats, we obtained values of 3.16 g/dL and 3.27 g/dL compared to 3.9 g/dL and 3.8 g/dL in Borrás Novel. For carbohydrates, we obtained values of 6.85 g/dL and 7.19 g/dL, and they reported 7.1 g/dL and 7.3 g/dL, respectively.

We did not find differences in macronutrients related to the newborn´s sex. However, there are studies that link male sex with higher carbohydrate values compared to female sex in term infants, which may be due to greater energy demands from male infants[5]. This fact could be important for optimizing individualized nutrition based on the sex of the premature infant.

Differences have been found in the type of delivery and the composition of macronutrients in HM. Being born vaginally will result in a higher fat content in colostrum compared to cesarean deliveries, significantly so. This data could not be confirmed in the reviewed literature, but it has been observed that there is a greater amount of carbohydrates and proteins in vaginal deliveries compared to cesareans[27,28].

We did not find differences between maternal age and macronutrients; however, the reviewed literature indicates that there could be an influence of maternal age on the composition of macronutrients. There is more fat in colostrum and carbohydrates in mature milk in older women (≥ 35 years) compared to younger ones (< 35 years)[29].

Although we did not find statistical differences in some of the studies conducted, we observed a trend that we believe could be confirmed with a larger sample size.

We consider the sample size to be a limitation of the study, as it may affect the representativeness of the relationship between some variables, where we only observed trends. Another limitation is that we did not collect data on the mothers' lifestyle and dietary habits, as well as their body mass index (BMI), which could influence the macronutrient composition of HM. The literature indicates that maternal BMI and HM fat content are positively correlated[30], and that both carbohydrate and protein concentrations increase in the HM of mothers with higher body weight[31]. However, it appears that maternal body composition plays a fundamental role in the nutritional composition of HM, more so than diet[32].

Conclusions

- Producing less than 140 mL/day on day 4 postpartum predicts lower production on day 15 (< 500 mL/day).
- Maternal age and type of delivery do not influence human milk production.
- Newborn´s sex and maternal age do not affect the macronutrient composition of human milk.
- There is higher fat contain in colostrum from vaginal deliveries compared to cesareans.

Data availability

The datasets generated and/or analyzed during this study are not publicly available due to the study's characteristics and informed consents but are available from the corresponding author upon reasonable request.

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Author contributions
MCFT conceived the idea and drafted the manuscript. LTL, SVM and NGC assisted with the literature search and data collection. APM was responsible for the statistical analysis, manuscript revision and drafting. MLC assisted in the manuscript revision and drafting. All authors reviewed and approved the final manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

Ethics approval and consent to participate
Approval for this study was obtained from the Galicia Research Ethics Committee, Santiago-Lugo, under registration number 2019/229, version 3.0. All experiments were performed in accordance with relevant guidelines and regulations.

Informed consent
Informed consent was signed prior to the start of the study.

Consent for publication
Necessary permissions have been obtained from the participants for the publication of data once the study is completed.

Additional information
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