Early Initiation of Milk Expression in Mothers of Very Low Birth Weight Infants

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PROTOCOL

Research Design

A randomized clinical trial with three treatment arms will be conducted to determine the efficacy of alternative timing of breast milk expression (mechanical pumping) on both short and long-term breast milk supply, and Mothers who have delivered a VLBW infant prematurely will be stratified by gestational age and then randomly assigned to one of three groups and asked to mechanically pump their breasts beginning at a specific time. Timing of lactogenesis stage II will be documented, and volume of breast milk produced will be measured for the first 6 weeks following delivery.

Sample and Setting

180 mothers who have been hospitalized anticipating delivery of an infant less than 32 weeks' gestation and weighing less than 1500 grams will be sampled by convenience from a maternity unit at Shands Teaching Hospital, a Level III tertiary care center. With a 5% anticipated drop-out rate, the final sample will include 180 mothers. The hospital is part of the University of Florida and includes a Level III (52 bed) NICU. The hospital catchment area encompasses North Central Florida and Southern Georgia, a predominantly rural and semi-rural population. 120 VLBW infants per year are delivered with 95% of these women electing to express breast milk for their infants. In our preliminary work, all but 1 mother consented for the study.

Inclusion criteria for the mothers are: 1) at least 18 years of age, 2) English speaking , 3) stated intent to breastfeed, 4) anticipating the birth of a very low birth weight infant (≤1500 grams) between 23-32 weeks gestation. Exclusion criteria are: 1) known illicit maternal drug use, 2) history of breast reduction or augmentation, 3) positive HIV status, 4) mother not transported to recovery by 45 minutes following delivery or 5) infant not expected to live over 2 weeks following delivery.

All infants born to mothers will be included in the sample (see Targeted Enrollment Table, for Infants). Mothers delivering twins will be included since milk volume in pump dependent mothers of multiple births have been shown to be similar to mothers delivering singletons (Bishara, Dunn, Merko, & Darling, 2009). Mothers will not be approached if they have receive pain medicine within the last 4 hours. Their mental and physical ability to consent will be verified with the nurse caring for them.

Sample Size Determination / Power Analysis

The primary foci of this study are amount of breast milk expressed after initiation and time to lactogenesis II. For the continuous longitudinal outcome amount of breast milk expressed, with 60 subjects randomized to each intervention group (early, intermediate, late initiation), we will have 85% power to detect at least a 0.5 standardized (sd units) effect size between the three intervention groups [assuming 13 time points (initial expression, days 1-7, 14, 21, 28, 35, 42)]; intra-class correlation no greater than 0.5; level of significance (α)=0.02, two-tailed]. A standardized effect size of 0.49sd (Cohen's d, "moderate" effect) is equivalent to a raw effect size of 125 ml (raw scale units) for expressed breast milk (assuming pooled sd for expressed breast milk is 250, from preliminary data. For number of hours to achieve lactogenesis II assuming α = 0.02 (Type I error rate), two-sided; independent sample t-test comparison of means (adjusted for multiple comparison between 3 groups); equality of variance between groups with 60 mothers per group we will have 85% power to detect a difference of 34.1 hours (effect size Δ=0.62s; s=55.0) between the groups.

For analysis of the continuous secondary efficacy outcome measure self-efficacy, we will have 85% power to detect a difference of 0.62 s between the three groups assuming α = 0.02 (Type I error rate), two sided; independent sample t-test comparison of means; equality of variance between groups accounting for multiple comparisons.

Randomization

Table 1. Randomization and Stratification of Mothers

Gestational Age	Group 1 Early Expressers	Group 2 Intermediate Expressers	Group 3 Late Expressers
23-27 weeks	0-60 minutes	61-180 minutes	181-360 minutes
28-< 32 weeks	0-60 minutes	61-180 minutes	181-360 minutes

Informed consent will be obtained prior to delivery. Table 1 (below) shows randomization/stratification of mothers into groups. Following the birth of an infant weighing less than 1500 grams, with a gestational age between 23-32 weeks, mothers who continue to meet inclusion criteria will be stratified according to the gestational age of the infant (23-27 weeks and 28-32 weeks). Gestational age will be determined through one of the following; 1st or 2nd trimester ultrasound or the average of the obstetric gestational age and the results of the Ballard exam. This stratification is based upon likely differences in breast milk production in mothers who deliver earlier in gestation due to decreased mammary gland development and decreased levels of prenatal hormones necessary for preparation of the breast for lactation.

Following stratification, mothers will be randomly assigned to one of three groups to begin milk expression at different time periods following delivery. Group 1 (early expressers) will begin milk expression 0-60 minutes following delivery, Group II (intermediate expressers) will begin 61-180 minutes following delivery, and Group III (late expressers) will begin 181-360 minutes following delivery. The selection of the time periods for the groups is based upon our preliminary data showing greater milk volume and earlier lactogenesis stage II in women who began milk expression within 1 hour following delivery compared to those who began milk expression 1-6 hours following delivery (Parker et al., in press).

Procedure

The procedures used in this study will be the same as those successfully used in our recent pilot study. Mothers will be randomized, assisted with their first mechanical milk expression session, and then asked to bring their expressed breast milk into the NICU for weighing. Additionally, mothers will be asked when they felt a sudden sensation of fullness in their breasts indicating the initiation of lactogenesis II.

Primary Aim

The primary aim of this study is to compare over; the infant's hospitalizationthe effect of timing of initiation of breast milk expression on timing of lactogenesis stage II and amounts of breast milk produced during the following time periods:

- (a) early (within 1 hour of delivery),
- (b) intermediate (within 2-3 hours) and
- (c) late (within 3-6 hours).

Once mothers are randomly assigned to one of the three intervention groups, the research coordinator, the PI, or a research assistant experienced with lactation will meet with the mother at the designated time, confirm her continued consent, and assist her with her first breast pumping session using a hospital grade breast electric breast pump. Mothers who have recently delivered premature infants often take time to recover from anesthesia and may be clinically unstable. Since these mothers cannot always express their breasts independently, the above research team members will assist these mothers with milk expression. All research team members who assist mothers with the initial milk expression session will either be certified lactation consultants or have extensive training in lactation and milk expression. Assistance may range from education to actually performing the milk expressing for the mother. In the pilot study, all mothers randomized to milk expression within one hour were able to have their breasts expressed within the first hour following delivery Since individuals assisting mothers with the initial breast expression may be required to have significant involvement in the milk expression process, they will be evaluated prior to assisting mothers and their skills will be monitored bimonthly by the research coordinator or PI to ensure that a safe and standardized procedure is used. Furthermore, a procedure checklist will be used to ensure consistent information and instruction is provided to all mothers (Appendix A). Following the initial milk expression session, mothers will receive the standard written and verbal pumping instructions provided to all of the mothers who are pumping breast milk for their infants (Appendix B). Information regarding maternal factors will be collected by a research team member (Appendix C)

Mothers will be visited in their hospital room by the research coordinator, PI, or a research assistant who has completed a formal lactation course approximately 24 hours after the initial breast pumping session to ensure they are successfully using the breast pump and to provide additional instruction if needed. Mothers will also have access to lactation services and nurses on the unit. There are currently 3 part time lactation specialists

employed in the hospital who provide services to all lactating mothers in the hospital. Lactation services are provided in the NICU upon request from the nurse or physician. This is congruent with all other breastfeeding mothers and those expressing milk for their infants in the NICU.

Mothers will be provided with a supply of pre-weighed and labeled milk vials in which they will place the milk following expression per standard NICU policy. During hospitalization or while visiting their infant in the NICU, mothers will have a hospital grade electric pump available for use. Upon discharge, all mothers will be provided the same hospital grade electric pump for use while their infant is in the NICU and they continue to lactate. Mothers will be asked to bring vials from all pumping sessions to the NICU, regardless of apparent results. While mothers are still hospitalized, the mother or nurse will bring the vials to the NICU following each expression, where they will be deposited in a refrigerator specified for storage of breast milk for all infants in the NICU. Once mothers go home, they will be instructed to store their vials of expressed breast milk in the refrigerator or freezer at home, and to bring vials to the NICU when visiting their infants (standard NICU policy). All breast milk will be weighed by a research assistant blinded to the group assignment of the mother. After milk is weighed, the milk will be available for infant consumption. Should breast milk be required for infant consumption prior to being weighed, the bedside registered nurse will remove the necessary breast milk with a syringe and record the exact amount of milk removed (mL of breast milk is equivalent to mg of breast milk). Mothers who continue milk expression will be reminded 3 times per week—through phone calls, texts or during visits to the NICU—to bring in their breast milk when they visit.

Measurement of milk volumes by weight will occur at the initial expression session, for the first 7 days, and for one 24-hour period on weekly until the infant is discharged from the hospital. (see Table 2 for a list of all instruments). If the infant breastfeeds during the 24-hour period, test weighing will be used to calculate breast milk consumed. Test weighing or the weighing of infants prior to and following breastfeeding, has been shown to be an accurate method of determining intake during breastfeeding in preterm infants (Haase, Barreira, Murphy, Mueller, & Rhodes, 2009; Hurst, Meier, & Engstrom, 2004). Prior to each breastfeeding session, infants will be weighed by the nurse assigned for patient care, and weight will include the clothing, diaper and/or blankets infant is wearing. Following completion of the breastfeeding session, the infant will be weighed again, clad in the same clothing as with initial weight. Infants will be weighed on the Baby Weigh scale (Medela, Inc, McHenry, Ill) to the nearest 0.2 g. The Baby Weigh scale is a portable electronic digital scale that is accurate to within 0.2 grams. The scale will be calibrated by the study coordinator prior to initiation of the study and after every 10 measurements, using 0.2mg, 1 mg, 10mg and 50 mg weights. All weights will meet or exceed ASTM (American National Standards Institute) specifications and will be calibrated by a NVLAP (National Institute of Standards and Technology) accredited lab. The difference between the pre and post-feeding weights will be recorded on the Breastfeeding Data Collection Form taped at the infant's bedside and will be added to the total amount the mother expressed during that 24-hour period (Appendix E).

Table 2. Instruments (Logs)

Variable	Instrument	Measurement
Maternal Factors	Log maintained by Project Coordinator	Age, parity, diabetes, pregnancy induced hypertension, mode of delivery, type of anesthesia, experience with breastfeeding, smoking, obesity, antenatal steroids
Lactation Factors (1-3 below)		
1. Onset of lactogenesis stage II	Log maintained by Project Coordinator	Date and time of sensation of breast fullness Corresponding breast milk measurement
2. Milk volume	Log maintained by Project Coordinator	Daily total breast milk weight for days 1-7 24 hour sample weekly
3. Milk volume alternative for breastfed infants	Log maintained by Project Coordinator	(Test weighing) - difference in infant's weight before and after feeding as indicator of breast milk intake.
Behavioral Factors	Log maintained only by mother	Date and time of pumping sessions, episodes of lactation support. **Kangaroo Care log** will be maintained separately (see below).
Kangaroo care	Log maintained by nurse or mother	Incidence and length of kangaroo care in min.
Infant breast milk intake (1-2		

below)		
1. Weekly percentage of breast milk received by infant during first 6 weeks of life	Log maintained by Project Coordinator	Data extracted from medical record
2. Percentage of breast milk feedings at discharge	Log maintained by Project Coordinator	Data extracted from medical record

Mothers in all groups will be instructed to record daily in the Maternal Behavioral Factors log book the following data: number of expression sessions per day, the date and time of each pumping session, and whether they received lactation assistance (Appendix F). The frequency of milk expression will be verified by the research coordinator by documentation of the date and time of each vial of milk brought to the NICU. Kangaroo care is encouraged in this NICU and nursing protocols have been established to provide support to all mothers regarding provision of kangaroo care. Infants are also encouraged to suckle at the breast during kangaroo care. Episodes and duration of kangaroo care between mother and infant will recorded on the Kangaroo Care log sheet (Appendix G) located at the infant's bedside by either the mother or the nurse. While control of these variables within the study is impossible, we will carefully document these behavioral factors to allow for inclusion in statistical analysis as adjustment variables.

Beginning 24 hours after delivery and continuing daily, mothers will be questioned (either in person or over the phone) regarding a sudden feeling of fullness in their breasts as an indicator of the onset of lactogenesis II (Appendix H). This has been a traditional method of determining the timing of lactogenesis stage II and has been shown to correlate with actual timing of this stage (Chapman, Perez-Escamilla, 2000; Perez-Escamilla & Chapman, 2000). Due to the effects of anesthesia, post op pain, and pain medication, mothers may have difficulty appreciating indicators of the onset of lactogenesis stage II, we will therefore confirm the reliability of self-report by determining whether >100mL of milk is produced per a 24-hour period (Neville, Morton, & Umemura, 2001). Since it is anticipated the onset of lactogenesis stage II will occur during the first 7 days, the number of hours post-delivery until lactogenesis stage II will be captured and recorded by research team members in the Milk Volume log.

Prior to discharge, at day 21 and 42, mothers will be asked to complete the MAACL-R. The MAACL-R is a standardized instrument with well-documented psychometric properties that assesses mood states by using positive and negative mood states scales for Anxiety, Depression, Hostility, Positive Affect and Sensation Seeking. It consists of 132 adjectives that are at or below an 8th grade reading level and takes approximately 3 minutes to complete. This test has been successfully used by the consultant for this project in lactating mothers of premature infants (Hill et al 2006; Lubin and Zuckerman, 1999). Mothers will be phoned by the research coordinator the day before the scheduled testing day to remind them to complete the test. The written test that will be completed following discharge will be provided to the mothers upon study entry. Mothers will return the test when they visit their infant and results will be hand scored and recorded on the MAACL-R Result Log by either the RA or the research coordinator. The test and grading sheet will be purchased with institutional funds upon initiation of this project.

Prior to discharge it will be determined whether mothers are continuing to express breast milk. This will be determined by whether mothers are bringing expressed breast milk to the NICU for infant consumption. If they are no longer expressing breast milk, they will be contacted to determine whether they would be willing to participate in a 10 minute interview concerning reasons for cessation of breast milk expression and their feeling concerning cessation of lactation. If interested, an appointment will be made for the interview during a time when the mother will be present in the hospital visiting her infant and a ~~RA who is a~~ licensed registered nurse will ask the mother (5) questions (see addendum S). Should the mother find this to be a stressful topic, the interview will cease immediately and a social worker employed in the neonatal intensive care unit experienced with caring for mothers of premature infants will be immediately notified and will assess whether further services are required.

The percentage of breast milk the infant receives weekly and the percentage of breast milk the infant is receiving upon discharge (mL/kg/d for the week prior to discharge) will be collected from the medical records (Appendix J). Neonatal nurses and physicians caring for the infants will not be told of the mother's assigned group.

Exploratory Aim 1

Exploratory Aim 1 is to examine the effect of timing of initiation of breast milk expression on infant breast milk intake (e.g., weekly percentage of breast milk the infant receives during hospitalization and amount of breast milk infant is being fed at discharge). This information will be collected from the infant's medical records.

Exploratory Aim 2
Exploratory Aim 2 is to examine the effect of timing of initiation of breast milk expression on milk volume and lactogenesis stage II in mothers who deliver at an earlier gestation (23-27 weeks) and those who delivery at a later gestation (28-32 weeks). Information will be collected according to the procedure in Aim 1.

Equipment
A simultaneous electric Symphony Breastpump ® (Medela, Inc.) will be used during hospitalization, and infant visitation and the same pump will be provided to all mothers enrolled in the study for home use while their infant is hospitalized. These are hospital-grade pumps that can be used for simultaneous breast milk expression (both breasts at the same time). Milk will be weighed on a Scout Balance electronic scale, a portable digital scale accurate to within 0.1 grams (Scout Balance, Florham Park, NJ). The scale contains an external calibration mechanism, stabilizes within three seconds, and will be calibrated by the research assistant prior to initiation of the study and after every 10 measurements using 0.1mg, 1mg, 10mg and 50 mg weights. All weights will meet or exceed American National Standards Institute (ASTM) specifications and will be calibrated by a National Institute of Standards and Technology (NVLAP) accredited lab. The scale will be placed on the same platform at all times and will be wiped clean with a moist cloth and dried between each weighing.

Statistical Analysis

Descriptive Statistics. Descriptive statistics will be calculated for all variables, as appropriate. Continuous demographic and baseline clinical variables will be compared using t-tests or Wilcoxon rank sum tests. Categorical variables will be compared using chi-square or Fisher's Exact Test. Sample distributions will be evaluated to ensure that distributional assumptions underlying the proposed statistical tests have been met.
Design/Analysis Issues.

Analysis sets. For primary analyses the intent-to-treat (ITT) sample will be used comprising all randomized mothers patients. Secondary per-protocol (completer) analysis will include mothers who were compliant with protocol requirements and for whom all required measurements have been made. ITT and per-protocol analyses will be compared to test the sensitivity of our conclusions to non-adherence/attrition.

Missing data. Every attempt will be made by the study coordinator to keep mothers in the study, with the help of the student research team members and to obtain required measurements in order to ensure the completeness of the study data. To minimize potential bias due to missing observations, mothers will be contacted by phone if vials of expressed breast milk are missed, and reasons for missing data will be carefully documented. Subject participation will only be terminated due to safety reasons for mother and infant, withdrawal of consent, or loss to follow-up. Several approaches to handle missing data in the ITT analysis set will be employed. For continuous variables using a single end-of-study value (e.g., amount of milk expressed at day 42), Little and Rubin's multiple imputation methods will be used (Little & Rubin, 1997). For analyses of longitudinal outcome data, we will use longitudinal methods (e.g. mixed models) capable of dealing with missing data (Hedeker & Gibbons, 2006).

Multiple outcome variables. In order to address possible Type I errors due to the number of outcome variables, we specify the primary outcome measures a priori corresponding to the stated a priori hypothesis and maintain the alpha (significance) level at 0.02. For analysis of secondary outcome variables, we will further adjust resulting p-values using Bonferroni-type corrections for multiple outcomes. Both unadjusted and adjusted p-values will be reported.

Analyses for Aim 1
The primary efficacy outcomes are amounts of breast milk expressed over the first 7 days post-initiation; days 14, 21, 28, 35, and 42 days post-initiation and weekly, and number of days to achieve lactogenesis stage II. To examine expressed breast milk amount over time (i.e. production of breast milk immediately after initiation and subsequently, volume measured daily over the first 7 days and on days 14, 21, 28, 28, and 42 post-initiation and weekly), we will compare the longitudinal profile of milk volume expressed in the three groups over the

study period using mixed effects models (MEM) analyses. These analyses will estimate the average change in breast milk volume within each group (early vs. immediate vs. late initiation) and individual change in breast milk volume for each mother. MEM analyses allow for missing data, measurement of study subjects at different time points during the study, and time varying covariates. MEM can also take into account the effect of clustering, i.e. correlation of repeated breast milk measurements within one subject (Hedeker & Gibbons, 2006; McCulloch & Searle, 2001). The efficacy variable expressed breast milk volume (mL) will be used as the dependent variable with treatment status (early vs. intermediate vs. late initiation), time and time-by-treatment interaction and gestational group (23-27 weeks vs. 38-32 weeks) as primary independent variables. In a second step, additional covariables will be included in the models to adjust for putative predictive variables such as age, mother's weight at delivery, diagnosis of preeclampsia or diabetes, parity, birth weight, weeks of gestation, behavioral factors including skin-to-skin contact ("kangaroo care"), lactation support and mean number of pumping attempts per day. Effect modifications of covariables will be examined through inclusion of a covariable-by-treatment interaction term in the multivariable models. For example, a significant age-by-treatment interaction would suggest that the relationship between treatment and milk volume is different for older mothers compared to younger mothers. Potential key intervening variables such as behavioral factors will be further examined using mediation analysis. Further, the relationship of maternal mood with amount of breast milk will be examined by inclusion of the MAACL-R scores obtained at discharge, days 21 and 42 in the longitudinal MEM model.

Additionally, unadjusted mean differences in volume of breast milk at day 1 through 42 will first be compared overall via one-way ANOVA (or equivalently non-parametric Kruskal-Wallis tests) between the three groups. Subsequently, pooled t-tests will be used to compare unadjusted means pairwise between the groups (using significance level $\alpha = 0.02$ to account for multiple comparisons). Mean differences for volume of breast milk at day 1 through 42 and number of days to achieve lactogenesis stage II will be adjusted for gestational group (strata) and putative covariables such as age, mother's weight at delivery, diagnosis of preeclampsia or diabetes, parity, birth weight, weeks of gestation, behavioral factors including kangaroo care and lactation support. Mean number of pumping attempts per day will be compared for each outcome measure (as dependent variable) using a general linear model (GLM) approach for the three milk initiation groups.

Furthermore, survival distribution for time from delivery to lactogenesis stage II will be obtained for each group using the Kaplan-Meier product limit method, and will be compared using the logrank test. Cox proportional hazards regression modeling (PHM) will be used with time from delivery to lactogenesis stage II as the dependent variable, and treatment (early vs. moderate vs. late initiation) and gestational group (strata) as the primary independent variables. Additional covariables and interaction terms will be added as described above for MEM analyses.

Analyses for Exploratory Aims

Aim 1. Volume of breast milk at discharge will be compared via one-way ANOVA; weekly percentage of breast milk during hospitalization will be compared between the three groups using chi-square tests.

Aim 2. In a first step, unadjusted mean time to lactogenesis stage II and mean volume of breast milk over the first 6 weeks (days 1 through 42) will be compared between the four groups: early timing of initiation of expression (TIE) / 23-27 weeks of gestation, late TIE / 23-27 weeks of gestation, early TIE / 28-32 weeks of gestation, and late TIE / 28-32 weeks of gestation, using one-way ANOVA. Subsequently, post hoc pooled t-tests (Tukey's) will be used to compare time to lactogenesis stage II and volume of breast milk means pairwise for the two early and the two late TIE groups between the two degrees of prematurity (23-27 vs. 28-32 weeks) and for the early-late TIE groups within each degree of prematurity. Adjusted means will be obtained and compared between the groups using a general linear models (GLM) approach with gestational age/degree of prematurity group as the primary independent variable and time to lactogenesis stage II (or amount of breast milk expressed at days 1-7) as the dependent variable. Additional covariables will be included in the models to adjust for putative predictive variables as described above.

Expected Outcomes

Based on our pilot study, it is reasonable to expect that mothers in Group 1 (earliest expression of milk) will have the earliest onset of lactogenesis stage II, produce the most milk overall and have infants who are provided increased breast milk during hospitalization and at discharge.

Data Entry and Management
All data will be entered into a REDCAP database having integrated data quality and consistency checks (e.g., data-range) as part of the data procedure. REDCap is a secure, web-based application for building and managing online surveys and databases. Using REDCap's stream-lined process for rapidly developing projects, projects are designed and created online from a web browser using the Online Designer or by constructing a 'data dictionary' template file in Microsoft Excel, which is uploaded into REDCap. Both surveys and databases can be built using these methods. REDCap provides automated export procedures for seamless data downloads to Excel and common statistical packages (SPSS, SAS, Stata, R), as well as a built-in project calendar, a scheduling module, ad hoc reporting tools, and advanced features, such as branching logic, file uploading, and calculated fields. The study database created in REDCap will be password-protected and housed on a designated database server, which is protected by a computer firewall. Data quality will be monitored and assured: 1) as reported; and 2) as entered into the database. For the former, all hardcopy forms will be visually inspected before data entry. Furthermore, a manual comparison of randomly selected data hardcopy forms with data output listing generated from the study database will be performed, and consistency checks will be generated by SQL or SAS programs as part of routine data cleaning procedures.

All subjects eligible for eligible for enrollment will be registered and entered into the study database designed by the program assistant. The system includes designated web servers and supporting database servers. Study data will be directly entered into the database via a secure internet connection. The system allows the PI to access data in real-time and to generate reports based on enrollment status, basic demographics, and data summaries. The web-based data system will also allow for the sharing of data in the future. The system will assign a global unique identifier to each enrolled subject that will serve as the unique subject id. Personal Health Information will not be stored in the study database. The study case report forms (CRFs) will be maintained in study specific folders. The CRFs are considered the primary data collection instruments for this study. When CRFs are not actively being processed, they will be secured in locked file cabinets. In addition to the use of passwords and other security measures, all documents containing identifying information are considered confidential materials and are safeguarded to the greatest possible extent. No individually identifying information will be released or discussed with anyone other than study staff.

Potential Problems and Alternative Strategies
The most likely potential problem is difficulty retaining subjects in the study between consenting prior to delivery and initial breast milk expression. Although our pilot study indicated this dropout to be highly unlikely, to minimize this problem a member of the research team will be available to the mother to help her pump the first time (at the time designated). Should she chose to pump at a different time (earlier or later), then she will receive the standard care available to her from the nursing staff and will no longer be eligible for the study.

Another problem related to retention is the continued willingness of mothers to express milk at the frequency required and for the duration of the study (at least 5 times a day until attainment of lactogenesis stage II). While this frequency may appear burdensome, these are the standard directions provided to mothers of VLBW infants receiving standard care i.e. who are not enrolled in the study.

A third potential problem is retention of the mother in the study for 6 full weeks post-delivery, when many women stop pumping due to lack of breast milk. To encourage mothers to continue trying to express their milk, each mother will be assigned to a graduate research assistant with experience involving the care of lactating women. This same student RA will contact the mother three times per week, either in person in the hospital or via telephone. The student will remind the mother to continue to pump at least 5 times per day and to bring in her breast milk with every visit. The role of student RAs in maintaining contact with mothers will decrease the likelihood of contamination of data collection procedures, since the PI or one of the co-investigators could inadvertently provide lactation consultation during the calls.

Potential Risks

Milk expression procedures for mothers in this study will be the same as if they were not in the study; therefore, there is no more risk for participants than there would be in day-to-day life. If lactation problems occur, including nipple pain, problems with the mechanical pump, or breast discomfort, this information will be communicated to a lactation consultant in the Neonatal Intensive Care Unit. Infants' involvement will be purely observational, and therefore there is no risk added by their inclusion in the study.

In this study, the mothers will likely be expressing their milk earlier than standard clinical practice dictates. In our pilot work, mothers who initiated milk expression within one hour of delivery had less pain and stress associated with the initial milk expression session than those who began milk expression 1-6 hours following delivery. Therefore, those in the Intermediate and Late Expressers groups are more likely to experience discomfort. However, this discomfort is expected to be fleeting and no different than the discomfort the mother would have experienced in usual clinical care. It is important to note that initial milk expression will be facilitated only by the research coordinator, the PI, or a research assistant experienced with lactation. Ongoing lactation support will be the same for all lactating mothers in the NICU and will include lactation services and support by the nursing staff.

The PI will notify maternal subjects of new information that may become available and which might affect their decision to remain in the study. Because the subjects will be low-risk, the categorization of level of risk is minimal or the probability of harm or discomfort is not anticipated or greater than those encountered during the routine care. **Alternative treatments** include discontinuation of milk expression.

All documents will be de-identified using a subject number and filed within a locked filing cabinet within the PI's research space.

Potential Benefits of the Proposed Research to Human Subjects and Others

Benefits are relevant to the NIH mission to enhance health and reduce the burdens of illness for preterm infants. This study is expected to make a significant contribution to understanding the effects of alternative timing of initiating milk expression following delivery in mothers of VLBW premature infants. This research will provide the knowledge to decrease time to lactogenesis stage II and increase milk production thereby increasing the amount of breast milk fed to VLBW infants and decreasing the complications and costs associated with premature births. These results can be translated into practice by influencing protocols regarding initiation of milk expression for mothers of VLBW infants in labor and delivery, post partum units, and NICUs.
Mothers of VLBW infants will benefit from this study by having a greater production of breast milk and an earlier lactogenesis stage II because they will begin milk expression earlier than standard care requires. Mothers will also benefit from this study because they will be given a hospital grade pump for home-use for the duration of the study

Conflict of Interest

There are no conflicts of interest to report