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ORIGINAL ARTICLE

Effect of early breast milk expression on milk volume and timing of lactogenesis stage II among mothers of very low birth weight infants: a pilot study

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Objective: The purpose of this randomized pilot study was to collect preliminary data regarding the feasibility and effects of early initiation of milk expression on the onset of lactogenesis stage II and milk volume in mothers of very low birth weight (VLBW) infants.

Study Design: Twenty women were randomized to initiate milk expression within 60 min (group 1) or 1 to 6 h (group 2) following delivery. Milk volume and timing of lactogenesis stage II was compared between groups using Wilcoxon's rank sum tests.

Result: Group 1 produced statistically significantly more milk than group 2 during the first 7 days ($P = 0.05$) and at week 3 ($P = 0.01$). Group 1 also demonstrated a significantly earlier lactogenesis stage II ($P = 0.03$).

Conclusion: Initiation of milk expression within 1 h following delivery increases milk volume and decreases time to lactogenesis stage II in mothers of VLBW infants.
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Introduction

The provision of breast milk for infant nutrition is widely endorsed on international and national levels[1] and is one of the goals of Healthy People 2020.[2] The potential for improved health benefits in the premature infant is significant, especially for those weighing <1500 g at birth, or very low birth weight (VLBW) infants. Breast milk has been shown to significantly decrease morbidity associated with prematurity, including feeding intolerance, late onset sepsis and necrotizing enterocolitis.[3–5] Other suggested benefits include decreased length of hospital stay and improved neurodevelopmental outcomes.[6,7]

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Providing breast milk to the VLBW infant is a challenging experience for many mothers, as it is fraught with numerous obstacles. For various reasons, the number of mothers of VLBW infants who initiate breastfeeding is substantially lower than mothers of term infants,[8] and these mothers are likely to discontinue breastfeeding or providing breast milk much earlier than mothers of term infants.[9] The result is that infants who would gain the most from breast milk are the ones deprived of its substantial benefits.

Lactogenesis stage II signals the change from production of small quantities of colostrum to copious amounts of breast milk. This stage occurs following delivery, due to a dramatic decrease in progesterone, removal of milk from the breast and maintenance of prolactin levels.[10,11] In term mothers, this stage generally begins by 30 to 48 h following delivery.[10]

Delay in achievement of lactogenesis stage II is common in all mothers, with 22 to 31% of women entering this stage after 72 h following delivery.[12,13] Delay has been associated with decreased success in later lactation in mothers of term infants.[14] Preterm delivery and a delay in breastfeeding have also been associated with later onset of lactogenesis stage II.[10,15,16] Mothers of preterm infants who experience delayed attainment of this stage have demonstrated decreased milk volumes at 24 h after birth.[17] If a delay is associated with inadequate volume of breast milk for feeding, breast milk may be replaced with formula or initiation of enteral feedings may be delayed.

Insufficient milk supply is commonly cited by mothers of VLBW infants as the reason they elect to discontinue breastfeeding their infants.[18] Breast milk volume is correlated with gestational age, so mothers of VLBW infants have a strong tendency to produce significantly less milk than mothers of term infants.[19,20] Milk production in mothers delivering prematurely may be adversely affected by decreased mammary gland development and decreased exposure to prolactin, cortisol and other hormones that occur during a full-term pregnancy.[15]

In term infants, maintenance of lactation is dependent on suckling frequency and intensity.[21] Due to developmental, physiologic and gastrointestinal immaturity, VLBW infants are

unable to feed directly from the breast, so mothers must artificially express breast milk to feed their infants.[22] Artificial forms of milk expression may not effectively emulate the sucking mechanics of breastfeeding, potentially limiting maintenance of lactation, and causing a delay in lactogenesis stage II.

Earlier initiation of the first feeding at the breast and duration of breastfeeding have been associated with several studies' results, indicating that a delay of over 1 to 1.5 h was related to shorter breastfeeding duration.[23–25] Timing of initial breastfeeding following term delivery has been shown to correlate with milk volume on day 5, and a delay in initial breastfeeding has been shown to delay lactogenesis stage II in term infants.[13] Earlier initiation of milk expression has also been associated with increased milk volume in mothers of VLBW infants.[26–29]

Unfortunately, mothers of VLBW infants often delay milk expression for hours to days following delivery. In an observational study, Hill *et al.*[30] found that the mean delay in initiation of milk expression was 34 h in mothers of VLBW infants. Studies assessing the association of initiation timing and milk volume showed a wide discrepancy in timing of initial expression, which often began over 24 h following delivery. The earliest expression initiation was reported by Furman *et al.*,[26] who found initiation prior to 6 h following delivery was positively associated with prolonged lactation over 40 weeks' postmenstrual age. These researchers, however, did not assess the effect of initiation timing on milk volume or lactogenesis stage II. Studies examining the association of milk expression within the first hour following delivery and milk volume in mothers of VLBW infants are lacking. Therefore, the purpose of our randomized pilot study was to evaluate the feasibility of initiation of milk expression $\leqslant 60$ min, compared with initiation between 1 and 6 h, following delivery in mothers of VLBW infants. We also sought to collect preliminary data regarding the effect of early initiation on the onset of lactogenesis stage II and milk volume.

Methods

Study population

A convenience sample of 20 pregnant women carrying a singleton fetus with an estimated gestational age <32 weeks and an estimated fetal weight of <1500 g were recruited for this feasibility study from a labor and delivery unit associated with a level III tertiary neonatal intensive care unit. Exclusion criteria consisted of (1) younger than 18 years, (2) no intention to breastfeed, (3) non-English speaking, (4) presence of major fetal anomalies, (5) illicit maternal drug use, (6) history of breast reduction or augmentation, (7) positive HIV status or (8) the fetus not expected to live over 2 weeks following delivery. Approval for the study was obtained from the Institutional Review Board at the University of Florida. Mothers were approached for enrollment in their antepartum hospital room, where they were invited to participate and sign a written consent form.

Randomization

Following delivery, mothers were randomized to one of the two groups using a sequentially numbered envelope system. Group 1 (early initiation) ($n = 10$) began milk expression within 60 min following delivery. Group 2 (late initiation) ($n = 10$) began milk expression between 1 and 6 h following delivery.

Milk collection and volume measurement

The initial milk expression session, facilitated by a registered nurse or member of the study team, lasted for 15 min. A simultaneous expression symphony breast pump (Medela, McHenry, IL, USA) was used for artificial expression of milk. The volume of milk expressed during this session was weighed on an electronic digital scale (Scout Balance, Florham Park, NJ, USA) to the nearest 0.1 g. Following the initial pumping session, mothers received standard written and verbal pumping instructions. This included information explaining that their breasts needed to be pumped at least eight times per day. Mothers were instructed to pump simultaneously for 15 min. Although mothers were instructed on the benefits of simultaneous pumping, if they elected to pump sequentially, they were instructed to pump for 10 min on each side. If milk was still flowing, mothers were instructed to continue pumping for 2 min after flow of milk ceased. In addition, each mother received a pamphlet with explicit instructions about milk expression, collection and storage as per the neonatal intensive care unit protocol. All mothers had access to a simultaneous electric symphony breast pump during hospitalization and infant visitation, and an equivalent breast pump for use at home. Mothers were instructed to store their expressed breast milk in a collection vial provided to them, and to date and time their collection vial of breast milk from each expression session. They were then instructed to bring the vials to the neonatal intensive care unit when visiting their infants, and the vials were stored in the hospital freezer.

Measurement of 24-h milk volumes were obtained on days 1 to 7, day 21 and day 42 by weighing each vial of expressed milk brought in by the mother on an electronic digital scale (Scout Balance) to the nearest 0.1 g and summing together all milk expressed during a 24-h time period. The weight of breast milk in grams is nearly equivalent to its volume in milliliters and is a precise method of determining the amount produced.[31] Mothers in both groups were instructed to record in a daily log book the date, time and duration of each pumping session, type of pump used and whether they received lactation consultation. Frequency, timing and length of Kangaroo care (holding of an infant dressed only in a diaper upon a mother's or father's bare chest) were also recorded by either the nurse or the mother during hospitalization. If the infant breastfed during the 24-h milk volume measurement session, intake

was measured by test weighing (weighing infants prior to and following breastfeeding), which has been shown to be an accurate method to determine intake during breastfeeding in preterm infants.[32]

Determination of lactogenesis stage II

Beginning at 24 h and continuing daily until initiation of lactogenesis stage II, mothers were questioned regarding if and when they noticed a sudden feeling of fullness in their breasts. Timing was recorded in 12-h increments as occurring (0800 to 2000 or 2000 to 0800 hours). This has been a traditional method of determining timing of lactogenesis stage II and has been shown to correlate with actual timing of this stage.[33]

Data analysis

Data entry and analysis were performed using SPSS-PC (SPSS, version 17, JPSS, Chicago, IL, USA). Demographic differences between groups were compared using *t*-tests for continuous data and χ^2 for nominal data. Due to non-normal distribution of the outcome data, mean milk volumes for each of the 24-h periods and timing of lactogenesis stage were compared using Wilcoxon's rank sum tests. As this was a feasibility study with a small sample size ($N = 20$), statistical significance was not anticipated, but rather trends toward statistical significance were projected. *P*-values of 0.05 or less were considered statistically significant.

Results

The convenience sample for this pilot study consisted of 20 mothers who delivered VLBW premature infants. Thirty-two mothers were consented for the study. Ten of these mothers were excluded following delivery due to delivery of an infant $\geqslant 32$ weeks, weighing $\geqslant 1500$ g ($n = 9$) or not expected to live over 2 weeks ($n = 1$). Two mothers delivered infants meeting inclusion criteria, but the study team was not notified in time to randomize prior to 1 h following delivery. Only one mother who was approached declined to participate in the study. Groups were demographically similar, and there were no significant difference between provision of Kangaroo care and expression frequency between groups (Supplementary Table 1). The mean gestational age of infants whose mothers enrolled in this study was 27.4 weeks with a mean birth weight of 994.2 g: 55% were Non-Hispanic White and 40% were Non-Hispanic Black.

Exploratory analysis of the study data showed that mothers in the early initiation group produced significantly more milk during the first 7 days ($P = 0.5$). Total milk volume expressed by mothers in the early expression group during the first week was over twice that of mothers in the late expression group, respectively (1374.7 vs 608.1 ml, $P = 0.05$). Mean milk volumes for early and late initiation groups are shown in Table 1.

Women in the early initiation group produced significantly more breast milk at 3 weeks ($P = 0.01$) following delivery compared with the late initiation group (Table 1). A total of 80% (8/10) of women in the early initiation group and 70% (7/10) in the late initiation group continued milk expression for 3 weeks, while 60% (6/10) of women in the early initiation group and 40% (4/10) in the late initiation group continued milk expression for 6 weeks. No statistically significant differences in length of milk expression between groups existed at either 3 weeks ($P = 0.69$) or 6 weeks ($P = 0.32$).

Table 1 Milk volume (ml)

Time	*Volume of milk*		
	Early initiation (n = 10)	*Late initiation* (n = 10)	
	M	*M*	*P*
Initial expression session	4.19	0.1	0.14
Day 1	19.2	0.7	0.06
Day 2	76.7	2.2	0.01
Day 3	142.3	45.4	0.14
Day 4	185.7	69.9	0.09
Day 5	282.0	85.8	0.06
Day 6	322.0	191.9	0.06
Day 7	355.0	188.8	0.1
Total at 1 week[a]	1374.7	608.1	0.05
	$N = 8$	$N = 7$	
3 weeks[b]	613.0	267.2	0.01
	$N = 6$	$N = 4$	
6 weeks[c]	451.0	209.95	0.07

[a]Sum of all milk volume for days 1–7.
[b]24 h milk volume at 3 weeks.
[c]24 h milk volume at 6 weeks.

Mothers in the early initiation group attained a statistically significant earlier lactogenesis stage II compared with mothers in the late initiation group (80.4 vs 136.8 h, $P = 0.03$). Substantial variation was noted over both groups with timing of lactogenesis stage II, ranging from 14 to 216 h with a mean of 108.6 h following delivery.

Discussion

To our knowledge, this is the first study investigating effects of breast milk expression within 1 h following delivery on milk volume and timing of lactogenesis stage II in women who deliver VLBW preterm infants. While results of this pilot study are tentative, findings suggest that initiation of milk expression prior to 1 h following delivery may increase milk volume and decrease time to lactogenesis stage II compared with initiation of milk expression between 1 and 6 h following delivery.

Mothers in the early milk initiation group had greater milk volumes at the initial milk expression session and at every 24-h period during the first 7 days following delivery. Total milk volume

during the first week was over twice as much for mothers in the early initiation group compared with those in the late initiation group. Differences in milk volume between groups were statistically significant for the first 7 days and on day 2. Due to the small sample size of 20 mothers, this pilot study was not powered to detect statistically significant differences between groups. However, clinically important differences in milk volume between groups and trends toward significance did suggest that a larger, adequately powered study may indicate statistically significant differences in milk volume between groups for all time points.

Results are congruent with other study findings of a relationship between earlier initiation of milk expression and increased milk volume.[28,29] This study is the first, however, to investigate specific timing of milk expression and the influence of milk expression within 1 h following delivery on milk volume in mothers of VLBW infants. Hill *et al*.[19] found initiation of milk expression in mothers of VLBW infants occurred 27.3 ± 14.9 h following birth and that timing of initial milk expression inversely correlated with milk volume at 1 week. Furman *et al*.[26] found a positive association between initiation prior to 6 h following delivery and prolonged lactation over 40 weeks' postmenstrual age. Hopkinson *et al*.[29] also reported a positive correlation between day of milk expression initiation and milk volume at week 2.

Thirty percent (3/10) of women in the late expression group expressed 0 ml of breast milk for 3 of the first 7 days. Many neonatal intensive care units initiate enteral feedings of VLBW infants within 24 h following birth. If mothers fail to express sufficient quantities of breast milk during this time, formula feedings may be substituted or feedings may be delayed until sufficient breast milk is available.

Tremendous variation in milk volume existed among individual mothers with total milk volume for the first week, ranging from 148 to 4021 ml. Little research exists regarding breast milk volume in mothers of VLBW infants during the first week following delivery. Henderson *et al*.,[34] found mothers of VLBW infants produced 264 to 4137.9 ml of breast milk during the first 7 days. Milk volume obtained from these mothers is less than those previously reported for mothers of term infants, suggesting that mothers of VLBW infants may produce less breast milk than mothers of term infants in the first 7 days following delivery.[31,35,36]

Mothers in the early initiation group had a significantly shorter time to onset of lactogenesis stage II than mothers in the late initiation group. This suggests that milk expression within the first hour following delivery may result in an earlier onset of lactogenesis stage II. Impaired lactogenesis stage II is associated with decreased milk volume in mothers of VLBW infants; therefore, earlier onset of lactogenesis stage II may be important in enabling mothers to provide sufficient breast milk for their infants during the first few days of life.[15]

Few studies have investigated timing of lactogenesis stage II in mothers of VLBW infants. This stage generally begins by 30 to 48 h following delivery in term mothers.[37] Women in this study initiated lactogenesis stage II an average of 108.6 h following delivery, indicating a delay in initiation of this stage in this sample. Such a delay is consistent with the findings of Cregan *et al*.,[15] who found 82% of mothers delivering at 31 to 35 weeks' gestation had impaired lactogenesis stage II. Similarly, Henderson *et al*.[34] found a delay in initiation of this stage by 1 day in mothers who delivered prior to 28 weeks' gestation.

The amount of breast milk produced by mothers of VLBW infants is often substantially reduced at 3 to 4 weeks following delivery, thereby potentially limiting mothers' ability to provide exclusive breast milk feedings for their infants.[19,27] This was apparent in this study with a substantial decrease noted in milk volume for those mothers who continued pumping for 3 and 6 weeks. Women in our early initiation group produced significantly more breast milk 3 weeks following delivery. This contradicts findings of Hill *et al*.,[19] who found that while earlier initiation of milk expression was correlated with early milk output (week 1), it was not correlated with milk output at weeks 2 through 6. Conversely, Hill *et al*.[27] found timing of expression initiation correlated with adequate milk production (defined as milk output of $\geqslant 3500$ ml per week) during weeks 2 through 6.

Later adequacy of milk production in mothers of VLBW infants is associated with milk volume during week 1, suggesting the importance of early strategies to facilitate adequate milk volume.[27,28] A majority of factors negatively influencing milk volume in lactating mothers are not amendable to change, so clinicians must focus on modifiable interventions to increase milk volume.[13] Although some study findings have confirmed that earlier initiation of milk expression following delivery improves milk supply in mothers of VLBW infants,[26,28,38] many mothers delay milk expression for hours to days following delivery, which may negatively impact milk volume. Mothers should be encouraged and provided with support to initiate milk expression as soon as possible after delivery, preferably within the first hour.

Certain limitations existed in this pilot study. This pilot study included a small sample size of 20 women and, therefore, a much larger, appropriately powered study is necessary to confirm our conclusions regarding benefits of milk expression within 1 h following delivery in mothers of VLBW infants. Further, *P*-values in this pilot study were not adjusted for multiple comparisons to avoid overly conservative conclusions.

Conclusion

This pilot study is the first to our knowledge to examine the effect of milk expression within 1 h of milk volume and lactogenesis stage II. Findings suggested that initiation of milk expression within 1 h following delivery in mothers of VLBW infants is feasible. This early milk expression may be an effective strategy to increase milk volume for the first 7 days and 3 weeks following delivery,

and to decrease time to lactogenesis stage II. Since early milk volume is associated with adequacy of later milk volume, strategies aimed at increasing milk volume during the early postpartum period are imperative for mothers of VLBW infants. Our findings suggest that mothers expressing breast milk within 1 h following delivery experience increased milk supply for the first week. To benefit from this potential increase, health-care providers should assist and support mothers to begin milk expression as soon as possible following delivery.

Conflict of interest

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Journal of Perinatology website (http://www.nature.com/jp)