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Breastfeeding of preterm newborn infants following hospital discharge: follow-up during the first year of life

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Abstract *This paper aims to evaluate the prevalence of breastfeeding among premature infants following hospital discharge. Cohort (< 33 gestation weeks) followed up to 12 months (adjusted age). Variables: breastfeeding, anthropometric measurements, social and family information. The proportion of breastfeeding during follow-up was calculated. Survival analysis was conducted to estimate breastfeeding duration. In total, 242 of the 258 infants (93.7%) returned to follow-up; 170 (69.9%) at 6 months and 139 (57.2%) at 12 months (adjusted age). A history of miscarriages (27.5%), stillbirths (11.7%), neonatal deaths (9.5%) and preterm births (21.1%) was noted in 65.5% of women. At hospital discharge: 5.5% received exclusive breastfeeding, 65.8% breastfeeding and formula, 28.6% formula. At month 1, 81.3% received breastfeeding, decreasing to 68.5% at month 2, 62.4% at month 3, 48.1% at month 4 and 22.4% at month 6 (adjusted age). The median of breastfeeding duration was 4 months. Breastfeeding occurred up to four months adjusted age in almost half of the population. Despite the need to improve these rates, the results could reflect the profile of the Child-Friendly Hospital Initiative Unit. Maintaining breastfeeding amongst preterm infants following hospital discharge is still a challenge, for both mothers and health professionals.*

Key words *Breastfeeding, Newborn, Premature*

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Introduction

Breastfeeding of preterm infants is a great challenge for health professionals involved in their care, due to the health impact in the short and long term[1]. The difficulties inherent to prematurity and mother's milk production are some of the factors that contribute to weaning.

Mother's milk is the most complete nourishment for newborn or infants[2], and the World Health Organization (WHO)[3] recommends exclusive breastfeeding up to the age of 6 months. In spite of the well-known advantages of mother's milk in relation to cognitive development, growth promotion and prevention of obesity and metabolic diseases[4-7], very preterm newborns, below 33 weeks gestation, which are at greater risk for morbidities, very often are deprived of breastfeeding for a series of reasons related to first period of their life and the need admit them to Neonatal Intensive Care Units. Mother's milk macronutrients are often insufficient to promote growth similar to intrauterine development in this population of newborns[8].

Several intrinsic and extrinsic factors hamper breastfeeding and its maintenance throughout the hospital stay and after discharge: the prolonged hospital stay, the physiological immaturity of these newborns, maternal stress induced by uncertainty of baby's survival, the difficult start of oral feeding, social and cultural factors that interfere the breastfeeding, decreased milk production due to the lack of stimulation of suction and hospital discharge before term age[1,8-10].

Despite this knowledge, breastfeeding rates among preterm newborn are still low[11]. Few studies report the duration of breastfeeding after hospital discharge, throughout the first year of life, among newborn below 33 weeks of gestation[12-15]. This knowledge is important for the promotion of breastfeeding maintenance strategies in this group of infants.

Strategies such as the "Child-Friendly Hospital" and the "Kangaroo Care" have proven effective in encouraging and facilitating breastfeeding among preterm babies' mothers[16,17], but are still insufficient to promote sustained breastfeeding in the group of very preterm newborns. Therefore, this study aimed to verify the prevalence of breastfeeding in a cohort of preterm newborn under 33 weeks gestation in a Child-Friendly Hospital.

Methods

This research was part of a cohort study to evaluate growth and development approved by the Ethics Committee of the Institution. In this cohort, all newborns with a gestational age under 33 weeks delivered in the Maternity Ward of the Instituto da Saúde da Mulher, da Criança e do Adolescente Fernandes Figueira in the period May 2005-October 2010, admitted to the Neonatal Intensive Care Unit of the Department of Neonatology and monitored in the Follow-Up Clinic were included[18]. Considering a prevalence of 50% of breastfeeding at 6 months of age, an error of 10% and a confidence level of 99%, the sample size calculated was 166 newborns. Newborns with congenital anomalies and congenital infections confirmed by serology were excluded.

Data for the analysis were obtained through a review of medical records, interview with parents for sociodemographic information and the child examination. In the medical records, information about morbidities during pregnancy, gender, gestational age, birth weight, delivery type, very severe clinical morbidities (Hyaline Distress Syndrome, sepsis, pneumonia, bronchopulmonary dysplasia, necrotizing enterocolitis, retinopathy of prematurity and ductus arteriosus), respiratory support and parenteral nutrition, as well as anthropometric measures at birth and at hospital discharge and the classification of weight adequacy for gestational age. In the interview with the parents, information was on mother and father schooling years, maternal age, presence of the father or stepfather within the family (father figure) and readmissions after hospital discharge.

Follow-up appointments were monthly during the first year of life, up to the adjusted age of 12 months. At each appointment, child food type's intake was recorded (exclusive breastfeeding, breastfeeding complemented with formula or exclusive formula appropriate for the child's age), as was the age of introduction of juices and solid foods, weight, height, head circumference and body mass index (BMI). The Z score for each measure was calculated. Fenton's reference chart was used until week 50, adjusted age[19], and after that, the WHO reference chart[20]. Measurements tools were a digital scale – Filizola (S Paulo – Brasil) with a precision of 5g for weight, an infantometer for height and an inextensible tape for head circumference. The same team of nurses at the Follow-Up Clinic performed measurements.

Statistical analysis

The means and proportions of gestational, social and family variables were evaluated to describe the population. The prevalence of breastfeeding was estimated considering the adjusted age for prematurity. The proportions of exclusive breastfeeding and the duration of breastfeeding were calculated. Afterwards, for the analysis, children were divided into two groups: those who breastfed exclusively or complemented with formula, and those who received only formula, throughout the follow-up during the first year of life. The proportions of each group were analyzed during the first 6 months adjusted age at each monthly appointment and then at 12 months adjusted age. A survival analysis, using the Kaplan Meyer curve was used to evaluate breastfeeding's duration, up to the adjusted age of 12 months. This study's dependent variable was the duration of breastfeeding, meaning exclusive and/or complemented with formula. Children with interrupted follow-up before the adjusted age of 13 months were considered censored data. The means of the Z scores for weight, height and head circumference and of the BMI of each child were calculated monthly up to month 6 and at month 12 adjusted ages and later compared using the Student's t-test.

Results

In the study period, 478 children were born at the Maternity Ward of the Fernandes Figueira Women, Child and Adolescent Health Institute; six were transferred to others units, 72 died in the neonatal period and 142 were excluded from the cohort because of congenital malformations and/or genetic syndromes.

Initially, 258 children were included in the study, characterizing a population of very low birth weight and preterm infants (mean gestational age of 29 weeks and mean birth weight 1,254.3g); 242 children (93.7%) returned to the first appointment at the Follow-Up Clinic, 170 (69.9%) were being followed at month 6 adjusted age and 139 (57.2%) at month 12 adjusted age. Neonatal variables and gestational information that characterize the study population are shown in Tables 1 and 2. Most mothers (95%) had a prenatal follow-up and 65.5% of women had a history of miscarriage (66/240 – 27.5%), stillbirth (28/239 – 11.7 %), neonatal death (21/220 – 9.5 %) or preterm birth (49/232 – 21.1 %), which characterizes an at-obstetric risk population.

Parents schooling was similar, with a mean of 9.8 years for mothers and 9.4 years for fathers. Mean maternal age was 26.7 years (ranging from 13 to 43 years), with only two mothers with less than 20 years of age (0.93%). The presence of a father figure, either biological father or stepfather was 87.9%, and in 69.7% of households, the father or the stepfather lived together or in family terms with the child. Readmissions after hospital discharge occurred in 33.2% of children during the first year of life.

At hospital discharge, 5.5% of the children were being exclusively breastfed; 65.8% were receiving mother's milk and formula, and 28.6% only formula. At month 1 adjusted age, 7.5% of the children were being exclusively breastfed; this proportion fell to 6.2% at month 2 and 4.3% at month 3 adjusted age. At month 6 adjusted age, 2 children were still being exclusively breastfed (1.2%). At month 1 adjusted age, 81.3% of the children were being breastfed. There was a gradual decline and, at month 6 adjusted age, about one fifth of the population (22.4%) was still breastfed besides the formula, as can be seen in Graphic 1. The median of the duration of any breastfeeding, by the Kaplan Meyer curve, was 4 months (CI 95%, 3.2–4.7 months), as can be seen in Graphic 2. At month 7 adjusted age, around 60% of the children were receiving other foods, besides breastfeeding or formula.

There was no significant difference in Z scores for weight, height and head circumference between the children who were breastfed exclusively or with formula complementation and those who received only formula, except for the mean weight Z score at month 5 adjusted age and height Z score at month 2 adjusted age, as can be seen in Table 3. In relation to BMI, there was no significant statistical difference between the means Z scores at months 6 and 12 adjusted age. BMI values found in the group who were breastfed and received formula were -0.78 ± 1.07 (range: -2.55 to 1.25) at month 6 adjusted age and -0.22 ± 1.02 (range: -1.46 to 1.10) at month 12 adjusted age. In the group who received only formula, BMI values were -0.58 ± 1.2 (range: -4.09 to 2.74) at month 6 adjusted age and -0.37 ± 1.21 (range: -3.4 to 2.9) at month 12 adjusted age.

Table 1. Description of the study population: demographics characteristics.

Variable	N	Mean (SD)	Range
Birthweight (g)	255	1254.3 (370.4)	460 - 2395
Birth HC (cm)	220	27.2 (2.5)	21 - 34
Birth Height (cm)	220	37.8 (4.08)	27 - 47
Birthweight Z score		-0.35 (1.04)	-2.91 - 3.78
Birth HC Z score	218	-0.01 (1.39)	-5.6 - 3.6
Birth Height Z score		-0.40 (1.31)	-4.2 - 2.6
Gestational Age (weeks)	256	29 (2.1)	28 - 32
APGAR 1stminute	233	6.3 (2.3)	1 - 9
APGAR 5thminute	234	8.3 (1.2)	2 - 10
Weight at discharge (g)	233	2210.8 (529.8)	1760 - 4260
Height at discharge (cm)	208	44.11 (3.1)	34 - 62
HC at discharge (cm)	209	32.5 (1.7)	26.5 - 41
Weight Z score at discharge	230	-1.78 (1.23)	-4.78 - 4.0
Height Z score at discharge	204	-1.71 (1.51)	-6.0 - 1.8
HC Z score at discharge	197	-0.5 (1.12)	-4.8 - 2.7
Variable	**N**	**Frequency (%)**	
Male gender	125/256	48.8	
Small for gestational age*	7/256	18.2	
Height restriction at birth*	57/256	22.1	

HC – head circumference. * Considering the cutoff point at the 10th percentile of Fenton growth chart[19].

Discussion

In this study, breastfeeding rates evidenced the difficulty in maintaining breastfeeding among mothers of preterm newborns with less than 33 weeks gestational age. Nevertheless, despite obstacles faced by these mothers, 81.3% of the children were being breastfed, even if not exclusively, at month 1 adjusted age, although this rate declined with the child's age. However, the maintenance of exclusive breastfeeding was much more difficult in this population, with less than 10% of the children in exclusive breastfeeding either at discharge or at month 1 adjusted age.

Low rates of breastfeeding are a concern for this population in Brazil and around the world, raising proposals of intervention to promote breastfeeding. In this context, the Kangaroo methodology has been a widely described proposal with effective results in the maintenance of breastfeeding among preterm newborns. However, few studies evaluate persistent breastfeeding among preterm newborn under 33 weeks gestation after the first month of life[12,14,15,21].

The evaluation of breastfeeding in a Brazilian cohort of preterm newborns, who had been hospitalized in a Kangaroo Ward showed that 94.9% of newborns were breastfeeding when discharged (56.2% exclusively breastfed), and that, at six months of age, 40.7% were still breastfed (14.4% exclusively breastfed[21]). In this study, where 55.5% of the initial population was evaluated at six months of age, the prevalence of breastfeeding was higher than that found in our study. This can be explained by the greater gestational age of newborns, where 76.6% had between 30-34 weeks of gestational age.

Santoro and Martinez (2007), studied a population of very low birth weight newborns, evaluating the effect of an intervention, which consisted in providing support to the mother that started at prenatal care appointments and a continuous support to breastfeeding, including the follow-up of the children. An increased rate of breastfeeding was observed in the group that received the intervention: in the first appointment, there was a proportion of 16.6% exclusive breastfeeding and 75% breastfeeding comple-

Table 2. Description of the study population: gestational and perinatal information.

Variable	N	Frequency (%)
Prenatal appointments	231	95,0
Antenatal corticosteroids	230/241	95,5
Multiparous	144/245	65,5
Alcohol use in pregnancy	17/225	16,5
Tobacco use in pregnancy	34/233	14,6
Premature rupture of membranes	61/221	27,6
Oligohydramnios	71/229	31,0
Twin pregnancies	95/253	37,5
Diabetes in pregnancy	19/236	8,1
Arterial hypertension in pregnancy	90/240	37,5
Cesarean section	152/241	63,0
Hyaline Distress Syndrome	160/225	71,1
Pneumonia	16/203	7,9
Bronchopulmonary dysplasia*	54/221	24,4
Intraventricular hemorrhage**	43/229	18,8
Necrotizing enterocolitis	3/206	1,5
Sepsis***	20/204	9,8
Ductus arteriosus****	88/226	38,9
Retinopathy of prematurity*****	49/142	34,5
Ventilation******	114/203	56,2
Parenteral nutrition	148/164	90,2

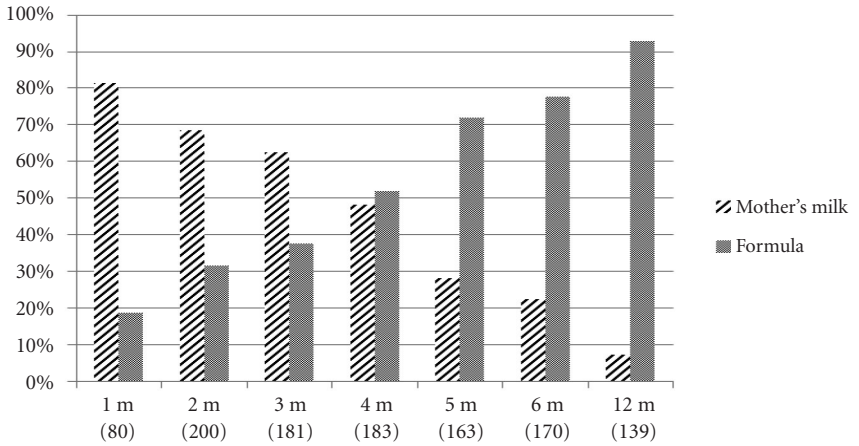
*Considering the need of oxygen with 28 days or more. ** 76.2% grade I. ***Considering positive blood culture. ****Spontaneous closure: 32/88 (36.3%); closure with Ibuprofen: 7/88 (7.9%); surgical closure: 12/88 (13.6%). ***** Majority grades I and II. ****** Mechanical ventilation with a mean duration of 7 days.

mented with formula in the intervention group, and 5.6% exclusive breastfeeding and 36.1% breastfeeding complemented with formula in the group that received a routine guidance service. The median duration of breastfeeding was 54 days (1.8 months) for the non-intervention group and 91 days (3 months) for the intervention group[22]. Even not having been admitted to a Kangaroo Ward, which wasn't available at the time, and not having received any specific intervention, which would be equivalent to the non-intervention group of the Santoro e Martinez[22] study, children's mothers in our study were able to maintain breastfeeding for 4 months. However, mothers in our study received support to breastfeed and all the guidance for stimulating breastfeeding, during hospital stay and after discharge, from the nursing team, speech therapists, Human Milk Bank team and at the Follow-Up Clinic, as this is a Friendly-Child Hospital. Probably, because of this approach, our study evidenced a high proportion of breastfeeding in the first month of adjusted age, even if not exclusive.

Freitas et al (2016) reported the duration of breastfeeding in preterm infants followed during the first year of life, dividing them into two groups: lower and greater than 32 weeks gestation[15], and later analyzing the duration of breastfeeding between the children who were in exclusive breastfeeding at the first appointment or not. They found a median of breastfeeding in the total population (lower and greater than 32 weeks) of 5 months, but in the group of lower gestation age the median was 4.2 months when in exclusive breastfeeding at the first appointment, and of 1.2 months when breastfeeding was complemented with formula at the first appointment.

A recent, temporal population-based study evidenced increased breastfeeding prevalence between the years of 1996, 2001 and 2009 in Feira de Santana, in the state of Bahia, with an annual increase in breastfeeding at the first hour of life, of the duration of exclusive breastfeeding in the first six months of life and in the duration of breastfeeding between 9 and 12 months of age[23]. Authors associated this improvement to the breastfeeding incentive strategies, as well as to lower proportion of teenage mothers and higher maternal schooling throughout this period[23]. While not comparable with our study, since this was a population-based study encompassing children of variable ages and many born at term, two of the findings on the changes that occurred over time cause a stir: lower proportion of teenage mothers and greater maternal schooling. In our population, almost all the mothers had more than 20 years of age and mean maternal schooling was 9.9 years, thus, factors that are favorable to the maintenance of breastfeeding.

There is evidence that mothers with a preterm delivery show a delayed onset of lactogenesis and a lower milk volume production[24]. Cregan et al (2002) showed that 82% of the mothers of preterm newborn have a restriction in the beginning of lactation in the fifth day of life and that these women had significantly less milk produc-



Graphic 1. Trend of the proportion of children who were breastfeeding (exclusive or complemented with formula appropriated for the age) and of the children who were receiving exclusive formula throughout the first six months and at twelve months of adjusted age

The numbers in parenthesis indicate the number of children who came to the appointment at the adjusted age indicated.

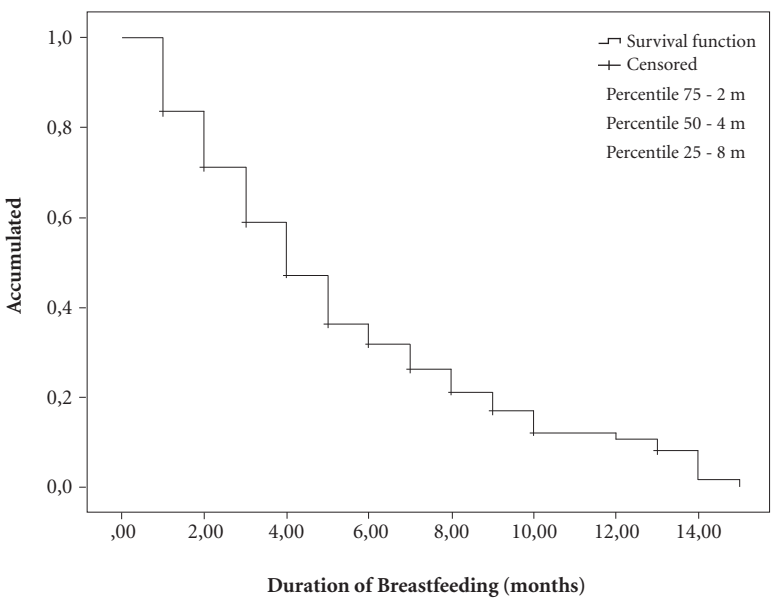


Chart 2. Duration of breastfeeding in months in the population of children who were born preterm under 33 weeks gestational age: Kaplan Meyer curve.

tion in the first 24 hours[25]. There is also evidence that women submitted to repeated cesarean sections have more difficulty in beginning breastfeeding[26], and cesarean section is associated with lower milk volume on the fourth day after birth[27]. In our population, the incidence of cesarean section was 65%. These factors, therefore, could also act as barriers to breastfeeding among mothers of preterm newborns: late lactogenesis in these mothers associated with the cesarean section

Table 3. Trend of Z scores for weight, height and head circumference in the study population during follow-up up to 6 months and at 12 months, considering the adjusted age for prematurity, and in accordance with groups which breastfed with formula complementation or received only formula.

Weight Z scores							
Age	Breastfeeding and Formula			Formula			p-value
	n	Mean (SD)	Min-Max	n	Mean (SD)	Min-Max	
1 month	64	1.10(0.79)	2.94-0.54	15	1.29(0.89)	2.75-0.34	0.4155
2 months	135	1.40(1.08)	3.95-1.35	61	1.16(1.08)	3.98-1.69	0.1514
3 months	109	1.19(1.34)	4.56-1.17	65	1.17(1.39)	4.80-1.53	0.9253
4 months	85	0.90(1.38)	4.19-1.74	91	0.81(1.44)	3.81-2.39	0.6730
5 months	**45**	**1.22(1.28)**	**3.94-1.36**	**114**	**0.71(1.38)**	**3.69-2.54**	**0.0337**
6 months	38	0.84(1.21)	3.44-0.78	127	0.76(1.33)	4.02-3.25	0.7404
12 months	9	0.37(0.81)	1.66-0.68	122	0.35(1.47)	3.63-4.24	0.9679
Height Z score							
Age	Breastfeeding and Formula			Formula			p-value
	n	Mean (SD)	Min-Max	n	Mean (SD)	Min-Max	
1 month	64	0.52(0.67)	160-0.90	15	1.15(1.26)	3.90-1.10	0.0791
2 months	**135**	**0.77(2.70)**	**4.60-1.35**	**61**	**1.36(1.14)**	**3.98-0.80**	**0.0421**
3 months	109	1.16(1.43)	4.70-1.80	65	1.51(1.18)	4.80-0.90	0.1056
4 months	85	1.10(1.50)	5.10-2.30	91	1.17(1.37)	4.50-2.00	0.7495
5 months	45	1.01(1.47)	4.06-2.20	114	0.82(1.49)	4.40-2.97	0.4767
6 months	38	0.49(1.40)	3.09-2.14	127	0.60(1.38)	4.19-2.36	0.6753
12 months	9	0.36(1.00)	1.58-1.05	122	0.14(1.44)	3.72-3.51	0.6540
Head circumference Z score							
Age	Breastfeeding and Formula			Formula			p-value
	n	Mean (SD)	Min-Max	n	Mean (SD)	Min-Max	
1 month	64	0.06(0.90)	2.10-2.70	15	0.23(0.76)	0.80-2.23	0.5009
2 months	135	0.09(1.00)	2.50-2.90	61	0.11(1.26)	5.70-1.80	0.2926
3 months	109	0.03(1.20)	3.00-3.90	65	0.22(1.18)	3.10-2.30	0.3239
4 months	85	0.19(1.49)	4.30-4.90	91	0.07(1.20)	3.30-2.60	0.5664
5 months	45	0.07(1.36)	3.60-1.72	114	0.02(1.25)	2.93-3.30	0.6963
6 months	38	0.42(1.46)	2.69-4.73	127	0.10(1.30)	3.08-2.84	0.2086
12 months	9	0.49(0.77)	0.81-1.30	122	0.33(1.40)	3.18-4.27	0.7501

would induce low milk production. In spite of having a low prevalence of exclusive breastfeeding in our population, this occurred in 7.5% of the children up to the age of one month; moreover, 81.3% of the children were breastfeeding at month 1 adjusted age, even if not exclusively. This proportion of exclusive breastfeeding or even the high proportion of complemented breastfeeding occurred soon after hospital discharge and may be reflecting the need of lower volumes of milk by the child at this moment, which the mother was still able to produce.

Other authors showed that delay onset of milk expression was significantly associated with the use of formula at hospital discharge[27], as well as the beginning of milk expression within the first hour of life is associated with increased volume of milk produced[24]. A study performed in a Brazilian cohort evidenced that guidance during prenatal care regarding the advantages of breastfeeding, of vaginal delivery and term gestation favored breastfeeding within the first hour of life[28]. In our study, as a function of the characteristics of the population of newborns with less than 33

weeks gestation and very low birth weight, there as a clinical contraindication to put those newborns at the mother's breast within the first hour of life. Several factors contribute to a lower volume of milk production in most of these mothers, associated with a probable delay in the onset of lactogenesis due to prematurity and all the difficulties of putting the newborn at the mother's breast, to the initial clinical morbidities and the immaturity of the preterm, who often receives hospital discharge before term age, explaining in part the low prevalence of exclusive breastfeeding in this population[1,24-28].

However, despite the low prevalence of breastfeeding during the first year of life, almost half of the children at four months adjusted age and one fifth of them at six months of adjusted age received their own mother's milk. Roussel et al (2012), studied the factors that influenced breastfeeding in a population of hospitalized newborns in an Neonatal Intensive Care Unit and showed that the main factor that influenced breastfeeding was the gestation due to assisted reproduction, with older women, and that women's age in this group influenced breastfeeding; moreover, this effect persisted up to hospital discharge[29]. Among women in our study, with an obstetric risk history, breastfeeding may have been very important, contributing to the maintenance of breastfeeding in part of the population.

In our population, 69.7% of fathers or stepfathers lived in family terms with the child. In the study of Menezes et al.[21], who evaluated the maintenance of breastfeeding in graduates of a Kangaroo Ward, 81.1% of fathers lived with the family. The skin-to-skin contact in the Kangaroo method creates favorable conditions to help women keep breastfeeding, and this practice is encouraged by the involvement of the partner in this approach[30]. A recent study showed that partner support since the onset of breastfeeding at the hospital increased the prevalence of exclusive breastfeeding at six months of age; 97% of mothers who received their partner support at the immediate post childbirth kept breastfeeding after hospital discharge, and 26% up to six months of age, as opposed to those who didn't have this support, where this prevalence was 10.1%[31]. Partner's involvement in the breastfeeding incentive process is important for the success, with fathers expressing their desire to participate in this process[32].

As to anthropometric measurements, there was no significant statistical difference in the Z scores of weight, head circumference and height throughout the first year of life between the groups who received only formula and breastfed complemented with formula, with only two exceptions: the mean weight Z score at month 5 in the breastfeeding group and the mean height Z score in the formula group in the first month. However, these findings were one-off, and in both moments, there was a greater dispersion of values in the formula group, as can be seen with the standard deviations. A relevant modification should be persistent and not one-off. Moreover, there was no repercussion on the values of the BMI at six and twelve months of age. It is important to stress that there were no significant differences between measurements of head circumference between the two groups, an indirect evaluation of brain growth.

This study's limitations were losses in the long-term follow up, which weaken the evaluation. Nevertheless, we had the return of 57% of the study population at twelve months of adjusted age. Another limitation was that we did not analyze the possible causes for weaning in this population, as it was not the objective of this study. It would be important, in a future study on breastfeeding among very preterm newborns, to evaluate the factors that favor breastfeeding in this population, as well as factors that can induce early weaning.

Conclusion

This study evidenced that, besides difficulties in maintaining breastfeeding among mothers of children born preterm, with a low gestational age and very low birth weight, it was possible to uphold breastfeeding, even if not exclusive, up to the four months of adjusted age in almost half of the population.

Nevertheless, it is necessary to reinforce that these rates must be improved with the organization of strategies to encourage breastfeeding in this population. It is very likely that these results are related to the profile of the Care Unit, which belongs to the Child-Friendly Hospital Initiative, staffed with a multiprofessional team geared to support breastfeeding.

The maintenance of breastfeeding after hospital discharge is still a challenge in the care of this population, for both the mothers and health professionals. Breastfeeding plays an important role in neuropsychomotor development and in the healthy growth of preterm newborns, as well as in the prevention of metabolic diseases and obesity.

Contributions

MDBB Méio participated in the design of the project, data collection and analysis, interpretation of the statistical analysis and elaboration of the manuscript. LD Villela participated in data collection, interpretation of the statistical analysis and elaboration of the manuscript. SCS Gomes Júnior participated in data analysis, interpretation of the statistical analysis and the elaboration of the manuscript. CM Tovar, participated in the preparation of the project and elaboration of the manuscript. MEL Moreira participated in the interpretation of the statistical analysis and elaboration of the manuscript.

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