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Feeding Imprinting: The Extreme Test Case of Premature Infants Born With Very Low Birth Weight

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Feeding imprinting, considered a survival-enabling process, is not well understood. Infants born very preterm, who first feed passively, are an effective model for studying feeding imprinting. Retrospective analysis of neonatal intensive care unit (NICU) records of 255 infants ($M_{\text{gestational age}} = 29.98 \pm 1.64$) enabled exploring the notion that direct breastfeeding (DBF) during NICU stay leads to consumption of more mother's milk and earlier NICU discharge. Results showed that DBF before the first bottle feeding is related to shorter transition into oral feeding, a younger age of full oral feeding accomplishment and earlier discharge. Furthermore, the number of DBF meals before first bottle feeding predicts more maternal milk consumption and improved NICU outcomes. Improved performance in response to initial exposure to DBF at the age of budding feeding abilities supports a feeding imprinting hypothesis.

Infants' thriving is dependent on their nurturance (Ehrenkranz et al., 2006; Hay & Lucas, 1999), both in the physical sense and in the psychological sense (Silberstein, Feldman, et al., 2009). It has been well established that mother's milk (MM) is the preferred nutritional composition for premature infants (Academy of Nutrition and Dietetics, 2015; Eidelman & Schanier, 2012; Lessen & Kavanagh, 2015). Mother's milk provides many benefits, including improved composition of intestinal microbiota (Sela & Mills, 2010), lower rates of sepsis (Furman, Taylor, Minich, Hack, & Chb, 2003) and necrotizing enterocolitis (Cristofalo et al., 2013; Schanler, Shulman, & Lau, 1999; Sullivan et al., 2010), lower long-term growth failure (Hintz, 2005), fewer hospital readmissions for illness in the 2 years after discharge (Vohr et al., 2007), and improved neurodevelopmental outcomes examined at 3 months through 15 years of age (Blaymore Bier, Oliver, Ferguson, & Vohr, 2002; Gibertoni et al., 2015; Isaacs, Fischl, Quinn, Chong, & Gadian, 2010; Vohr et al., 2007). Infants born at term typically receive MM through oral feeding. In order to be able to orally feed, an infant must be capable of complex integration of controlled and regulated activity of multiple anatomic structures including the lips, jaw, cheeks, tongue, palate, pharynx, and larynx. In addition, coordinated rhythmic sequences of sucking, swallowing, and breathing are required, as well as the ability to sustain an alert behavioral state (Amaizu, Shulman, Schanler, & Lau, 2008; Delaney & Arvedson, 2008). This complex process, which requires neurological (Silberstein, Geva, et al., 2009) and physiological maturation of the relevant organs (Delaney & Arvedson, 2008), begins to be developmentally possible at approximately 33 weeks gestational age (GA; Bache, Pizon, Jacobs, Vaillant, & Lecomte, 2014) or earlier (Amaizu et al., 2008).

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Infants born preterm have limited and altered experiences in their path toward achieving normal feeding. They are challenged by the need to manage oral motor coordination at an earlier age than infants born at term (Bu'Lock, Woolridge, & Baum, 1990), and they often lack the ability to coordinate the sucking–swallowing–breathing cycle during feeding (Lau, Alagugurusamy, Schanler, Smith, & Shulman, 2000). Additional challenges in feeding preterm infants may include difficulties in feeding tolerance (Dollberg, Kuint, Mazkereth, & Mimouni, 2000) and the need to fortify MM in order to meet the special nutritional needs of premature infants (Cohen & McCallie, 2012). Neonatal intensive care unit (NICU) staff and parents seek more accurate protocols in order to facilitate the transition to oral feeding in infants born preterm.

Infants born prematurely are less likely to receive MM than infants born at term age (Donath & Amir, 2008; Flacking, Nyqvist, & Ewald, 2007). Providing MM for the premature infant is a challenging task for mothers due to various reasons, one of them being the delay or lack of direct breastfeeding (DBF; feeding directly at the breast; distinguished from feeding expressed breast milk by bottle or other means), owing to the complexity and difficulty involved in the process of oral feeding. This delay brings to more challenges, including reliance on breast pumps and diminished milk supply, in addition to a stressful environment in the NICU (Callen & Pinelli, 2005). However, there are no definite criteria that signal infant readiness to feed orally (Ross & Browne, 2002), and as studies examining the main predictors of the transition process from gavage to oral feeding show inconsistent findings (Jackson, Kelly, Mccann, & Purdy, 2015), the process of oral feeding initiation and progression is extremely challenging and somewhat unclear.

Transitioning from gavage to full oral feeding is indeed one of the most important tasks infants must accomplish in the NICU. It is a criterion for discharge (American Academy of Pediatrics, 2008), and is a demanding undertaking for many preterm infants (Silberstein, Geva, et al., 2009), often causing a delay in attaining full oral feeding skills and a prolonged hospitalization in the NICU (Bakewell-Sachs et al., 2009). Feeding difficulties frequently linger after discharge and often elicit secondary issues, compromising the feeding dyad relationship (Silberstein, Feldman, et al., 2009).

Bottle feeding is considered less effortful than DBF for infants (Ahluwalia, Morrow, & Hsia, 2005; Lagan, Symon, Dalzell, & Whitford, 2014) and particularly for infants born preterm (Briere, 2015). Consequently, mothers report that bottle feeding with expressed MM is often encouraged by NICU staff, with the implication that DBF is an additional step forward, which will take place following discharge (Niela-Vilén, Axelin, Melender, & Salanterä, 2014). Moreover, bottle feeding is encouraged with a promise of a faster discharge, despite the lack of direct evidence to support this claim (Briere, 2015). Once at home, with no guidance, mothers often find it difficult to establish DBF (Niela-Vilén et al., 2014). Nevertheless, because most studies examining the benefits of MM for preterm infants do not distinguish between the nutritional benefits of MM provision and the additional benefits of its "mode of administration" through DBF (Eidelman & Schanier, 2012; Furman et al., 2003; Gibertoni et al., 2015; Isaacs et al., 2010), it is still unknown if indeed bottle feeding with MM results in better NICU outcomes than DBF.

Furthermore, there is a need for research focusing on the first steps of feeding, as it may be that the first feeding phase may comprise a feeding imprinting process and may be hyperpotent in establishing a preferred feeding mode. As oral feeding abilities become developmentally possible at approximately 33 weeks GA (Bache et al., 2014), infants born earlier than that may serve as an effective test case to explore the notion of feeding imprinting. Infants born preterm experience feeding as a major challenge. This may suggest a more pronounced sensitivity to the imprinting process relative to infants born at term, who manage the feeding process more easily, regardless of mode of initiation. Second, infants born very preterm may be studied at ages that precede initiation of oral feeding, as well as during a more prolonged phase of acquiring effective oral feeding ability, thereby offering an effective model to study this process effectively in human infants.

Lorenz's imprinting term describes the process by which newly hatched goslings identify and bond to the first object they see as their mother (Lorenz, 1937). This primary input alters the infant's brain, affecting the density of postsynaptic density of axospinous synapses in the left hyperstriatum ventral, thought to form the neural basis for recognition memory (McCabe & Horn, 1988). Full-term human infants also go through a behavioral imprinting process in the early hours of life, mediated by oral tactile sensory stimuli, normally fixating to the mother's nipple (Mobbs, 1989). Mobbs, Mobbs, and Mobbs (2016) recently proposed that the oral tactile imprint to the breast serves as the foundation for optimal breastfeeding and latching, thereby serving the first stage of emotional development, preceding attachment, and suggesting feeding imprinting as a relevant construct in exploring human newborns.

This process of latching to the breast does not typically occur in the first hours in the lives of infants born very prematurely, as the ability to safely feed orally develops at approximately 33 weeks GA (Bache et al., 2014; Delaney & Arvedson, 2008). Hence, once oral feeding does begin, feeding imprinting may still occur and serve an important role in establishing DBF and in supporting its potential impact. Imprinting is also thought to strengthen as exposure dose increases (McCabe & Horn, 1988). Indeed, Pineda (2011a) found that mothers who employed DBF in the NICU were more likely to provide MM at discharge and that the duration of MM feeding in the NICU was associated with DBF. Importantly, age at first DBF attempt and whether the first oral feeding attempt was at the breast were found as potential factors in the duration of MM feeding in the NICU (Pineda, 2011a). These findings suggest that whether the first oral feeding is through DBF or with a bottle may make a difference in infants born very preterm.

Some of the variables shown to be significant predictors of the transition process from gavage to oral feeding include GA (Dodrill, Donovan, Cleghorn, McMahon, & Davies, 2008), birth weight (Jackson et al., 2015), number of oral feeding attempts (Pickler, Best, & Crosson, 2009), behavioral state (Kirk, Alder, & King, 2007), and medical conditions (Jackson et al., 2015). Yet, to date, the nature of the first exposure to oral feeding, namely, whether the first oral feeding is done using a bottle or at the breast, and its relationship with the length of the transition period, has not been explored. Given that the first exposure may serve a pivotal role in facilitating the vital transition to oral feeding in infants born very preterm, such a study may serve to validate the notion of feeding imprinting and offer factors affecting it in human infants.

Method

Study Design and Setting

This retrospective analysis study took place in a Level III, 40-bed NICU at Sheba Medical Center, Ramat Gan, Israel. The study was approved by the human subjects committee at the study site. Informed consent was waived because the data were retrieved using chart review of information which was not deemed to be sensitive. The philosophy of the Newborn Individualized Developmental Care and Assessment Program (Als, 1986) was gradually introduced at the study site at the beginning of the study period. Parents were encouraged to actively participate in infant care at all hours. Skin-to-skin care was promoted, as well as MM pumping and non-nutritive sucking. DBF was promoted starting at 33 weeks postmenstrual age (PMA), aided by a lactation consultant, yet exclusive DBF was nonexistent, and all oral supplementation was conducted via bottle. For purposes of the current study, all selected cases were fed a predetermined volume every 3 hr. Based on previous feeding interventions with premature infants (Yildiz & Arikan, 2012), the minimal sample size required for the detection of differences between the two groups is 33 participants in each cell (Cohen, 1988). We therefore collected data from a large cohort ($N = 340$) to enable the exploration of varying attributes that occur at a minimal rate of 10% of the sample.

Data Extraction

Based on a protocol for retrospective studies (Gearing, Mian, Barber, & Ickowicz, 2006), type, volume, and mode of infant feeding were documented by the staff at the study site, using a patient data management system (iMDsoft MetaVision®, Tel Aviv, Israel). Proper data extraction was supervised by the hospital's information technology advisor and directly transferred to Microsoft Excel (Version 14.0.7177.5000; Microsoft Office Professional Plus 2010), limiting manual processing.

Data Analysis

The cohort was divided according to the criterion of having DBF exposure or not having such exposure. To enable the exploration of group differences, a multivariate analysis of variance (MANOVA) was conducted. Exploration of the relations between in vitro fertilization (IVF), twinhood, and the inclination to DBF was conducted using a logistic regression. Exploration of the relation between GA and the inclination to DBF was conducted using a chi-square analysis. A multivariate analysis of covariance (MANCOVA) was conducted to determine differences between the DBF groups in NICU outcomes using IVF and twinhood measures as covariates. Feeding imprinting hypothesis was explored by dividing the portion of the cohort that did have DBF exposure in the NICU according to the criterion of having exposure to DBF prior to bottle feeding or not having such exposure. In order to determine differences between the groups, a MANOVA was conducted. Exploration of differences between the groups in NICU outcomes was conducted using a MANCOVA, with intrauterine growth restriction (IUGR), birth weight, and Clinical Risk Index for Babies II (CRIB) II score as covariates.

In order to explore the factors involved in feeding imprinting, stepwise multiple regressions were conducted predicting length of transition period, age at full oral feeding accomplishment, and age at discharge using weight at birth, GA, sex, CRIB II score, Apgar 1, Apgar 5, singleton or twin, IVF, IUGR, maternal age, primiparous or multiparous, age at first bottle feeding, age at first DBF, number of DBF meals prior to first bottle feeding, and percentage of MM consumed as possible predictors. A similar stepwise regression was conducted to explore predictors of age at first bottle feeding. In order to explore the relations between maternal age, parity, and the inclination to DBF, a MANOVA was conducted. All analyses were conducted using SPSS (Version 20.0; IBM® SPSS® Statistics, Armonk, NY. USA).

Participants

Participants included 340 infants who were admitted to the NICU at Sheba Medical Center between January 1, 2012 and April 30, 2015 and were born earlier than 32 weeks GA, excluding infants who presented congenital malformations (n = 18), intraventricular hemorrhages Grades III or IV (n = 11), necrotizing enterocolitis (n = 10), infants that died during hospitalization (n = 46), and infants receiving different feeding protocols (n = 13), resulting in a final sample of 255 infants. Demographic characteristics are presented in Table 1. The Sheba Medical Center is the largest public hospital in Israel, with a Level III NICU unit, catering to the greater municipal area of central Israel. The mean education level of mothers represents attaining a degree at the undergraduate level. According to the Central Bureau of Statistics (Yafe, 2013), this level of education is characteristic of 57% of women at this age range in Israel. Based on the above needed sample size calculation, this sample size was designed to enable sufficient power to explore moderating factors such as maternal age, parity, and IVF.

Measures

Seven dependent measures were collected: *first nutritive DBF*, defined as the first time an infant suckled directly at the breast and some milk was transferred. Infants feeding directly at the breast were weighed before and after breastfeeding to assess milk intake; the *number of DBF meals prior to the first bottle feeding* was noted; *first bottle feeding*, defined as the first time an infant was fed with a bottle, irrespective of whether the bottle contained MM or formula; *transition period* calculated from the day at which an infant was first fed with a bottle, until the day in which full oral feeding was accomplished; *percent nutritive DBF in NICU* was calculated as the percent of meals in which nutritive DBF occurred of all feedings from the first DBF attempt until discharge. Every nutritive DBF attempt was counted, even when supplementation via bottle or enteral feeds was necessary; *percent MM consumed in NICU* was calculated out of the total amount of milk consumed by an infant from the time of admission until discharge; *MM provision at discharge* was noted if the infant received MM during the last day in the NICU, whether by bottle or at the breast; *nutritive DBF at discharge* was noted if the infant was directly breastfed during the last 2 days in the NICU; finally, *infant medical risk* was measured by the CRIB II (Parry, Tucker, & Tarnow-Mordi, 2003; Figure 1).

Table 1
Demographic Characteristics of the Cohort

	M	*SD*	Range
Birth weight (g)	1,286.96	311.22	478–2,023
GA (weeks) birth	29.98	1.64	24.6–31.6
CRIB II	6.28	2.98	1–19
Apgar 1	7.50	2.09	0–10
Apgar 5	9.09	1.25	4–10
Maternal age (year)	31.92	5.72	20–51
Maternal education (year)	15.37	3.01	12–30
Paternal age (year)	34.26	6.01	21–52
Paternal education (year)	14.55	2.94	8–29
No partner	7.8%		
Male/female	49.4%/50.6%		
Singleton/twin	43.1%/56.9%		
Twin death	2.35%		
Primipara/multipara	45.5%/54.5%		
IUGR	10.2%		
IVF	43.14%		

Note. GA = gestational age; CRIB = Clinical Risk Index for Babies; IUGR = intrauterine growth restriction; IVF = in vitro fertilization.

Results

Sixty-six percent (n = 169) of infants were directly breastfed at least once during their NICU stay. A

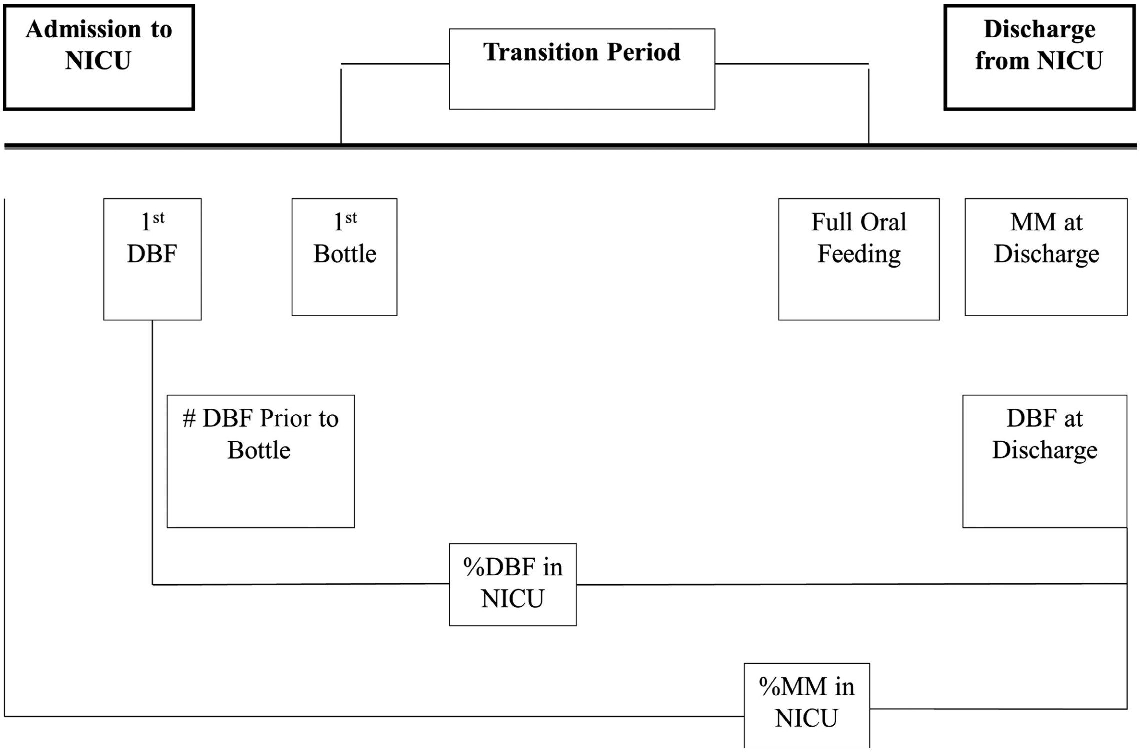

Figure 1. Research paradigm: Timeline of data collection and dependent measures. NICU = neonatal intensive care unit; DBF = direct breastfeeding; MM = mother's milk.

MANOVA and a chi-square analysis were conducted to determine differences in perinatal measures between infants who were DBF at least once during their NICU stay (DBF group) and those who were not (no DBF group), exploring the notion that these characteristics possibly affect the

Table 2
Demographic Characteristics DBF Versus No DBF Groups

	DBF group (n = 168)		No DBF group (n = 87)		
	M	*SD*	*M*	*SD*	*p*
Birth weight (g)	1,299.09	315.94	1,263.53	302.31	.388
GA (weeks) birth	29.96	1.61	30.02	1.72	.772
CRIB II (score)	6.26	2.93	6.31	3.09	.887
Apgar 1 min	7.42	2.07	7.65	2.13	.403
Apgar 5 min	9.06	1.18	9.14	1.37	.628
Maternal age (year)	31.63	5.57	32.49	5.99	.264
Maternal education (year)	15.62	3.04	14.85	2.89	.073
Paternal age (year)	34.27	5.81	35.11	5.16	.318
Paternal education (year)	14.71	2.82	14.19	3.18	.247
No partner	5.96%		9.12%		.327
Male/female	54.8/45.2%		42.5/57.5%		.064
Singleton/twin	49.4/50.6%		31/69%		.005**
Twin death	2.98%		1.15%		.364
Primipara/multipara	45.2/54.8%		46/54%		.908
IUGR	8.93%		12.64%		.355
IVF	33.93%		50.57%		.010**

Note. DBF = direct breastfeeding; GA = gestational age; CRIB = Clinical Risk Index for Babies; IUGR = intrauterine growth restriction; IVF = in vitro fertilization.
$^{**}p < .01$.

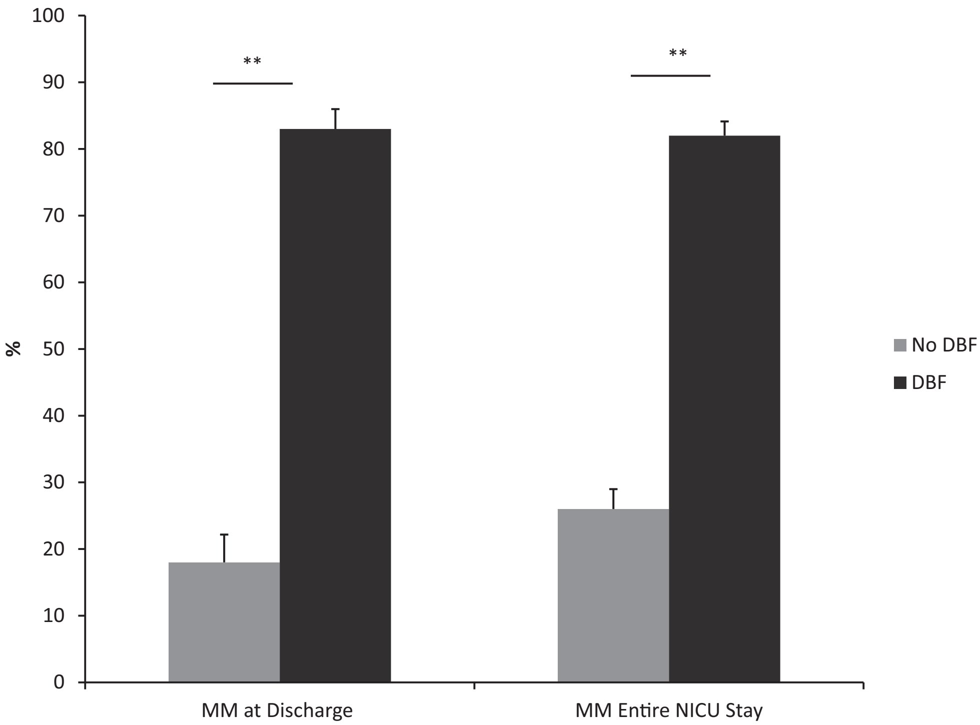

Figure 2. MM provision in DBF versus no DBF groups. NICU = neonatal intensive care unit; DBF = direct breastfeeding; MM = mother's milk.
$^{**}p < .01$.

inclination to DBF (see Table 2). The no DBF group was found to have a higher percentage of twins (68.97%) compared to the DBF group (50.56%; $p < .005$), as well as a higher percentage of infants conceived via IVF (50.57%) compared to the DBF group (33.93%; $p < .01$). Due to the high percentage of twins among infants born to mothers who conceived via IVF (75.76%) compared to mothers who conceived spontaneously (43.66%), a logistic regression analysis was conducted to explore if and how the two findings are related. A test of the full model against a constant only model was statistically significant, indicating that the predictors as a set reliably predicted the likelihood to DBF, $\chi^2(3) = 11.345$, $p < .01$. Prediction success overall was 65.9%, however, a Nagelkerke's R^2 of .06 indicated that the model accounts for 6% of the variance. The Wald criterion demonstrated that only twins who have been conceived via IVF have a higher likelihood of belonging to the no DBF group ($p = .001$), whereas infants who are twins but conceived spontaneously ($p = .089$; *ns*) and singletons who have been conceived via IVF ($p = .327$; *ns*) do not.

In order to explore the notion that GA does not affect the inclination to DBF, a chi-square test was conducted to examine the relation between extremely premature infants (24–28 weeks GA) and belonging to the no DBF group. The relation between these variables was not significant, $\chi^2(1) = 0.13$, $p = .723$; *ns*, indicating that extremely premature infants are not more likely to be in the no DBF group.

In order to explore the notion that DBF in the NICU may be a factor in NICU outcomes, a MANCOVA was conducted, controlling for factors that differed between the DBF and no DBF groups, that is, twinhood and IVF. The NICU feeding method outcome relations to DBF indicated that infants in the DBF group were more likely to receive MM at discharge ($M = 83 \pm 38\%$) compared with infants in the no DBF group ($M = 18 \pm 39\%$), $F(1, 251) = 148.61$, $p < .001$, $\eta^2 = .37$ (see Figure 2), and received a higher percentage of MM during their NICU stay ($M = 82 \pm 24\%$) compared to infants in the no DBF group ($M = 26\% \pm 33$), $F(1, 251) = 223.58$, $p < .001$, $\eta^2 = .47$ (see Figure 2). No group differences were noted in length of the transition period, PMA at full oral feeding accomplishment, and weight and PMA at discharge.

Of the infants in the DBF group, 60.36% ($n = 102$) were DBF prior to being bottle fed. In order to explore the importance of feeding imprinting, that is, the importance of being exposed to

DBF on the first oral feeding exposure, as compared with being exposed to DBF after having been exposed to bottle feeding, a MANOVA and a chi-square analysis were conducted to explore perinatal differences between infants who were first DBF prior to being bottle fed (DBF initial exposure group [DBF-IE]; n = 102, 40% of the total sample) and infants who were DBF later during their NICU stay (bottle IE group [bottle-IE]; n = 66, 25.88% of the total sample). In comparing the demographic characteristics of IE-type groups, a selective bias was noted. The bottle-IE group was found to have a higher percentage of infants diagnosed with IUGR (18.18%) compared to the DBF-IE group (2.94%), $\chi^2(1, 168) = 11.45$, $p < .001$. Accordingly, infants in the bottle-IE group were found to have a lower birth weight (M = 1,190.55 ± 303.18 g) than infants in the DBF-IE group (M = 1,369.32 ± 305.3 g), $F(1, 163) = 13.82$, $p < .001$, $\eta^2 = .08$, and higher CRIB II scores (M = 6.86 ± 2.86) than infants in the DBF-IE group (M = 5.86 ± 2.92), $F(1, 163)$, $p < .03$, $\eta^2 = .03$, suggesting a more complicated course for participants in the bottle-IE group.

A MANCOVA was then conducted, controlling for IUGR, birth weight, and CRIB II scores in order to explore differences in NICU outcomes between the two groups. The analysis showed that infants in the DBF-IE group had a shorter transition period (M = 9.22 ± 5.29) than infants in the bottle-IE group (M =11.53 ± 6.66), $F(1, 162) = 6.74$, $p < .011$, $\eta^2 = .04$, they accomplished full oral feeding at a younger PMA (M = 35.36 ± 0.85) than infants in the bottle-IE group (M = 35.76 ± 1.39), $F(1, 162) = 7.51$, $p < .007$, $\eta^2 = .044$, and were discharged at a younger PMA (M = 36.80 ± 0.96), almost a week earlier, than infants in the bottle-IE group (M = 37.44 ± 1.72), $F(1, 162) = 11.43$, $p < .001$, $\eta^2 = .066$. In addition, once DBF was initiated, infants in the DBF-IE group had a higher percentage of DBF meals (M = 4.2 ± 3.6%) compared to infants in the bottle-IE group (M = 2.8 ± 3.5%), $F(1, 162) = 6.42$, $p < .028$, $\eta^2 = .038$ (Figures 3 and 4). No differences were found in the percentage of MM throughout the NICU stay, DBF and MM provision at discharge, and weight at discharge between the groups.

In order to explore the factors involved in feeding imprinting, stepwise multiple regressions were conducted predicting (a) length of transition period, (b) PMA at full oral feeding accomplishment, and (c) PMA at discharge, using weight at birth, GA, sex, CRIB II score, Apgar 1, Apgar 5, singleton or twin, IVF, IUGR, maternal age, primiparous or

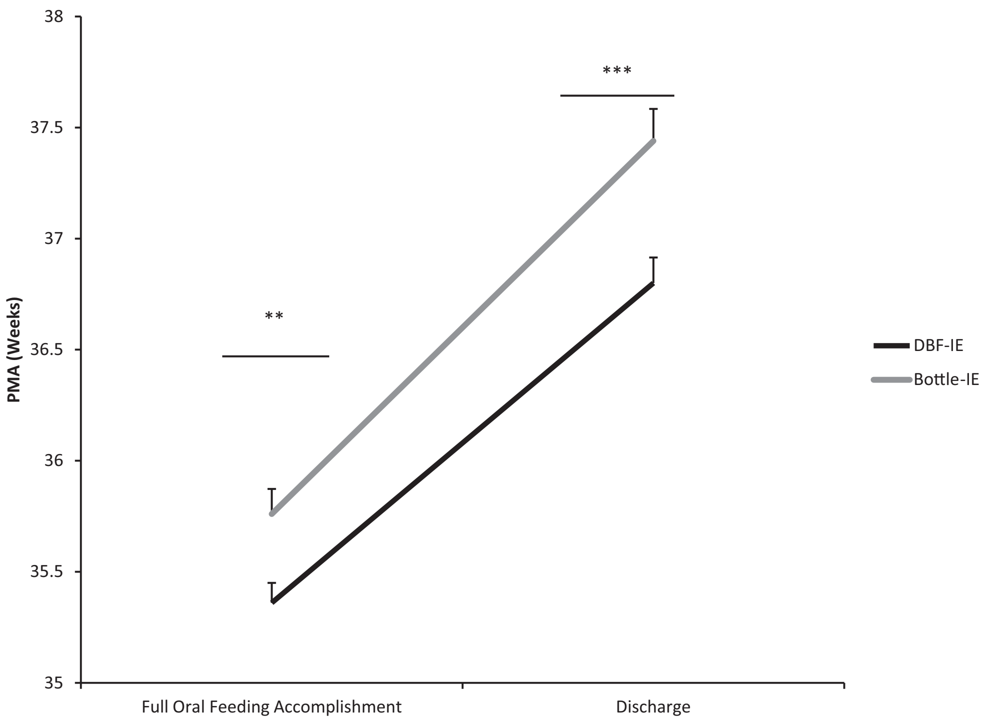

Figure 3. PMA at full oral feeding accomplishment and discharge of DBF-IE versus bottle-IE groups (adjusted means). PMA = postmenstrual age; DBF = direct breastfeeding; IE = initial exposure.
**$p < .01$. ***$p < .001$.

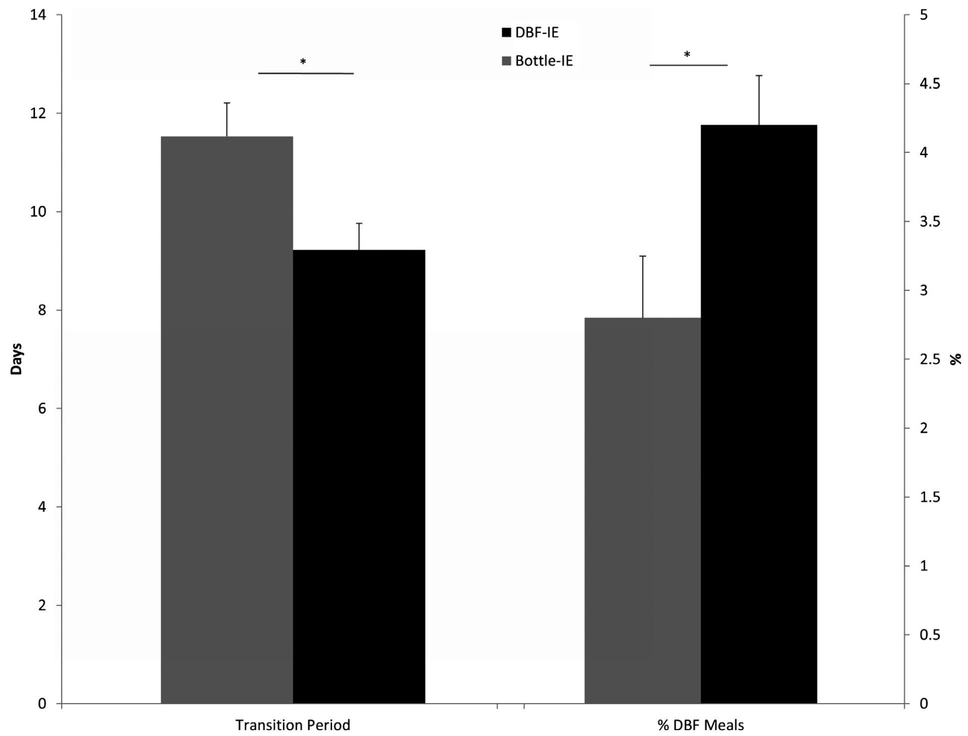

Figure 4. Transition period and DBF meals in DBF-IE versus bottle-IE groups (adjusted means). DBF = direct breastfeeding; IE = initial exposure.
$*p < .05$. $**p = .011$.

multiparous, PMA at first bottle feeding, PMA at first DBF, number of DBF meals prior to first bottle feeding, and percentage of MM consumed. Comparable models were explored with one caveat: In Regression Analyses 1 and 2, predicting length of transition period and PMA at full oral feeding accomplishment, the percentage of MM consumed up to full oral feeding accomplishment was entered, whereas in Regression 3, predicting PMA at discharge, percentage of MM consumed up to discharge was entered.

The data contained approximately normally distributed measures and had met the assumptions of collinearity, homogeneity of variance and linearity, and independent errors (Durbin–Watson: Regression 1 = 1.702, Regression 2 = 1.673, Regression 3 = 1.86). Analyses of standard residuals indicated two outliers in Regression 1, one outlier in Regression 2, and five outliers in Regression 3. The outliers were not included in the analyses. The final step of each regression is presented in Table 3.

Testing for predictors of length of the transition period, birth weight, IUGR, maternal age, and the number of DBF meals prior to bottle feeding were entered into the regression equation. Other variables were not found to predict the length of the transition period and were therefore not included. According to the multiple correlation coefficients (R^2 change), birth weight accounted for approximately 20.2% of the variance, IUGR accounted for approximately 4.9% of the variance, maternal age 4.2% of the variance, and number of DBF meals prior to bottle feeding accounted for additional 2.3% of the variance.

Similarly, testing for predictors of PMA at full oral feeding accomplishment yielded comparable variables, with PMA at first bottle feeding accounting for approximately 50.3% of the variance, birth weight for 7.8% of the variance, maternal age 2%, GA 1.7%, CRIB II score 2%, and number of DBF meals prior to bottle feeding accounted for additional 0.8% of the variance.

Testing for predictors of PMA at discharge showed that PMA at first bottle feeding accounted for approximately 46.9% of the variance, birth weight for 7.0%, IUGR for 2.5%, number of DBF meals prior to bottle feeding 1.6%, and PMA at first DBF accounted for additional 1.6% of the variance. No correlation was found between PMA at first bottle feeding and the number of DBF prior to bottle feeding ($r = -.099$, $p < .116$; *ns*).

In order to evaluate which variables predicted PMA at first bottle feeding, a stepwise regression

Table 3
Stepwise Regressions Predicting Length of Transition Period, PMA at Full Oral Feeding Accomplishment, and PMA at Discharge

Dependent variable	Independent variables entered	*B*	*t*	R^2	*F*	R^2 change	*F* change	*df*1	*df*2
Length of transition period (days)	(Constant)	24.850	10.152**	.316	28.035**	.023	8.014**	4	243
	Birth weight	−0.006	−4.695**						
	IUGR	5.047	4.078**						
	Maternal age	−0.228	−3.948**						
	DBF before bottle (count)	−0.400	−2.831**						
PMA at full oral feeding accomplishment	(Constant)	−4.440	−1.646	.647	73.602**	.008	5.759*	6	241
	PMA at first bottle feeding	0.973	11.541**						
	Birth weight	−0.001	−2.268*						
	Maternal age	−0.038	−4.488**						
	GA	0.267	4.588**						
	CRIB II	0.164	3.781**						
	DBF before bottle (count)	−0.051	−2.400*						
PMA at discharge	(Constant)	2.380	0.780	.596	71.762**	.016	9.764**	5	243
	PMA first bottle feeding	1.035	11.558**						
	Birth weight	−0.001	−3.386**						
	IUGR	0.862	3.896**						
	DBF before bottle (count)	−0.101	−4.114**						
	PMA first DBF	0.011	3.125**						

Note. DBF = direct breastfeeding; GA = gestational age; PMA = postmenstrual age; IUGR = intrauterine growth restriction.
**p* < .05. ***p* < .01.

Table 4
Final Step of Regression Testing for Predictors of PMA at First Bottle Feeding

Dependent variable	Independent variables entered	*B*	*t*	R^2	*F*	R^2 change	*F* change	*df*1	*df*2
PMA at first bottle feeding	(Constant)	30.018	33.185**	.368	35.004**	.012	4.535*	4	245
	IUGR	0.532	5.514**						
	GA birth	0.131	4.838**						
	CRIB II	0.056	3.546**						
	Apgar 5	−0.037	−2.130*						

Note. GA = gestational age; CRIB = Clinical Risk Index for Babies; IUGR = intrauterine growth restriction; PMA = postmenstrual age.
**p* < .05. ***p* < .01.

was conducted using a comparable regression model (see Table 4). IUGR, GA, CRIB II, and Apgar 5 scores were entered into the regression equation. The rest of the variables were not found to predict PMA at first oral feeding and were therefore left out. IUGR accounted for 29.5% of the variance, GA accounted for 2.4%, CRIB II score 3.7%, and Apgar 5 score accounted for additional 1.2% of the variance. Experience with DBF did not account for PMA at first bottle feeding.

Finally, in order to evaluate the relations between maternal age, parity, the inclination to DBF, and NICU outcomes (i.e., age at full oral feeding accomplishment, length of transition period, and age at discharge), a MANOVA was conducted. The analysis indicated that the interaction between the number of previous pregnancies and DBF was significant, $F(3, 236) = 2.77$, $p < .05$; Wilk's $\Lambda = .966$, partial $\eta^2 = .34$, as well as the interaction between maternal age and DBF, $F(3, 236) = 2.984$, $p < .05$; Wilk's $\Lambda = .963$, partial $\eta^2 = .037$, indicating that the older and more experienced the mother who directly breastfed, the better infant outcomes were. More specifically, univariate tests show that the older the mother, the shorter the transition period, $F(1, 238) = 4.778$, $p < .05$, partial $\eta^2 = .02$, and the younger the infant at full oral feeding accomplishment, $F(1, 238) = 4.267$, $p < .05$, partial $\eta^2 = .018$. There was no main effect of the number of previous gestations on NICU outcomes, $F(1, 238) = 2.198$, $p = ns$; Wilk's $\Lambda = .973$, partial $\eta^2 = .027$, indicating that in exploring maternal maturational factors, it is not the number of previous gestations alone that affects NICU outcomes.

Discussion

The present study sought to revisit the construct of feeding imprinting by using the test case of very preterm infants given the stronger implications feeding trajectories serve in this cohort. Key findings of this study suggest that a feeding imprinting process occurs in very preterm infants, with DBF imprinting leading to improved NICU outcomes. More specifically, findings showed that DBF imprinting was related to an earlier and quicker attainment of full oral feeding, a higher percentage of DBF throughout the NICU hospitalization period, and an earlier discharge from the NICU. In addition, similar to other imprinting phenomena, a dose–response function was seen, such that the number of DBF meals prior to first bottle feeding predicted the length of the transition period to full oral feeding, PMA at full oral feeding accomplishment, and PMA at discharge. This finding is consistent with the classic Sluckin and Salzen's (1961) framework by which imprinting in precocious organisms (in their case, birds) occurs in a sensitive time period and is strengthened by the amount of experience or exposure to the stimulus (Sluckin & Salzen, 1961). This conclusion should be treated with caution, as potential mediators and moderators may be effective in this process.

In considering the exposure notion, current data with infants born very preterm seem to take apart some of the unique contributions of each process in neonatal feeding, thus enabling the exploration of the role of exposure to content, administration mode, and timing of the exposure within the sensitive time period. Specifically the frequency of DBF meals prior to first bottle feeding was found to be a significant predictor of length of transition period, and PMA at full oral feeding accomplishment and discharge, but the percentage of MM consumed was not found to predict any of these variables. This finding uncovers the benefits of DBF in the process of oral feeding acquisition, thus suggesting that the known nutritional benefits of MM to the infant (Andreas, Kampmann, & Mehring Le-Doare, 2015; Eidelman & Schanier, 2012) are augmented by early exposure to DBF.

This was shown in two ways: First, infants who were DBF in the NICU received more MM throughout their NICU stay and at discharge compared to infants who were not DBF in the NICU, thereby replicating previous work by Pineda (2011a). Second, infants who were DBF imprinted obtained the ability to fully feed orally in a shorter amount of time compared to infants who were bottle imprinted, and accomplished full oral feeding at an earlier PMA, resulting in higher rates of DBF and a NICU discharge earlier by almost a week.

What accounts for this effect? It appears that maturation does not account for the DBF effect. Overall, PMA at first oral feeding was found to be a significant predictor of PMA at discharge and PMA at full oral feeding accomplishment, suggesting that earlier exposure to oral stimulation, regardless of stimulation type, possibly facilitates the acquisition of feeding skills. Importantly, however, earlier exposure did not predict the length of the transition period, indicating that there is more to the acquisition period than timing and earlier onset of exposure.

Ease of feeding may partly apply to the DBF effect. DBF has been considered by some as more strenuous than bottle feeding for infants in general (Ahluwalia et al., 2005; Lagan et al., 2014) and specifically for infants born preterm (Briere, 2015). Yet, data regarding very low birth weight (VLBW) infants have shown a greater stability of oxygen saturation and breathing during DBF when compared with bottle feeding (Blaymore Bier et al., 1993; Dowling & Thanattherakul, 2001).

Consequently, the process seems to be dependent on neonatal tailored exposure, specifically to tactile sensory stimuli, achieved through Merkel cell mechanosensors in the buccal mucosa (Mobbs et al., 2016). The oral tactile imprint is a learned form of perceptual recognition via Merkel cell mechanosensation which governs the imprinting process (Maksimovic, Baba, & Lumpkin, 2013). Other effects of DBF include somatosensory exposure along with an opportunity for bonding. Specifically, skin-to-skin contact, which naturally comes into play during DBF, has been shown to activate oxytocin release and reduce stress and anxiety responses in parents of preterm infants (Cong et al., 2015).

DBF could therefore set the beginning of the oral feeding journey with a more positive experience leading to a quicker learning process with more successful results, with more intensive exposure resulting in a more efficient transition. Indeed, the regression models underscored the number of DBF meals prior to the first bottle feeding as an important predictor of the length of the transition period to full oral feeding, PMA at full oral feeding accomplishment, and PMA at discharge.

Importantly, of all the variables found as predictors of these outcome measures, the number of DBF meals prior to the first bottle feeding is the only one over which caregivers may have some control. Being able to affect NICU outcomes directly is extremely significant, as most infant variables in the NICU are uncontrollable (i.e., birth weight, GA, health condition, etc.).

Pending replication, these findings may easily be translated into practice for all dyads with some caveats. Current data uncovered some demographic biases of infants' likelihood of receiving DBF. First, we found that infants born as part of a multiple were less likely to be DBF than singletons, in a manner compatible with some previous research (Geraghty, Pinney, Sethuraman, Roy-Chaudhury, & Kalkwarf, 2004; McDonald et al., 2012; Yokoyama et al., 2006) but not with others who examined VLBW infants born as part of a multiple birth (Pineda, 2011b; Smith, Durkin, Hinton, Bellinger, & Kuhn, 2003). The different finding may be explained by a higher rate of twins in the current cohort as compared with the latter cohorts (20% and 24.6%, respectively; Pineda, 2011b; Smith et al., 2003; compared to 53% twins in the present cohort) and higher birth weights, more mature GA, and sociodemographic advantages compared to the current cohort, differences that may have contributed to their high DBF rates (Smith et al., 2003). Further research is recommended in order to clarify this issue.

Another factor affecting the likelihood of DBF was conception via IVF. We found that infants conceived via IVF were less likely to be DBF than infants conceived spontaneously. This finding is partially concordant with data in other cohorts who do not necessarily comprise of infants born very preterm, showing that mothers who conceived via IVF were less likely to exclusively DBF their infants yet were as likely to provide some DBF (Castelli et al., 2015; Fisher et al., 2013), if not more so (Hammarberg, Fisher, Wynter, & Rowe, 2011), than mothers who conceived spontaneously.

Both conception of twins and conception via IVF involve significant segments of populations of infants born preterm. However, because conception via IVF often results in multiparous gestations, it is important to explore the interaction between these two factors as a selected group may explain these effects. Current data enabled us to explore this interaction. A logistic regression analysis showed that twins conceived through IVF were less likely to receive DBF compared to spontaneously conceived singletons, an effect not seen among non-IVF twins or IVF singletons. This then may point to a subgroup of mothers, mothers who conceived via IVF and gave birth to twins, who may require a targeted attention, as they are possibly challenged by their increased demands.

Finally, with regard to demographic characteristics that possibly play a role in the feeding imprinting process, maternal age predicted the length of the transition period and PMA at full oral feeding accomplishment. Note that the current study included mothers aged 20–51 and thus did not include teenage mothers. Findings showed that the older the mother, the shorter the transition period and the younger the infant is at full oral feeding accomplishment. Furthermore, the older and more experienced the mother who directly breastfed was, the better infant outcomes were. Note that solely having had previous pregnancies did not affect the NICU outcomes measured. This possibly underscores the role of maternal experience and maturation as a broader process that reflects a more complex process than solely the mother's personal direct experience. Given the wide age range of motherhood these days, the current finding suggests that an exploration of the topic is required.

Being a retrospective analysis, this study was limited by the inability to influence data collection. Data that were not available to be tested for mediatory or moderator effects on this study's findings include mother-related feeding measures (e.g., skin-to-skin care, prior maternal experience in DBF, and parental involvement in infant care), data regarding respiratory and feeding complications, and input from members of the multidisciplinary team at the NICU. Further research in the field may gain from addressing these variables.

Overall, current findings are suggestive of feeding imprinting in human infants by showing that DBF can help in facilitating infant thriving, thereby possibly contributing to public policy development and assisting NICU staff in forming evidence-based guidelines for team and parents regarding infant feeding in the NICU. There are a few protocols available to guide clinicians, yet they are not always soundly based on research evidence (Pickler, Reyna, Wetzel, & Lewis, 2015), and they are only partly adopted by NICU staff (Jackson et al., 2015). Pending replication, current results may yield future public policy changes in oral–motor intervention protocols for premature infants, as the data refuted the claims that infants who are DBF in the NICU are discharged later or at a lower weight than infants who are fed by bottle solely. This may imply that NICU staff may encourage mothers of premature infants to DBF their infants as many times as possible prior to the first bottle feeding, even if exclusive DBF is unattainable. That is, findings support the notion of feeding imprinting in late term age, highlighting the importance of further research into this vital process, not only for supporting infant survival and growth, but also for supporting infants' physical and emotional thriving later on.

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