Research Summary
NCT03370757

1. **Protocol Title** – Very Low Birth Weight (VLBW) Preterm Infant Health Outcomes with *Bundled Care* in the Neonatal Intensive Care Unit: A RCT

2. **Purpose of the Study** – Explore the impact of bundling nursing care activities on the overall health of VLBW preterm infants who receive bundled care in a Level IV Neonatal Intensive Care Unit (NICU). Specific study aims include:

   Aim 1. Evaluate differences in infant health when diaper changes occur at 3- versus 6-hours during 3-hour bundled care.
   - **RQ1.** What caregiving activities other than diaper changes are included in *bundled care events* and do they differ when diaper changes are bundled every 3 versus 6 hours.
   - **RQ2.** Does infant physiological stability (∆ heart rate, ∆ respiratory rate, ∆ oxygen saturation, # apnea # bradycardias, # desaturations, feeding tolerance) differ ***between 3- and 6-hour bundled care diapering***?
   - **RQ3.** Does infant weight gain over time differ between 3- and 6-hour bundled care diapering?
   - **RQ4.** ***Within the 6-hour bundled diaper care group***, does infant physiological stability (∆ heart rate, ∆ respiratory rate, ∆ oxygen saturation) differ between bundled care with and without a diaper change (3 versus 6-hours)?

   Aim 2. Evaluate differences in infant skin health between 3- and 6-hour bundled care diapering at two sites (buttocks and chest).
   - **RQ1.** Does skin transepidermal water loss (TEWL) differ over time and at the end of 6 weeks between 3- and 6-hour bundled care diapering on the chest and buttocks?
   - **RQ2.** Do neonatal skin condition scores differ over time and at the end of 6 weeks between 3- and 6-hour bundled care diapering?
   - **RQ3.** Does skin pH differ over time and at the end of 6 weeks between 3- and 6-hour bundled care diapering?
   - **RQ4.** Does the skin microbiota differ over time and at the end of 6 weeks between 3- and 6-hour bundled care diapering?

   Aim 3. Evaluate the acceptability and feasibility of conducting a bundled care intervention where the frequency of one care activity, diaper care, is standardized.
   - **RQ1.** What are the perceptions of parents and providers about the criteria necessary for conducting a diaper change in the NICU?
   - **RQ2.** What are the perceived barriers of parents and providers to longer timeframes between diaper changes?

   Exploratory health outcomes will examine the incidence of late onset sepsis and urinary tract infections in both bundled care groups.

3. **Background & Significance** –

Progress in perinatal medicine has resulted in an increased survival rate of very preterm infants (typically ≤ 1500 grams and < 34 weeks). However, preterm infants' growth and developmental outcomes vary with illness severity and degree of neurological insult (Boyle et al., 2012; Ge et al., 2013) and unpredictable variations in outcomes exist even among healthy preterm infants (Schneider et al., 2014; Vohr, 2014). Preterm infants also have poorer

orientation, self-regulation, and reflexes and more excitability, hypotonia, and hypertonia at 40 weeks postmenstrual age (PMA) when compared to term infants (Pineda et al., 2013). The variations in preterm infants' outcomes have led to the supposition that the NICU environment may negatively impact the health and development of these infants and significant research has been devoted to examination of light, noise, and caregiving interventions (Boo et al., 2002; Brandon et al., 2017; Brandon et al., 2002; Morag & Ohlsson; 2013).

In addition, individualized developmental care has become the norm in most NICUs since developmental care was first promoted by Dr. Heidelise Als in the 1980s (1982). A body of literature has emerged to support the observation of infant behavioral organization (infant cues) in response to the stress of the NICU environment to guide caregiving to minimize infant stress and optimize infant outcomes. The overall goal of individualized developmental care is to promote the short- and long-term health and development of high risk infants including interventions that promote the well-being of an infant's family (Macho, 2017). Consequently, many NICUs have implemented neuroprotective strategies to reduce over simulation, promote sleep and facilitate brain development in all high-risk infants (Gressens et al., 2002; Lucas-Thompson et al., 2008).

One neuroprotective strategy includes the grouping of care activities around a single caregiving event described as *"clustering"* or *"bundling care"*. Individualized developmental care dictates that a *bundled care event* is halted if an infant displays significant behavioral stress cues. Yet, the number and type of caregiving activities that are included in *bundled care* and the timeframe between *bundled care events* has not been systematically studied. While painful and intrusive interventions like heel sticks are often described as a source of infant stress (Lucas-Thompson et al., 2008) routine interventions like diaper changes can also be a high stress event (Mörelius et al., 2006). Current bundled care typically includes a diaper change, but the timeframes between bundled care events differ based upon infant illness severity, postmenstrual age, and individual NICU practice standards. Currently no operational definition exits around the expected length of time that categorizes a *bundled care event* from a *non-bundled care event*. In our pilot work of five extremely low birth weight (ELBW) infants we identified a caregiving event as including at least two nursing care activities and lasting at least five minutes. Over the first 14 days of life in the NICU, 161 *bundled care* events were identified, averaging 4.7 events per day per infant. The average duration for *bundled care* events greater than 5 minutes was 10.8 minutes. The time between *bundled care* events was 2.9 hours.

The inclusion and exclusion of certain care activities in any individual care event is often dictated by the infant's treatment plan or needs, but some activities like diapering may be optional. Understanding the impact of when to include optional, yet stress provoking interventions, will allow us to minimize overall environmental stress in hospitalized VLBW infants. It is unclear how the different times used in bundled care affect overall infant health including skin health in the diapered area. Therefore, given the lack of data around bundled care, we aim to explore the impact of bundling diaper care activities on overall health of VLBW preterm infants. We will include a focus on skin health because it is important to understand the benefits of decreased infant stress and any potential skin health trade-offs associated with longer versus shorter time between diapering care. Findings from this study will allow us to better understand the relationship between neonatal skin health while providing developmentally appropriate bundled care.

4. Design & Procedures

a. Design and Setting. A randomized controlled designed will be used to evaluate the effect of *bundled diaper care* on overall infant and skin health. Infants will be recruited from the Level IV Intensive Care Nursery (ICN) at Duke University Hospital. The ICN is a Level IV, 67-bed nursery and one of the area's leading referral centers for infants born prematurely. The

Duke ICN has an average daily census of 90% and annually admits 150 VLBW infants. The ICN practices individualized developmental care with standard bundled care intervals that vary based upon an infant's birth weight and feeding status (every 3, 4, or 6 hours). Infants who are admitted or transferred to one of the ICN three stepdown units will also be considered if eligible and will continue to receive the intervention when transferred including the Special Care Nursery at Durham Regional Hospital. The principal investigator has been a clinical nurse specialist affiliated with the ICN since 1993 and has the support of the nursery medical and nursing team to conduct this research including the Division of Neonatology, the ICN Intensive Care Developmental Care Team and the Medical Director.

b. Participants. A maximum of 80 VLBW infants will be recruited to ensure at least 25 infants in each study group with complete data. Ten parents of infant participants (5 in each study group) and 10 health care providers will participate in interviews about the acceptability and feasibility of the diapered bundled care intervention. The goal of this study is to enrich the literature with estimation of values and variances of the outcomes measured as well as how these values change over time. With a total sample size of 50 VLWB infants, we are confident that we will be able establish effect to inform a larger subsequent study. Because most of the infants are expected to receive care up to 6-weeks, we expect little missing data or participant attrition.

Infant Inclusion criteria are:

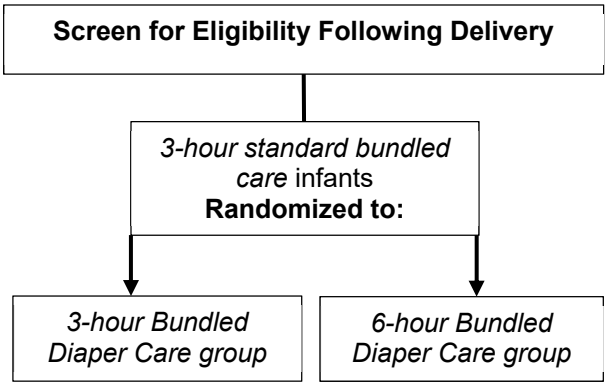
1. Must be participating in the ICN standard 3-hour bundled care
2. Must wear a breathable diaper
3. Must be ≤ 32 weeks gestation at birth
4. Infants are expected to remain hospitalized for at least 4 weeks

Infant Exclusion criteria are:

1. Neonatal Abstinence Syndrome
2. Humidified incubator
3. Diaper rash
4. Pre-existing or genetic skin conditions
5. Severe illness requiring 1:1 nursing care: e.g. minimal stimulation protocol, high frequency ventilation, vasopressor drug support, or body cooling.

Infants will be recruited and enrolled as soon as they meet study criteria (**see Figure 1. Study Schemata**). Data collection will begin following study enrollment and will continue until 6 weeks following randomization (**see Table 1**).

Figure 1. Study Schemata



c. Intervention: Infants will be randomly assigned and stratified by birth weight (≤ 800 grams, >800 grams to 1150 grams, > 1150 grams) to either 3- or 6- hour bundled diaper care. All infants will receive bundled care per unit standards until they are eligible for *3-hour bundled care*. Infants typically transition to *3-hour bundled care* once they are receiving interval feedings every three hours. This transition usually occurs before 3 weeks chronological age in infants >800 grams at birth and by 6 weeks

for infants ≤ 800 grams. Other care activities included in the standard 3-hour bundle vary based upon infant treatment needs, but usually include a diaper change. The only difference in a 3-hour and 6-hour bundled care event will be the timing of the diaper change. Infants will receive a diaper change at either 3 or 6 hours. While 3-hour bundled care is standard of care, this is a clinical environment in which caregiving cannot be expected to occur exactly every three hours to the minute, therefore both expected and actual times for the bundled care events will be collected to include in data analysis as needed. Infants who are receiving the 6-hour bundled diaper care will continue to receive 3-hour care interventions for all other interventions or as dictated by infant care needs. At the 3-hour caregiving interval in which the 6-hour bundled care infants are not due for a diaper change, the nurse will peek into the diaper to ensure there are no stool present. If stool is present, a diaper change should occur and the deviation from the protocol noted in the bedside documentation.

d. Procedures: A neonatal provider will give approval for participation in the study. Parents will be approached for consent after eligibility screening is completed (≤ 32 weeks gestation). The principal investigator is a clinical nurse specialist and nurse scientist who has been affiliated in the Intensive Care Nursery since 1993. Study personnel will explain the study, allow time for questions, have a parent sign the consent form, and a copy will be given to the parent. Parents will provide consent for their infant's participation in the study and will give permission for themselves to participate in an interview about the study, if selected. Five parents from each group will purposively selected to participate in an interview. Parents will be selected to include parents with infants < 1000 grams at birth and parents with infant ≥ 1000 grams at birth.

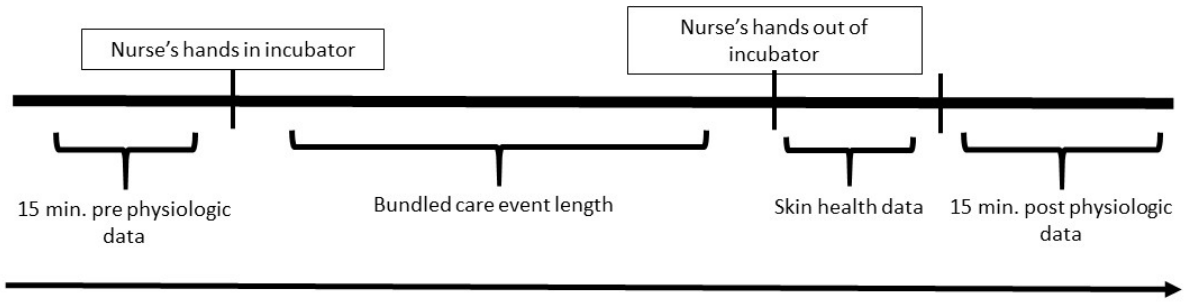
Following consent, the infant will be monitored until all inclusion and exclusion criteria are met. At that time randomized to an intervention group and data collection will be initiated. The intervention will continue for six weeks at which time collection of data will stop and bundled diapered care will resume according to unit protocol. If the infant no longer meets inclusion criteria the intervention will be discontinued and routine data collection will be discontinued. The exclusion criteria will be noted, e.g. use of diaper cream, and data collection regarding any new illness characteristics or treatments will continue. If an infant requires a diaper change due to a stool or other needed procedure this will be recorded on a bedside card.

Ten nurses will be recruited for participation in interviews around intervention acceptability at the end of the study.

e. Data Collection Measures

Study data will include maternal and infant demographics and medical history extracted from the medical record to describe the sample and for needed covariate data, physiologic data (e.g. heart rate, respiratory rate, oxygen saturation), and biomarkers of skin health (pH, transepidermal water loss, NSCS, microbiome). See **Figure 2** below for the timeframe for the collection of physiologic data and contents and length of bundled care. **Table 1** for the constructs, measures, and timing and frequency of data collection.

Figure 2. Timeline



Data Collection Timeline for Bundled Care and Physiologic Data

i. **Medical Record Data:** Participant participation is documented in the electronic medical record and data extraction from the electronic medical record can be accessed through the Duke Health System EPIC or through a Virtual Pin Network. Flow sheets, lab results and provider notes will be reviewed for the required data.

Table 1. Data Collection

Construct	Measure/ Instrument	Variable	Time frame
	Descriptive data to describe the sample		
Maternal history	EMR[1]	Age, medical hx. of skin disease,	Enrollment
Infant characteristics	EMR	GA[2], birth weight, diagnoses, antibiotics, delivery mode, lab values as required to describe skin health (e.g. albumin, protein, bilirubin) All bundled care event time frames from study entry to study discharge (expected vs. actual bundled times) During bundled care—respiratory support, changes in support, FiO_2 changes, average Daily FiO_2, caffeine	Enrollment, 3x/week, or as available
	Aim 1. Infant Health		
Bundled care	Observation, videotape & EMR	Specific interventions (e.g. suctioning, positioning, offering pacifier, diapering) Length of bundled care events Time between bundled care events	Observer 3x/week for the 3-hour diaper group and 4s/week for the 6-hour group to allow for one within group comparison; Videotaped 1x/week Daily; retrieved 3x/week
Physiologic stability	GE monitor	1-minute values of heart rate, respiratory rate and oxygen saturation 15 minutes before and after each observed bundled care event	3x/week for the 3-hour diaper group and 4s/week for the 6-hour group to allow for one within group comparison
	EMR	# bradycardia events daily	Daily; retrieved 3x/week
	EMR	# apneic events daily	Daily retrieved 3x/week
	EMR	# desaturation events daily	Daily retrieved 3x/week
	EMR	Feeding tolerance (emesis, gastric residual volume-ml, feeding interruptions)	Daily retrieved 3x/week
Weight gain	EMR	Daily weight in grams	Daily retrieved 3x/week
	Aim 1. Covariates		
Development	EMR	Postmenstrual age (PMA)	
Severity of illness	Technology Burden	Total Score 0-100	Retrieved 3x/week to coincide with observation
	EMR	Time in minutes since the end of the last bundled event	With each observation

	Aim 2. Skin Health (buttocks and chest)		
Acid mantle development	pH	0.00 to 14.00.	3x/week with observation
Water loss	TEWL Dermalab	TEWL[3]	3x/week with observation
Skin status	NSCS[4]	NSCS-range 3-9	Twice daily retrieved 3x/week
Microbiome		Microbiota pattern	3x/week with observation
	Aim 2. Covariates		
	EMR	PMA	
	EMR	Delivery mode	
	EMR	Time since last bath	3x/week with observation
	EMR	Daily urine output	3x/week with observation
	EMR	Time in minutes since the last bundled event	
	EMR	Stool pattern (categorical)	3x/week with observation
	EMR	Feeding type (formula vs. breast and route; enteral vs. nipple)	3x/week with observation
	Bedside checklist	Diaper size and brand	3x/week with observation
	Stool	Scavenged	1x/week as available
	Aim 3. Feasibility		
Intervention acceptability and feasibility	Interviews	Parent perceptions Provider perceptions	End of infant data collection; At study end
	Exploratory Health Outcomes		
Late onset sepsis	EMR	Presence or absence	Two weeks after end of the study
Urinary tract infection	EMR	Presence or absence	Two weeks after end of the study

[1]EMR=electronic medical record; [2]GA=gestational age at birth; [3]TEWL=transepidermal water loss; [4]NSCS=neonatal skin condition score.

ii. **Physiologic measures:** Heart rate, respiratory rate, and pulse oximetry will be measured continuously with a GE Cardiopulmonary monitor (standard monitor at DUH) with acquisition from the server, sent to the PI weekly for all enrolled subjects in a data secured zip file. 1-minute values for each 24-hour observation in which a bundled care data collection occurred will be extracted and the required time frames will be extracted for analysis.

iii. **Bundled Care:** The content of bundled care will be determined through the observational coding of all caregiving activities during weekly observations (3 times/week for 3-hour diapered and 4 times/week for the 4-hour diapered intervention group; see intervention section above) and 12-hour videotaping (1 time per week for each group) of bundled care events. The length of time for each bundled care event will be operationally defined as beginning when the nurse's hands enter the incubator and will end when the nurse's hands leave the incubator following caregiving.

iv. **Microbiome**: One microbiome sample will be taken from the buttocks immediately after the diaper is removed and before the skin is cleaned, if there is NO stool present. A second swab will be collected from the chest at the same time. The following procedure will be used for all microbiome samples. Needed supplies will include a tube of sterile solution of PBS + 0.1%Tween 20 and a Nova Biostorage FLOQ swab with cuvette.
    a. Remove the swab from the package
    b. Insert the tip of the swab into the sterile PBS + 0.1% Tween 20 solution until moistened.

c. Before the buttocks is cleaned, swab the left and right buttocks with two back and forth strokes on each buttocks for a total of four strokes.
d. Place the swab tip into the cuvette and bend at scored break point until the tip remains untouched in the cuvette
e. Seal the cuvette, and label it with the subject number, site, visit number, date, time, initials of the individual taking the swab.
f. Place swabs into a -20°C (±5°C) freezer and store until end of study.

v. **pH:** The Extech PH100 meter will be used to measure skin pH in the range of 0.00 to 14.00. The small flat surface electrode provides a non-intrusive accurate measure of pH. The instrument's probe will be placed at the center of the test site (either chest or diaper) and measurements will be taken. Each measurement will be performed by holding the probe flush over the skin at the test site. The measurement is taken instantly and will be recorded into RedCap via iPad.

vi. **Trans Epidermal Water Loss**: TEWL values will be collected using the Dermalab. We will use non-invasive skin probes to perform each assessment of transepidermal water loss (TEWL). TEWL will be measured using the DermaLab® TEWL probe (Cortex Technology, Hadsund, Dermark), which consists of an open probe with paired sensors placed at different distances from the skin. Humidity and temperature are measured in each sensor to calculate vapor pressure gradients. The difference between two vapor pressure gradient measures is representative of TEWL at that point on the skin (Grove, Grove, Zerwick, & Pierce, 1999). Environmental humidity levels will also be measured using the DermaLab® before obtaining the TEWL measures. The average humidity readings from the two sensors will be recorded as environmental humidity. Individual probe covers will be used for each infant to prevent any cross contamination between patients. Each measurement will be performed by holding the probe flush over the skin at the test site for at least sixty (60) seconds and the average value (in g/m2*hr) will be recorded. Probe covers are used to prevent cross contamination between patients, in addition, the probe will be thoroughly cleaned with 70% ethanol prior to and between participants to minimize cross-contamination or microbial transmission. The ethanol will be allowed to air dry prior to use of the probe.

vii. **Neonatal Skin Condition Score (see Figure 2):** The NSCS will be collected twice daily (day and night shift by staff nurses) while infants are participating in the study. The NSCS is a reliable and valid measure of skin condition (Lund & Osborne, 2004)

Figure 2. NEONATAL SKIN CONDITION SCORE (NSCS)
Dryness
1 = Normal, no sign of dry skin
2 = Dry skin, visible scaling
3 = Very dry skin, cracking/fissures
Erythema
1 = No evidence of erythema
2 = Visible erythema, <50% body surface
3 = Visible erythema, ≥50% body surface
Breakdown
1 = None evident
2 = Small, localized areas
3 = Extensive
Note: perfect score = 3, worst score = 9.

For any score of 2 under dryness, erythema or breakdown, that includes the diapered area, the study will be discontinued.

viii. **Parent and Provider Interviews:** Parents will be asked to participate in an interview about the acceptability of the intervention after their infant completes the study. Ten parents (5 in each study group) will be interviewed. The interview is not expected to last more than 30 minutes, but will largely be under the direction of the parent (see Appendix A for the interview guide). Ten providers to include at least two neonatologists, two neonatal nurse practitioners and six bedside nurses, will be asked to participate in an individual interview at the time of study completion about the acceptability and feasibility of the intervention (see Appendix B for the interview guide).

5. Selection of Infant Subjects

Infant Inclusion criteria are:

a. Must be participating in the ICN standard 3-hour bundled care
b. Must wear a breathable diaper
c. Must be ≤ 32 weeks gestation
d. Infants are expected to remain in ICN at least 2 weeks

Infant Exclusion criteria are:

1. Neonatal Abstinence Syndrome
2. Humidified incubator
3. Diaper rash
4. Pre-existing or genetic skin conditions
5. Severe illness requiring 1:1 nursing care: e.g. minimal stimulation protocol, high frequency ventilation, vasopressor drug support, or body cooling.

6. Subject Recruitment and Compensation

Study personnel will check the daily admission log for eligible patients. Clinical staff known to the parent/guardian of the potential subject will introduce the research study to the infant's family. If the parent/guardian expresses interest in the study, the research staff will approach him/her to explain the study and request consent. There will be no compensation for study participation, however infants will be given a developmental toy at the end of the study.

7. Consent Process

Subject's Capacity to Give Legally Effective Consent

For infants, consent will be obtained from the parent or guardian following approval of the infant's provider using see Section 14 of the e-IRB submission form and complete the questions in that section. The PI, research coordinator or other study personnel (who is either an RN or has a PhD (or both) and is trained in study and consent procedures) will conduct the consent process in the study as outlined by GCP guidelines.

Parents will be approached for pre-consent if their infant is ≤ 32 weeks gestation. The infants' medical provider, or a member of the Neonatal Perinatal Research Unit will seek permission from the mother or father for the research staff to discuss the study with them. Then, research staff (i.e. PI, study coordinator or research assistant) will contact the parent in person while visiting the ICN. If contact cannot be made with parents during visiting hours, parents will be contacted via phone. Parents will be approached at the bedside of their infant. Parents will be offered the option to discuss the study at their infant's bedside or in an available conference or meeting room on the unit. If others are present, the parent is asked whether she would prefer for the research member to discuss the study in front of her visitors or at another location (e.g. unit conference room behind a closed door).

We estimate that the consent process will take about 30-60 minutes. Parents will be allowed as much time as needed to decide whether or not to enroll the infant in the study. Parents will be encouraged to ask questions at any time before their infant's participation in the trial. The study team will present the consent form in its entirety, with special emphasis on the voluntary nature of research, and the ability of subjects to withdraw from the study at any time. No one who is obtaining consent is directly involved in the supervision of the providers who are being asked to participate in the study or involved in the direct care of the infants whose parents are being asked to participate in the study. The research staff will closely follow the IRB and GCF guidelines..

Spanish speaking parents/guardians of eligible infants will be consented using two translators that are not involved in the study, one to present the consent form in Spanish and one to assure that what is presented is correct. The translator will translate the entire English consent form verbally to the potential study subject. The translator will relay questions to the study coordinator from the subject and the subject will have questions answered prior to signing the consent document. For Spanish speaking parents/guardians, the parent/guardian and witness will be asked to sign the Spanish short form. The witness and the person obtaining consent must sign the English consent form.

Providers will be consented using the same IRB and GCF guidelines described above. Provider consent should take 15-30 minutes.

8. Risk/Benefit Assessment

Risks associated with the intervention and data collection will be minimal. All of the study interventions including a less frequent diaper change are considered acceptable standard of care. The skin condition will be monitored twice daily to identify any potential change in the skin of the diapered area and if the NSCS is ≥ 5 due to dryness, erythema or skin breakdown in the diapered are, the intervention will be discontinued, the infant's provider will be consulted regarding the need for treatment and the infant will be monitored until resolution of the problem. The infant stress associated with stimulation due to data collection should be minimal. Data collection processes for the biomarkers should not cause discomfort and handling will be kept to a minimum. However, all research staff will have neonatal experience and be trained in developmentally appropriated infant handling and positioning.

The small risk of loss of confidentiality will be reduced by assigning code numbers to the samples. The key linking the identity of the subject to the code number will be separately maintained in the Research Office, under lock and key.

There may be a direct benefit to the subjects participating in the study including less physiological stress during caregiving or improved skin health. However, actual benefits will not be clear until future larger studies are conducted. By improving our understanding of the relationship between bundled diaper care and infant health outcomes more targeted interventions may be developed for general health, development and skin care.

9. Costs to the Subject

There will be no cost to study participants.

10. Data Analysis and Statistical Considerations

Initially, we will conduct descriptive analyses, including examination of means and proportions and the measures of variability for each variable overall and by bundled care groups. The descriptive analyses will be followed by modeling approaches to respond to the research questions. Tests of statistical significance will be two-sided and the threshold for statistical significance will be $P < 0.05$.

Aim 1. Evaluate differences in infant health when diaper changes occur at 3- versus 6-hours?

RQ1. What caregiving activities other than diaper changes are included in bundled care events and do they differ when diaper changes are bundled every 3 versus 6 hours
Analysis: Each day bundled care is observed, a list of activities will be marked as occurred (1) or did not occur (0). Each infant will have 18 days of data denoting whether an activity occurred or did not occur. Hierarchical clustering of repeated data with an appropriate algorithm that producing stable and accurate clusters will be used will be to determine the groupings of activities that occur in the sample. The frequency of the clusters between the intervention groups will be compared.

RQ2. Does infant physiological stability (Δ heart rate, Δ respiratory rate, Δ oxygen saturation, # apnea # bradycardias, # desaturations, feeding tolerance) differ between 3- and 6-hour bundled care diapering?
Analysis: Heart rate, respiratory rate and oxygen saturation values captured a minute apart for each observed bundled care with a diaper change will be graphically displayed in various constructions to be able to compare the general trends of change in these outcomes between intervention groups, among periods of the bundled care (before, during and after) and over weeks. Further, we will create a daily average of each outcome by period (before, during, and after). Using a repeated measures analysis, we will test for difference among the periods and intervention group and all pairwise period comparisons between groups. PMA and infant illness severity (Technology Burden Score) will be used as a covariate. We will control for multiple comparisons.

A Poisson Generalized linear model for repeated measure will be use to estimate the daily number of apnea, bradycardias, and desaturations events over the 18 days of bundled care observations with a diaper change. The need for a random intercept or slope will be assessed.

$$\text{Log}(\#\text{ of Apnea Events}_{ij}) = b_0 + b_1[\text{I}(6\text{HrBC}_i)] + b_2(\text{Day}_{ij}) + b_3[(\text{I}(6\text{HrBC}_i))\times(\text{Day}_{ij}) + b_4(\text{PMA}_{ij}) + b_5(\text{TechBur}_{ij}),$$

where *Log((# of Apnea Events$_{ij}$)* is the logarithm of the number of apnea events for individual *i* at day *j*. b_0 is the mean log of apnea events for participants who receive 3-hour diaper changes

and are mean postmenstrual age and with mean technology scores at the start of the study; b_1 is the change in log number of apnea events for those receiving 6-hour diaper changes; $I(6HrBC_i)$ is an indicator for participants receiving bundled care and 6-hour diaper changes; b_2 is the change in log of the number of apnea events for each day of bundled care; Day_{ij} is the indicator of the day the number of apnea events was recorded; b_3 is the additional change in log of the number of apnea events over days for those receiving bundled care and 6-hour diaper changes; PMA_{ij} is the difference from the baseline mean postmenstrual age of the *ith* participant at the *jth* day; $TechBur_{ij}$ is the difference from the baseline mean technology burden score for the *ith* participant at the *jth* day; b_4 and b_5 are the expected changes in log number of events for postmenstrual age and technology burden scores, respectively. The estimate of interest is b_3. The feeding tolerance outcome will be estimated in a similar manner using the appropriate distribution of the outcome and link function for the model.

RQ3. Does infant weight gain over time differ between 3- and 6-hour bundled care diapering?
Analysis: The trajectories of weight measured across the 18 days of bundle care will be fitted using a linear mixed model with a random intercept. We will first determine the best trajectory fit for weight over the 18 days. Once we select an appropriate trend for the data a model similar to the one described below will be estimated.

$$Weight_{ij} = b_0 + b_{i0} + b_1[I(6HrBC_i)] + b_2(Day_{ij}) + b_3[(I(6HrBC_i))\times(Day_{ij}) + b_4(PMA_{ij}) + b_5(TechBur_{ij}) + \varepsilon_{ij},$$

where $Weight_{ij}$ is the weight for individual *i* at day *j*. b_0 is the mean weight of participants who receive 3-hour diaper changes and are mean postmenstrual age and with mean technology scores at the start of the study; b_{i0} is the individual baseline weights; b_1 is the change in weight for those receiving 6-hour diaper changes; $I(6HrBC_i)$ is an indicator for participants receiving bundled care and 6-hour diaper changes; b_2 is the change in weight for each day of bundled care; Day_{ij} is the indicator of the day the weight was recorded; b_3 is the additional change in weight over days for those receiving bundled care and 6-hour diaper changes; PMA_{ij} is the difference from the baseline mean postmenstrual age of the *ith* participant at the *jth* day; $TechBur_{ij}$ is the difference from the baseline mean technology burden score for the *ith* participant at the *jth* day; b_4 and b_5 are the expected changes in weight for postmenstrual age and technology burden scores, respectively; and ε_{ij} represents variance components to be estimated. The estimate of interest is b_3.

RQ4. Within the 6-hour bundled diaper care group, does infant physiological stability (Δ heart rate, Δ respiratory rate, Δ oxygen saturation) differ between with and without a diaper change (3 versus 6-hours)?
Analysis: One day of the week for those individuals assigned to the 6-hour diaper changes will be randomly selected to have heart, respiratory, and oxygen rates at 3-hour without a diaper change to compare to the same day's outcomes at the 6-hour diaper change. The values captured a minute apart for each observed bundled care will be graphically displayed in various constructions to be able to compare the general trends of change in these outcomes within the 6-hour diaper change group, among periods of the bundled care (before, during and after) and over weeks. Further, we will create a daily average of each outcome by period (before, during, and after). Using these computed values, we will create a weekly average of the 3 outcomes by period (before, during, and after). Using an analysis for repeated measures from a matched sample, we will test for difference among the periods and intervention group and all pairwise period comparisons between treatment groups. We will control for multiple comparisons.

Aim 2. Evaluate differences in infant skin health between 3- and 6-hour bundled care diapering at two sites (buttocks and chest)?

RQ1. Does skin transepidermal water loss (TEWL) differ over time and at the end of 6 weeks between 3- and 6-hour bundled care diapering on the buttocks and chest?
Analysis: The trajectories of TEWL measured across the 18 days of bundle care will be fitted using a linear mixed model with a random intercept. We will first determine the best trajectory fit for TEWL over the 18 days. Once we select an appropriate trend for the data a model similar to the one described for weight will be estimated. We will remove technology burden scores and add time varying covariates for time since last bath, urine output, stool pattern, and feeding type indicator.

RQ2. Do neonatal skin condition scores differ over time and at the end of 6 weeks between 3- and 6-hour bundled care diapering?
Analysis: We will assess the distribution of the neonatal skin condition score. Based on the distribution we will select the most appropriate link function to fit a generalized linear model with repeated measures. The models to be estimated will be similar to the example model we presented for number of apnea events, including the appropriate link function and distribution. Further, we will remove technology burden scores and add time varying covariates for time since last bath, urine output, stool pattern, and feeding type indicator.

RQ3. Does skin pH differ over time and at the end of 6 weeks between 3- and 6-hour bundled care diapering on the buttocks and chest?
Analysis: We will assess the distribution of the pH of chest and buttocks. Based on the distribution, we will select the most appropriate link function to fit a generalized linear model with repeated measures. The models to be estimated will be similar to the example model we presented for number of apnea events, including the appropriate link function and distribution. Further, we will remove technology burden scores and add time varying covariates for time since last bath, urine output, stool pattern, and feeding type indicator. Separate models will be estimated for pH of the chest and pH of the buttocks.

RQ4. Skin Microbiota
Analysis: Mr. DNA in Shallowater, TX will provide the microbiome sample analysis services to determine composition of skin microbiome by 16S rRNA pyrosequencing. Funding for the microbiome analyses will come directly from Kimberly Clark's budget. Pyrosequencing data will be analyzed with a workflow based on QIIME software[1]. Data will be compiled and relative percentages of microbes will be determined for each individual sample. Data will also be compiled at each individual taxonomic level according to the NCBI taxonomy criteria. Significance differences in relative abundance of specific taxa between the intervention groups will be calculated using the Kruskal Wallis test.

To assess the extent to which taxonomic diversity at the family and genus level can be used to distinguish microbial communities associated with skin swab samples; Principal Component Analysis (PCoA) will be used. Rarefaction, Chaol, Ace and Shannon indexes will be performed on the data to evaluate if diversity and richness of populations are different among subjects within the different intervention and over time. Demographic and other clinical data will be evaluated for basic correlation with any observed microbiome changes.

Exploratory Health outcomes

The time to the occurrence of urinary tract infections and late onset sepsis will be measured from the start of the study to two weeks after study completion. Because of the small

sample size, we estimate the risk of urinary tract infections and late onset sepsis by intervention using the log rank test.

Aim 3. Analysis of interview transcripts will entail the review of transcripts to develop codes and the subsequent application of codes to all transcripts. Coded data will be reviewed to identify themes that characterize parents' and providers' of the acceptability and feasibility of the intervention. Data analysis activities will be supported by the use of Atlas.ti, a software program that supports the systematic coding and review of data. Rigor throughout the coding process will be maintained through a memo writing to recording thoughts, decisions, progression of code list, and identification of categories, and verifying coding results and interpretation with members of the research team.

Data and Safety Monitoring

There will be minimal risk associated with the study. The only study intervention is the timing of the diaper change. A record of study data for each subject will be maintained in the Research Office. Study records will be labeled with a code number. The key linking the identity of the subject to the code number will be separately maintained in the Research Office, under lock and key. No data that includes subjects' names, addresses, medical record number or other identifiers will leave Duke. Based upon the executed DTA with Kimberly Clark, microbiome samples will be sent via secure transfer for data analysis. The DTA will be uploaded under the full protocol section of the IRB.

Privacy, Data Storage, and Confidentiality

Study records (hard copy and electronic) and biologic samples will be labeled with a code number to reduce the small risk of loss of confidentiality. The key linking the identity of the subject to the code number will be separately maintained in the School of Nursing Research Office, under lock and key. No data that includes subjects' names, addresses, medical record number or other identifiers will leave Duke. All data will be collected and de-identified for data analysis. Data will be downloaded into a secure file and de-identified and stored on a secure server for analyses at Duke School of Nursing. All completed study documents will be stored in the study office within Duke School of Nursing in a locked cabinet.

Microbiome samples will be processed for total genomic DNA using standardized procedures by Mr. DNA in Shallowater, TX. All samples send to Mr. DNA will be de-identified. Processed samples will be destroyed by Mr. DNA within two years of completed analysis. Unused and unprocessed samples will be stored long-term at -80°C in a locked room at in Julia Walker's lab.

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