Study Title: The Stimulation To Induce Mothers (STIM) Study: A Parallel Group Randomized Controlled Trial

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Study Protocol

CLINICAL STUDY PROTOCOL

The Stimulation Therapy to Induce Mothers (STIM) Study: A Multicenter Parallel Group Randomized Controlled Trial

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Synopsis

Primary Objective

To determine whether intrapartum nipple stimulation therapy with or without synthetic oxytocin changes the likelihood of achieving a spontaneous vaginal delivery compared to receipt of synthetic oxytocin infusion without nipple stimulation to induce labor.

Secondary Objectives (if applicable)

The secondary objectives are as follows:

1. To determine if women who perform intrapartum nipple stimulation to induce labor have differences in other obstetric and maternal outcomes such as operative vaginal delivery, cesarean delivery, labor duration, postpartum hemorrhage, infection, and morbidities such as higher-order perineal lacerations, ICU admission, or death.

2. To determine if women who perform intrapartum nipple stimulation report differences in pain scores during labor, labor agentry and satisfaction scores, postpartum depression scores, and breastfeeding success compared to women who receive only intrapartum exogenous oxytocin infusion.

3. To determine if women who perform intrapartum nipple stimulation to induce labor have similar fetal and neonatal outcomes compared to women who receive only intrapartum exogenous oxytocin infusion.

4. At Yale, to measure circulating plasma and urine concentrations of proteins, microRNA, and small molecules using unbiased “omics” approaches, comparing patients undergoing induction of labor via intrapartum nipple stimulation versus continuous exogenous oxytocin infusion, un-ripened/unlabored control patients, and patients in spontaneous labor.

5. To measure milk maturation biomarkers and milk composition, comparing patients undergoing induction of labor via intrapartum nipple stimulation versus continuous exogenous oxytocin infusion.

Study Design

This is a multi-site, pragmatic, open label parallel group randomized clinical trial of pregnant women at 36 weeks of gestation and greater to compare the effectiveness of inpatient nipple stimulation therapy with or without adjunctive synthetic oxytocin (intervention) versus immediate synthetic oxytocin infusion without nipple stimulation (comparator) during labor induction at Yale New Haven Hospital, Weill Cornell Medicine and Northwestern Memorial Hospital.

Study Population

Nulliparous women with live singleton gestations of 36 weeks and greater and are planned to receive synthetic oxytocin infusion for induction of labor.
Number of Participants A total of 988 participants will be enrolled across all study sites with roughly even distribution.
Number of Study Sites The trial will be conducted at 3 sites: Yale New Haven Hospital (York Street Campus), Weill Cornell Medicine and Northwestern Memorial Hospital.
Primary Outcome Variables The primary outcome is spontaneous vaginal delivery.
Secondary and Exploratory Outcome Variables (if applicable) The following secondary maternal outcome variables will be explored: operative vaginal delivery, cesarean delivery, time interval from randomization to delivery, postpartum hemorrhage, infection, 3rd or 4th degree perineal lacerations, maternal death, maternal intensive care unit admission. The following secondary patient-centered outcome variables will be explored: labor pain, feelings of control during labor, satisfaction with the birthing process, ability to express and/or collect colostrum and/or breastmilk intrapartum, depression score, and breastfeeding success. The following secondary perinatal outcome variables will be explored: perinatal death, Apgar score <3 at 5 minutes of life, umbilical artery acidemia, neonatal intensive care unit admission, and a composite neonatal severe morbidity measure. In addition, breastfeeding as the sole source of nutrition (BSSN) at the time of delivery hospitalization within the first 72 hours and maximal percent newborn weight loss up to 14 days post-birth will be examined. In the Yale biospecimen sub-cohort, we will measure circulating plasma concentrations of proteins and microRNA and urine metabolites of prostaglandins using unbiased “omics” approaches, comparing patients undergoing induction of labor via intrapartum nipple stimulation versus continuous exogenous oxytocin infusion. The change in these labor biomarkers will also be evaluated in a cohort of patients presenting in early spontaneous labor who will be evaluated as a control. In the milk biospecimen sub-cohort, we will measure milk conductivity and sodium levels to estimate milk maturation (MM) before and after birth. When milk volume allows, we will also measure milk composition (macronutrients) and will use an unbiased approach to study mammary gland function using milk RNA sequencing. We will compare patients

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undergoing induction of labor via intrapartum nipple stimulation versus continuous exogenous oxytocin infusion.
Visit Schedule Table (Optional)
Study Flow Chart (optional)

Table of Contents

1 – Introduction

1.1 Introductory Statement

This protocol describes the background, design and organization of the trial. It will be reviewed and approved by the Data Safety and Monitoring Board and the Institutional Review Board (IRB) prior to trial initiation. Any changes to the protocol during the study will require approval by the IRB.

2 – Background

2.1 Background/prevalence of research topic

More than one-quarter of the almost four million U.S. women who give birth annually undergo labor induction (1). Generally, labor induction is indicated when the benefits of expeditious delivery outweigh the risks of continuing pregnancy. Additionally, labor induction is increasingly seen as a potentially therapeutic intervention even in the absence of any pregnancy complications for nulliparous women after 39 weeks of gestation (2) (3). However, compared to physiologic labor, induction is often associated with a longer latent phase which can have untoward consequences, including the need for cesarean delivery when an induction attempt fails. (4) Further, it has been shown that women who are induced are less likely to be satisfied with their birth experience compared to those with physiologic labor. (5) Women are increasingly interested in a less medicalized birth experience and desire more control during labor. (6) It has also been suggested that women who are induced or receive exogenous oxytocin may have less optimal breastfeeding outcomes. (7)

Nipple stimulation therapy has been purported as a natural and inexpensive non-medical method for inducing labor. Nipple stimulation was historically used for medically inducing contractions for the purpose of contraction stress testing (CST) (8) (9) (10). But, it is also a biologically plausible method of labor induction that may be more physiologic and allow labor induction to be more efficient and have other benefits. These may include effects related to improved uterine contractility (e.g., decreased postpartum hemorrhage risk), improved patient satisfaction, and stimulation of endogenous oxytocin which may improve breastfeeding and positive emotional responses.

Most prior studies have tested nipple stimulation as a tool to induce physiologic labor at home or did not have an appropriate comparison group. (11) (12) Data on nipple stimulation versus exogenous synthetic oxytocin infusion (the appropriate clinical alternative) are much more limited, but promising. In the two studies (13) (14) analyzed together in a Cochrane review (11) , there was a nonsignificant trend towards reduction in cesarean delivery with nipple stimulation (n=99, RR 0.60, 95% CI 0.31-1.18), no cases of hyperstimulation, and no significant difference in the number of women with an unfavorable or unchanged cervix. Chayen et al. (13) detected a significantly shorter time to onset of contractions and achievement of 200 Montevideo units without differences in rates of failed induction, tetanic contractions, or fetal bradycardia. Unfortunately, interpretation of the existing data is critically limited by small sample sizes.

Oxytocin is a nine amino acid neuropeptide primarily made in the hypothalamus, and to a lesser extent by other tissues including the endometrium, decidua, amnion, and placenta

(17). Within the nervous system, oxytocin signaling interacts with other neurotransmitter pathways including serotonin and endogenous opioids; surges in oxytocin during parturition have been associated with aspects of maternal adaptation including stress reactivity, mood, and lactation (18). Whether exogenous oxytocin, which theoretically does not cross the blood-brain barrier, interacts with these pathways while stimulating uterine contractions is unknown. Given these unknowns and their potential long-term effects on both maternal and fetal wellbeing, some have argued that exogenous oxytocin use should be used judiciously until more research can be performed on the long-term outcomes associated with exogenous oxytocin (18). These questions suggest that stimulation of endogenous oxytocin may be preferable for some women if it is also effective for labor induction and vaginal birth.

Although oxytocin is one of the most used medications in obstetrics, the kinetics of oxytocin during spontaneous labor and the dosages used for labor induction are based on a few small older trials, and still poorly understood. In an early series evaluating oxytocin signaling during spontaneous labor, among laboring women, plasma oxytocin concentrations were 27.6 pg/mL prior to labor and rose to 45.2 pg/mL in “advanced labor” (19). Among women in spontaneous labor, oxytocin concentration demonstrated minimal change over the course of labor from a mean of 47.2 pg/mL in early labor (less than 4cm dilated) to 45.8 pg/mL when the woman was found to be 10 cm dilation.(20) In contrast, oxytocin concentrations seem to rise in a dose-dependent manner in women being induced with exogenous oxytocin, with a mean plasma concentrations of 110 pg/mL in those receiving infusion at a rate of 10-16 mU/min. (20) (21) (22) It has been shown that over the course of labor, there is decreased responsiveness to oxytocin infusion which may be due to down-regulation of oxytocin receptors [17-20]. This phenomenon may contribute to the longer labor durations and labor dystocia patterns seen in induced labor when compared to physiologic labor. Interestingly, in women undergoing breast stimulation for CST, oxytocin concentration initially doubled from baseline after initiation of breast stimulation but then rapidly plateaued [21]. This study was limited in that it only evaluated oxytocin concentration over 40 minutes and labor was not reported. However, it is plausible that the kinetics of oxytocin concentrations among women who undergo nipple stimulation may be more similar to those experiencing physiologic labor rather than those who are induced with exogenous oxytocin. Lastly, physiologic stimuli, such as parturition or suckling, result in pulsatile release of oxytocin hormone into the peripheral circulation. On the other hand, exogenous synthetic oxytocin is usually administered as a continuous infusion. Previous trials have compared pulsatile versus continuous administration of exogenous oxytocin, and found that less total exogenous oxytocin was required with pulsatile administration, which suggests that it may have behaved as if it was more physiologic.(23) (24) (25) However, pulsatile administration of exogenous oxytocin did not improve clinical outcomes compared to continuous infusion, and it is plausible that even pulsatile administration is not enough to adequately emulate endogenous oxytocin.

In conclusion, despite excellent theoretical acceptance of nipple stimulation for labor induction from patients and providers (26), we lack robust data demonstrating its efficacy and safety. A well-designed randomized clinical trial is needed, particularly in comparison to the standardly accepted clinical alternative (exogenous oxytocin). Further, more data related to oxytocin concentrations and dose response are needed to inform our understanding of the biology of oxytocin during labor induction, especially when comparing exogenous oxytocin versus stimulation of endogenous oxytocin.

It is thought that breast stimulation induces uterine contractility by increasing the level of endogenous oxytocin hormone (15) (16). However, the exact mechanism remains unclear.

To our knowledge, only one previous published randomized clinical trial tested breast stimulation by electric breast pump (Medela, Inc) (13) which showed that the time to reach the active phase of labor was significantly reduced among women who underwent nipple stimulation compared to those who received oxytocin in the hospital setting. There was no difference in the length of labor between groups once women were in the active phase of labor or mode of delivery, but the trial included just 62 women. Reassuringly, only one of the 30 women assigned to breast stimulation requested discontinuation due to nipple soreness after 5.5 hours of intermittent stimulation. There were no serious maternal or perinatal adverse events reported in the trial.

At Yale, our research team recently completed our external pilot feasibility and acceptability randomized clinical trial (Yale IRB #2000029909, Clinicaltrials.gov NCT04756089). In this pilot trial, 24 women were randomized in a 3:1 ratio to nipple stimulation with a breast pump or exogenous oxytocin infusion. Of the 18 women randomized to breast pump stimulation, one woman withdrew due to delay of clinical care and received oxytocin (cross-over rate of 1/24 (4.2%)). Of the 6 women randomized to exogenous oxytocin infusion, one woman did not receive it due to spontaneous labor progress. All other participants followed their assigned intervention (22/24, 92%). The 17 women who performed breast pump stimulation stimulated for a median duration of 198 (IQR 125-291) minutes. There were no serious adverse events for either the mothers or their fetuses/infants.

As part of the pilot feasibility study, our team measured oxytocin in patients performing nipple stimulation and receiving IV oxytocin. We found that patients receiving nipple stimulation achieved regular contractions despite a smaller change in plasma oxytocin compared to patients receiving IV oxytocin to achieve the same contraction pattern. This suggests that nipple stimulation may be activating additional pathways in addition release of endogenous oxytocin.

3 – Rationale/Significance

3.1 Problem Statement

Over 1 million women have their labor induced in the United States each year, and synthetic oxytocin infusion is the most common method used. However, compared to spontaneous labor, medical induction is resource-intensive, has increased obstetric risks, and is associated with less successful breastfeeding. In contrast to endogenous oxytocin hormone which is released in a pulsatile fashion in the brain, synthetic oxytocin is continuously infused intravenously, resulting in important limitations related to efficacy, safety, and cost.

3.2 Purpose of Study/Potential Impact

It has been purported that nipple stimulation may help induce labor and has been successfully utilized for inducing contractions for the purposes of contraction stress testing. It may be an attractive option for induction of labor for both de-medicalization of birth, patient satisfaction, cost reduction, and other clinical benefits. The purpose of this clinical trial is to determine whether nipple stimulation can increase the likelihood of a spontaneous vaginal delivery, and therefore decrease the likelihood of an operative vaginal or cesarean delivery. We also seek to examine whether intrapartum nipple stimulation can decrease labor duration and improve other patient-centered outcomes such as patient pain score, patient agentry, patient satisfaction, depression scores, and breastfeeding success. Further, we aim to examine whether breast stimulation is associated with any significant differences in fetal or neonatal outcomes.

We propose a multicenter randomized trial at Yale, Weill Cornell Medicine and Northwestern Universities to compare inpatient nipple stimulation therapy via electric breast pump versus immediate synthetic oxytocin infusion without nipple stimulation for nulliparous women undergoing labor induction. This trial of 988 nulliparous women will provide adequate statistical significance to detect clinically meaningful differences in delivery mode and breastmilk as the sole source of nutrition for newborns. Successful completion of this proposal will provide rigorous data to help us show how this novel and potentially cost-effective method can radically change the way we induce labor and positively impact breastfeeding success and early infant nutrition through lactation.

At Yale, we will additionally measure oxytocin concentration among those who are being induced with nipple stimulation versus exogenous oxytocin, which has not been measured or reported by our groups to our knowledge. In our preliminary findings in 20 patients (n=10 per arm), we found no significant rise in oxytocin among patients who performed nipple stimulation therapy despite achieving adequate contractions, suggesting that other pathways are activated to promote contractions in these patients. Importantly, the biological measurements taken in this study will be correlated with clinical outcomes, which will further inform our understanding of the biology of oxytocin during induction of labor. If regular contractions are achieved with nipple stimulation, comparison between oxytocin levels in the

two groups will help us to understand whether absolute oxytocin concentration is predictive of ultimate mode of delivery. For example, if in more patients, consistent with preliminary findings, breast stimulation is found to result in equal rates of vaginal birth but lower concentration of oxytocin, this would provide evidence that endogenous oxytocin signaling acts through other methods such as affecting oxytocin receptor availability or interaction with other hormones. On the other hand, if oxytocin concentrations are similar or greater in women undergoing breast stimulation, it would suggest that oxytocin concentration is the main driver in successful versus unsuccessful inductions of labor. Similarly, if breast stimulation is found to result in lower oxytocin concentrations and not to be as effective as exogenous oxytocin, this would provide evidence that concentrations do need to reach some critical threshold to effect myometrial contractility.

Because, in our preliminary studies, we have found less rise in maternal oxytocin among patients undergoing nipple stimulation, it is possible that other pathways are being activated in these patients. We intend to explore those other pathways using “omics” approaches to look at plasma proteins and microRNAs and urine prostaglandin metabolites. These studies have the potential to identify the mechanism(s) by which nipple stimulation induces labor, may point to differences with IV oxytocin that lead to the increased morbidities associated with prolonged and failed induction of labor, and may elucidate novel pathways involved in the stimulation of labor, which would contribute to our understanding of induction of labor. In order to understand the mechanisms by which nipple stimulation therapy induces labor, we will also compare the measurements of these biological variables in subjects prior to induction of labor and in control patients in early spontaneous labor.

Lastly, lactation is activated and triggered when a very tightly regulated cascade of events occurs at the end of the pregnancy and during labor, but may be negatively impacted by obstetric interventions. Secretory activation of the mammary gland initiates mature milk production in epithelial cells (usually occur 48-72h postpartum) and then milk ejection is triggered by oxytocin hormone and nipple stimulation (e.g., infant suckling or breast pump). Factors such as nulliparity, labor induction, and cesarean delivery have been shown to increase the risk of early lactation difficulties. Delayed secretory activation is a key predictor of suboptimal breastfeeding outcomes. It is biologically plausible that with IV oxytocin the normal secretory activation process for lactation is altered. Using milk biomarkers, we will examine the effect of nipple stimulation induced labor on lactation initiation in the first 48-72 hours after birth.

3.3.1 Potential Risks

Risk related to study interventions

To promote additional safety related to study interventions, both study intervention arms (immediate intrapartum nipple stimulation therapy via electric breast pump versus immediate synthetic oxytocin infusion without nipple stimulation) will require continuous fetal cardiotocography during intervention use to monitor fetal heart rate patterns and contraction frequency. There will be clear recommendations in the study protocol for titration of both interventions to ensure that the frequency of contractions is within the recommended goal range. Therefore, no serious or life-threatening adverse events directly related to the study intervention are expected. To prevent the risk of nipple soreness or pain related to the incorrect use of an electric breast pump, each subject assigned to the study intervention will undergo a tutorial prior to the initiation of an electric breast pump to review correct breast pump flange fit and placement. Lanolin ointment will also be provided to subjects. The device manual or Instructions of Use will be provided to each subject to be the primary source of information of risks of the device provided by the study, should they choose to use it. The subject will also have the option to use their own home electric breast pump if they desire.

Risks related to biospecimen collection

There is a small risk associated with biospecimen collection. The risks include discomfort from the needle stick and/or IV catheter for venous blood draw, the possibility of pain or bruising at the site of the blood draw, the possibility of feeling lightheaded, and the rare risk of infection at the site of blood draw. There are no significant risks to providing mid-stream urine collection or collecting urine from an indwelling foley catheter. If the patient is having intermittent bladder catheterization, additional catheterization events will not be performed purely for study purposes.

There is minimal risk in milk collection including temporary discomfort in the breast/nipple for a short time after pumping. We anticipate that the small volume of milk (0.5ml up to twice a day) collected for the study will not affect infant growth or interfere with the nutritional needs of full-term infants.

Risk related to study interactions and subject confidentiality

There will be multiple procedures in place to minimize the risk of subject confidentiality, and will be outlined in the study protocol. Subject confidentiality will be held in strict trust by all members of the research teams. Subject medical record review will be limited to just the elements needed to complete the study. Only authorized HIPAA and GCP trained study team members will be allowed to extract research data from medical records and enter it into the *REDCap* database.

At each study site, subject records are maintained in the Epic System® EMR and all research data will be collected in and maintained in *REDCap*, an established, secure, web-based capture and management tool developed at Vanderbilt University). Any paper records (e.g. signed consent forms) will be kept in a locked cabinet within a locked office that is secured always and located on the main campus within the departmental research administrative office space at each study site to ensure confidentiality of subject data.

Data use agreements will be obtained and approved across all three institutions: Weill Cornell as the primary grant institution where the PD resides, Yale University (grant sub-award institution), and Northwestern University (grant sub-award institution).

The IRB may inspect study records at any given time. In addition, all scientific publications will refer to the subjects by study identifiers only. At no time will any of the subjects in the study be identified. Identifiable study information will be maintained for up to ten years after the research is complete. After that time, it will be destroyed or de-identified. The principal investigator will keep a link that identifies subjects to coded information, but this link will be kept secure and available only to the PIs or selected members of the research team.

All samples collected at WCM will be de-identified. A secure, access-restricted linkage key connecting study IDs to patient identifiers will be maintained solely by the local research teams. Dr. Golan's lab will only receive de-identified samples and coded data labeled with the study ID, and will have no access to patient identifiers. Results generated by Dr. Golan's lab will be uploaded to the WCM REDCap database using the matched study IDs provided with the samples, ensuring all data remain de-identified and participant confidentiality is maintained throughout the study.

3.3.2 Potential Benefits

There are potential direct medical benefits to individuals participating in the study if intrapartum nipple stimulation therapy via electric breast pump does shorten the duration of labor induction, reduce the risk for operative delivery and other obstetric complications, and improves lactation outcomes. Further, there are potential direct medical benefits to the newborns of individuals participating in the study if intrapartum nipple stimulation therapy via electric breast pump does improve lactation and infant feeding. Some of these outcomes, if beneficial, may also have indirect benefits on medical costs. Further, aside from potential direct medical benefits to the subjects, the subjects will know that they are contributing to the better understanding of labor induction and lactation and potentially improving to the methods used through this proposal.

4 - Study Objectives

4.1 Hypothesis

Our central hypothesis is that nipple stimulation with an electronic breast pump to induce labor increases spontaneous vaginal delivery, shortens duration of labor induction, improves patient-reported pain scores during labor, improves patient satisfaction and sense of self agentry with the birthing process, and improve scores on the postpartum depression scale. We also hypothesize that participants assigned to nipple stimulation therapy during labor will be more likely to use breastfeeding as the sole source of nutrition at hospital discharge, which will be associated with improved maternal perception of milk supply, less severe newborn weight loss, and sustained breastfeeding for the recommended 6 months. Secondarily, we hypothesize that intrapartum nipple stimulation will not increase fetal or neonatal morbidity outcomes. Last, by examining cost of care and health-related quality of life, we hypothesize that performing nipple stimulation therapy during labor will be more cost-effective than the comparator after considering their overall impact on labor and delivery, breastfeeding, and early infant nutrition and care in the first 6 months.

We also hypothesize that nipple stimulation therapy invokes the wide cascade of events that promote labor to a greater extent than IV oxytocin alone. We hypothesize that oxytocin in the plasma of patients performing nipple stimulation will rise less than among subjects receiving IV oxytocin at the time when the subject is first contracting regularly. We hypothesize that other proteins associated with labor (including but not limited to IL6, IL1beta, plasminogen activator inhibitor 1, C reactive protein, ICOS ligand, and ERP29 will rise more in the circulation of subjects receiving nipple stimulation therapy compared with IV oxytocin. We hypothesize that urinary metabolites of prostaglandin E2 and F2alpha will be higher in subjects receiving nipple stimulation therapy. We hypothesize that, on a systematic level, plasma changes among subjects partaking in nipple stimulation will differ from subjects receiving IV oxytocin. We hypothesize that nipple stimulation therapy will have earlier secretory activation as measured by milk sodium and conductivity) in the early days postpartum, compared to individuals receiving IV oxytocin alone.

4.2 Primary Objective

To determine whether intrapartum nipple stimulation changes the likelihood of achieving a spontaneous vaginal delivery compared to receipt of exogenous oxytocin infusion to induce labor.

4.3 Secondary Objectives (if applicable)

The secondary objectives are as follows:

1. To determine if women who perform intrapartum nipple stimulation to induce labor have differences in other obstetric and maternal outcomes such as operative vaginal delivery, cesarean delivery, labor duration, postpartum hemorrhage, infection, and morbidities such as higher-order perineal lacerations, ICU admission, or death.

2. To determine if women who perform intrapartum nipple stimulation report differences in pain scores during labor, labor agentry and satisfaction scores, postpartum depression scores, and breastfeeding success scores compared to women who receive only intrapartum exogenous oxytocin infusion.

3. To determine if women who perform intrapartum nipple stimulation report differences in their intention to breastfeed postpartum and their breastfeeding success scores, as well as continuation of breastfeeding for 6 months postpartum. In addition, to determine if neonates of women who perform intrapartum nipple stimulation have differences in breastfeeding as the sole source of nutrition (BSSN) at the time of their birth hospitalization discharge within the first 72 hours and their maximal percent weight loss up to 14 days post-birth compared to neonates of women who receive only intrapartum exogenous oxytocin infusion.

3. To determine if women who perform intrapartum nipple stimulation therapy to induce labor have similar fetal and neonatal outcomes compared to women who receive only intrapartum synthetic oxytocin infusion without nipple stimulation (comparator).

4. To examine the cost-effectiveness of inpatient nipple stimulation therapy via electric breast pump, in comparison to synthetic oxytocin infusion without nipple stimulation (comparator).

5. In a sub-cohort of women who are enrolled in the trial at Yale, to measure circulating plasma concentrations of proteins and microRNA and urinary prostaglandin metabolites using unbiased “omics” approaches, comparing patients undergoing induction of labor via intrapartum nipple stimulation versus continuous exogenous oxytocin infusion.

6. To compare the molecular changes observed with nipple stimulation therapy oor continuous exogenous oxytocin infusion with changes seen in early spontaneous labor.

7. In a sub-cohort of women who provide milk samples, we will compare milk composition before and after delivery for the nipple stimulation therapy group to see how the milk is changing and we will compare milk biomarkers after birth between patients undergoing induction of labor via intrapartum nipple stimulation versus continuous exogenous oxytocin infusion.

5 - Study Design

5.1 General Design Description

The proposed study is a multicenter, pragmatic, open label, parallel group randomized trial of nulliparas to compare the effectiveness of intrapartum nipple stimulation therapy with or without adjunctive synthetic oxytocin (intervention) versus immediate synthetic oxytocin

infusion without nipple stimulation (comparator) during labor induction. Potential subjects will be recruited from the Labor & Birth units at three study sites: Yale New Haven Hospital in New Haven, Connecticut, Weill Cornell Medicine in New York, New York and Northwestern Memorial Hospital in Chicago, Illinois. We aim to test the central hypothesis that intrapartum nipple stimulation increases spontaneous vaginal delivery, shortens labor duration, improves other patient-centered outcomes, improves breastfeeding success, and does not increase adverse neonatal and maternal outcomes. We chose a randomized controlled trial, the 'gold standard' of clinical research design, with the goal of obtaining the highest quality evidence to inform clinical practice. Randomly allocating subjects to different interventions minimizes selection bias and results in groups that are comparable with regards to important confounding variables, both measured and unmeasured. Blinding of subjects, maternity care providers, and investigators is not feasible given the intervention being studied (nipple stimulation). With the limitations of the existing data, we estimated our sample size and plan our data analysis based on two-tailed tests. This will ensure that we identify the optimal management strategy for labor induction that increases spontaneous vaginal delivery, irrespective of the direction of our findings.

We will follow the Consolidated Standards of Reporting Trials (CONSORT) guidelines wherever appropriate in the conduct and reporting of this trial. (27) We will use computer-generated random sequences to assign participants to the two interventions with stratification by study site and membrane status (intact versus ruptured). Labor management will be similar in the two groups except for use of nipple stimulation (intervention group) versus immediate synthetic oxytocin infusion without nipple stimulation (control). Analysis will follow the intention-to-treat principle.

5.1.1 Study Date Range and Duration

The study is expected to start in October 2021 at Yale, July 2023 at Northwestern and Spring 2024 at Weill Cornell Medicine and will be conducted over 7 years, with the final participant expected to be enrolled in June 2026. Final data collection and analysis is expected to be completed by June 2028.

5.1.2 Number of Study Sites

The trial will be conducted at three sites: Yale New Haven Hospital (York Street Campus), Weill Cornell Medicine and Northwestern Memorial Hospital.

5.2 Outcome Variables

5.2.1 Primary Outcome Variables

For the main trial, the primary outcome is spontaneous vaginal delivery, defined as a delivery that occurs without the use of forceps, vacuum, or cesarean. This is because spontaneous vaginal delivery is the most desirable outcome for laboring women

5.2.2 Secondary and Exploratory Outcome Variables (if applicable)

The following secondary maternal outcomes will be explored:

- Operative vaginal delivery (defined as delivery with the assistance of forceps or vacuum) and indication
- Cesarean delivery and indication
- Interval from randomization to delivery
- Postpartum hemorrhage (defined as a cumulative blood loss of ≥1,000 mL within 24 hours after the birth process)
- Severe postpartum hemorrhage (defined as any of the following: transfusion; non-elective hysterectomy; use of ≥2 uterotonic medications other than oxytocin; other interventions such as uterine compression sutures, uterine artery ligation or embolization, hypogastric artery ligation, balloon tamponade)
- Suspected intraamniotic infection, intrapartum chorioamnionitis, or postpartum endometritis (defined as maternal fever ≥38° Fahrenheit with planned or initiated administration of therapeutic antibiotics) after randomization and prior to delivery hospitalization discharge
- Third- or fourth- degree perineal lacerations
- Lactational mastitis (assessed up to 12 weeks postpartum from breastfeeding survey performed at 4-12 weeks postpartum and medical records)
- Maternal death (assessed until delivery hospitalization discharge)
- Admission to Intensive Care Unit (assessed until delivery hospitalization discharge)
- Subject-reported pain experienced during childbirth using the visual analog scale (28) , measured at 2 hours after start of intervention
- Subject-reported feelings of control during labor using the Labor Agentry Scale (29) , assessed during the hospital postpartum stay
- Subject-reported satisfaction with the birthing experience using the validated Birth Satisfaction Scale-Revised (BSS-R) (30) (31), assessed during the hospital postpartum stay
- Subject-reported ability to express and/or collect colostrum and/or breastmilk intrapartum
- Subject-reported perception of milk supply using the validated Perception of Insufficient Milk Supply (PIMS) (40), 2 weeks postpartum

- Subject-reported depression score using the validated Edinburgh Postnatal Depression Scale, assessed (32), 4-12 weeks postpartum
- Subject-reported breastfeeding success using the validated Maternal Breastfeeding Evaluation Scale (MBES) (33), assessed 4-12 weeks postpartum
- Subject-reported breastfeeding success by assessing infant feeding method (breastmilk, formula, or combination) via questionnaire, assessed 6 months postpartum

Among the women enrolled in the translational sub-study at Yale, the following secondary outcomes will be explored:

- Plasma proteomic profiles (measured by mass spectrometry) after 2 hours of treatment comparing patients receiving IV oxytocin and nipple stimulation. Plasma samples from patients who have not undergone cervical ripening will be collected as additional control to account for differences achieved from cervical ripening alone. Plasma samples from patients in early spontaneous labor will be obtained to compare how closely nipple stimulation therapy and IV oxytocin approximate the molecular phenotype of spontaneous labor.
- Changes in plasma proteomic profiles from baseline (prior to treatment), 2 hours, and the time of adequate contractions in each treatment group
- Plasma micro-RNA profiles (measured by bulk RNA sequencing) after 2 hours of treatment among patients receiving IV oxytocin, nipple stimulation, and patients in early spontaneous labor.
- Changes in plasma micro-RNA profiles from baseline (prior to treatment), 2 hours, and the time of adequate contractions in each treatment group
- Urine prostaglandin profiles (measured by mass spectrometry) at 2 hours comparing patients receiving IV oxytocin, nipple stimulation, nipple stimulation who cross-over to IV oxytocin, un-ripened control patients, and patients in spontaneous labor.
- Changes in urine prostaglandin profiles from baseline (prior to treatment), 2 hours, and the time of adequate contractions in each treatment group

Among the women enrolled in the translational sub-study for milk collection, the following secondary outcomes will be explored:

- Changes in milk sodium and conductivity levels from before delivery (in the nipple stimulation therapy group only), and 12, 24, 48 hours after delivery. For mothers hospitalized for a longer period, additional samples at 72 hours will be collected or later up to twice a day.
- Milk macronutrient composition at 48 hours after delivery in each treatment group.
- Milk RNA profile at 48 hours after delivery in each treatment group.

The following secondary neonatal outcomes will be explored:

- Intrapartum fetal death or neonatal death (assessed until birth hospitalization discharge or death)
- Neonatal Apgar score ≤3 at 5 minutes of life
- Umbilical cord arterial pH <7.0 or base excess >12 mmol/L; or umbilical cord venous pH <7.0 or base excess >12 mmol/L if arterial blood sample not available
- Any admission to Neonatal Intensive Care Unit (assessed until birth hospitalization discharge or death)
- Admission to the Neonatal Intensive Care Unit >24 hours (assessed until birth hospitalization discharge or death)
- Composite neonatal severe morbidity measure including any of the following: Intrapartum fetal death or neonatal death; intubation, continuous positive airway pressure (CPAP) or high-flow nasal cannula (HFNC) for ventilation or cardiorespiratory support within first 72 hours of life; neonatal encephalopathy as defined by Shankaran et al (34) ; seizures; need for hypothermic treatment (cooling); sepsis (defined as presence of a critically ill infant in whom systemic infection is suspected with a positive blood, cerebrospinal fluid, or catheterized/suprapubic urine culture, or in the absence of positive culture(s), clinical evidence of cardiovascular collapse or an unequivocal X-ray confirming infection); pneumonia confirmed by X-ray or positive blood culture; major birth injury (brachial plexus injury, bone fractures, other neurologic injury, retinal hemorrhage, facial nerve injury); meconium aspiration syndrome; intracranial hemorrhage or subgaleal hemorrhage; and hypotension requiring pressor support
- Breastfeeding as the sole source of nutrition (BSSN) at the time of birth hospitalization postpartum discharge within the first 72 hours. This timepoint is chosen because it is the most likely to be causally linked to the study intervention, avoids the risk of attrition bias, and predicts the likelihood of sustained breastfeeding for the recommended 6 months postpartum. BSSN is defined as the infant receiving breastmilk without any supplementary formula or water within the first 72 hours of life. infant feeding method (breastmilk only, mixed feeding which includes both breastmilk and infant formula, or infant formula only) is standardly documented in the EMR at least daily until day of birth hospitalization discharge.
- Maximal percent newborn weight loss up to 14 days post-birth. Maximal percent newborn weight loss is defined as the difference between birth weight and the lowest weight recorded subsequently up to 14 days post-birth, calculated as a percentage of the birth weight, as is typically done daily in clinical practice. Participants will sign a medical release form allowing the study team to obtain/access newborn data in this timeframe, not only from the site’s electronic medical record but also from outside

practices/institutions. For the assessment of maximal newborn weight loss, it is standard hospital practice at study sites to regularly weigh each newborn during their birth hospitalization. Infants are weighed naked using a digital scale, and the weight is expressed in kilograms. At the study site, newborns are weighed at birth and then at least daily until day of discharge. The first weight measured after birth is usually performed after at least 6 hours post-birth. Weighing is typically discontinued if the newborn regains his or her birth weight prior to discharge. Infant birth weights are recorded in a standardized fashion in specific EMR flowsheets at study sites. The date and time of each weight are recorded which allows calculation of precise age.

- The following cost outcomes will be assessed: 1) cost of relevant care, and 2) health-related quality of life. Both outcomes will be measured for the mother-infant pair.
    - **Cost of care** will include direct medical cost, direct non-medical care, and indirect cost. Direct medical cost will encompass hospital facility costs and provider professional fees associated with relevant care (maternal labor and delivery hospitalization, newborn's initial birth hospitalization, and infant's postnatal pediatric visits). Direct non-medical cost will account for breast pump use at home, infant formula use, and transportation to relevant care. Indirect cost (i.e., maternal productivity loss) will measure participants' work loss in the postpartum period. As the project will span multiple years, we will use consumer price index to inflation adjust all cost estimates to a constant year U.S. dollars
    - **Health-related quality of life** for participants will be measured by the EuroQol five dimensions (EQ-5D) instrument (41). EQ-5D is a validated instrument for measuring and valuing health. Participants' response to the five dimensions of the EQ-5D descriptive system will be converted to a utility score using a validated U.S. population-based algorithm. Utility score takes values ranging from 0 to 1, with 0 referring to a health state equivalent to death and 1 equivalent to perfect health. EQ-5D is a recommended and widely used instrument for measuring utility score in clinical trials and in maternal health research. For infants, the South Africa EQ-TIPS♥™ Paper Proxy 1 questionnaire (previously known as Toddler and Infant (TANDI) Health Related Quality of Life instrument) (42) will be completed by participants (as proxy reporters for their infants). This questionnaire has been shown to be valid and reliable for use in very young children (42). It contains a six-dimension descriptive system (movement, play, pain, social interaction, communication, and eating) with three severity levels (no, some, or a lot of problems), along with a visual analogue scale (VAS) assessing an infant's overall health. As utility score based on the EQ-TIPS♥™ descriptive system has not been developed yet, we will linearly transform the VAS score to a utility score on a 0 to 1 scale.

5.3 Study Population

Pregnant women who are nulliparas with live gestations of 36 weeks and greater who are planned to receive (but have not yet received) exogenous oxytocin infusion for induction of labor. While pregnant women represent a vulnerable population, they represent the study population of interest.

5.3.1 Number of Participants

The sample size for the trial is based on the primary outcome of spontaneous vaginal delivery. Therefore, the sample size of n=988 (shared between Yale, Weill Cornell Medicine and Northwestern) is selected for this proposed study to detect a 10% absolute increase while accounting for an expected 5% attrition rate. We then estimate, on the basis of the sample size for the primary outcome, the power we will have to detect clinically significant differences in the secondary outcomes.

For the translational sub-study at Yale, we will seek consent to obtain plasma and urine from patients enrolled in the main study (n=192). In addition, plasma will be obtained from 48 patients in spontaneous labor.

For the milk collection sub-study at Yale and Weill Cornell Medicine, to detect a 10 mM difference in sodium concentration between the intrapartum nipple stimulation therapy group and the immediate synthetic oxytocin infusion group at 48 hours postpartum, with a standard deviation of 20 mM, 80% power, and a significance level of 0.05, a sample size of 32 participants per group is required. Accounting for an expected 30% dropout rate, we plan to recruit 46 participants per group.

5.3.2 Eligibility Criteria/Vulnerable Populations

We will use broad inclusion criteria to ensure generalizability of our results.

Inclusion criteria

- Age 18 years and older
- Nulliparous women (a prior pregnancy that ended at <20 $^{0/7}$ weeks of gestation is acceptable)
- Gestational age 36 $^{0/7}$ weeks and greater at enrollment
- Singleton gestation (a multiple gestation reduced to a singleton, either spontaneously or therapeutically, before 14 $^{0/7}$ weeks of gestation is acceptable)
- Planned to undergo initiation of exogenous oxytocin infusion by their maternity care provider
- Cervix dilated <6 cm within one hour of enrollment (if membranes intact) OR ruptured membranes with less than 3 contractions in a 10-minute window averaged over 30 minutes prior to enrollment (regardless of cervical exam)

- Ability to give informed consent

Exclusion criteria

- Unable to understand English or Spanish
- Presence of tachysystole (defined as more than 5 contractions in 10 minutes averaged over 30 minutes), recurrent variable or late fetal decelerations, and bradycardia in the prior 30 minutes before enrollment
- Non-vertex presenting fetus at time of enrollment
- Planned for cesarean delivery or contraindication to labor by institutional policy (e.g., placenta previa, vasa previa, active genital herpes infection, previous transmural myomectomy)
- Multi-fetal gestation (e.g., twins, triplets, and higher-order multiples)
- Intrauterine fetal death
- Major fetal anomaly suspected prenatally (defined as a fetal anomaly with anticipated neonatal intensive care unit admission)
- Suspected alloimmunization (given the increased likelihood for anticipated neonatal intensive care unit admission)
- Known severe fetal growth restriction (estimated fetal weight <3rd percentile) or abnormal umbilical artery Doppler studies (given the increased likelihood for anticipated neonatal intensive care unit admission)
- HIV infection (nipple stimulation is not encouraged given the recommendation for these mothers not to breastfeed)
- Participation in another interventional study that influences management of labor and delivery or perinatal morbidity or mortality
- History of mastectomy or other contraindication to manual nipple stimulation or use of electronic breast pump
- Known allergic reactions to components of the electronic breast pump or to synthetic oxytocin intravenous solution
- Significantly impaired consciousness or executive function (e.g., intubated or sedated)

For collection of plasma and urine from patient participants in early spontaneous labor (defined as <6 cm dilated with intact membranes), inclusion criteria will be:

- Age 18 years and older
- Nulliparous

- Gestational age 36 0/7 and greater
- Singleton gestation
- Ability to give informed consent
- Presenting in early spontaneous labor defined as <6cm dilated with intact membranes.
- Patient and primary obstetric provider intend for patient to continue spontaneous labor

For collection of plasma and urine from patient participants in early spontaneous labor (defined as <6 cm dilated with intact membranes), exclusion criteria will be:

- Patient has performed nipple stimulation in the preceding 24 hours, received outpatient prostaglandin therapy, or had “membrane stripping” in the preceding 24 hours
- Patient is expected to have labor complications preventing continued spontaneous labor, such as arrest of dilation or fetal intolerance of labor

6 - Methods

6.1 Treatment

6.1.1 Intervention administration and schedule

Subjects will be encouraged to perform nipple stimulation with the aid of an electronic breast pump to promote standardization of the stimulation. The subject will be provided with a hospital-grade breast pump. The subject may choose to use their own personal non-hospital breast pump instead. Subjects can also alternatively choose to perform nipple stimulation by hand if they desire. Subjects will receive a 5-10 minute tutorial on either method by their labor nurse and supported by their labor nurse with the additional assistance of hospital lactation consultants if needed during their stimulation process. All labor nurses and hospital lactation consultations are educated on proper breast pump use.

6.1.2 Method of Assignment/Randomization

Enrolled subjects will be randomly assigned in a 1:1 ratio to both study groups. A web-based randomization sequence will be prepared using blocks of variable sizes, stratified by study site and amniotic membrane status (intact versus ruptured), and maintained centrally by the study statistician. The advantage of this method is that it provides a good probability of balance, and future assignments are unpredictable. A subject’s group assignment will be obtained only after the subject is confirmed to continue to meet inclusion criteria, and a study number is entered and locked in using *REDCap* (Research Electronic Data Capture), an established, secure, web-based capture and management tool developed at Vanderbilt University. Although blinding of both subjects and their obstetrical clinicians would be ideal, blinding is clearly not possible in this trial. We will minimize systematic bias by applying the

same standard procedures for other labor and management strategies between groups at each study site. Further, the group assignment of subject will not be taken into account by trained study staff collecting maternal and neonatal outcomes. Importantly, the main study outcomes of spontaneous vaginal delivery and breastfeeding as the sole source of infant nutrition at hospital discharge are objective measures.

6.1.3 Concomitant therapy

There are no restrictions on use of concomitant medications. However, for those who are randomized to nipple stimulation, the use of exogenous oxytocin intravenous solution will not be permitted until at least two hours of nipple stimulation alone has been attempted, unless this is decided by the treating obstetric provider. Stimulation may be discontinued at any time that the treating obstetric provider decides. If there are any signs of fetal heart rate abnormalities on the cardiotocography, the obstetric provider should provide usual care measures such as discontinuation of stimulation, lateral positioning, intravenous hydration, etc. Initiation of exogenous oxytocin intravenous solution prior to two hours of nipple stimulation alone will be considered a cross-over.

6.2 Assessments

Obstetric and perinatal clinical outcomes will be assessed and abstracted through chart review of the electronic medical record of both subjects and their infants by certified trained research members according to the outcome definitions listed in the protocol. Postpartum assessments will be conducted at 2 weeks postpartum and 4-12 weeks postpartum. While the ideal time period is 6-8 weeks postpartum, such a short time-window for follow-up may result in less-than-ideal protocol adherence, and 12 weeks is still within the typically described postpartum period (the “4th trimester”). In addition, economic costs and infant feeding method will be assessed at 6 months postpartum.

To assess patient-centered outcomes, several study questionnaires will be conducted:

1. To assess subject-reported pain experienced during childbirth, the visual analog pain scale (28) will be administered at three different timepoints: (1) prior to initiating study intervention (to obtain baseline); (2) two hours after initiating study intervention; and (3) during postpartum stay prior to delivery hospitalization discharge.

2. To assess subject-reported satisfaction with the childbirth process, the Birth Satisfaction Scale-Revised (BSS-R) (30) (31) will be administered during postpartum stay prior to delivery hospitalization discharge.

3. To assess subject-reported feelings of control during childbirth, the Labor Agentry questionnaire (29) will be administered during postpartum stay prior to delivery hospitalization discharge.

APPROVED BY THE YALE UNIVERSITY IRB 8/11/2025

4. To assess subject-reported feelings of depression after childbirth, the Edinburgh Postnatal Depression Scale (EPDS) (32) will be administered at two different timepoints: (1) prior to initiating study intervention (to obtain baseline) and (2) at 4-12 weeks postpartum (ideally 6-8 weeks postpartum). The baseline EPDS will be reviewed, and the total score will be tabulated in real-time. The EPDS at 4-12 weeks postpartum will be completed by the subject online or with a certified study team member via telephone. A certified study team member will check daily (Monday-Friday) to review any completed online surveys and tabulate scores during these daily (Monday-Friday) reviews. If the EPDS at 4-12 weeks postpartum is completed with the subject via telephone, the total score will be tabulated at the conclusion of the telephone encounter. Any score ≥10 on either the baseline or 4-12 weeks postpartum EPDS will be reported to the subject's primary obstetric provider team as this is the threshold that suggests increased risk of depressive illness.

5. Prior to delivery hospitalization discharge, subjects will be asked if they had any intrapartum expression of colostrum or breast milk, the quantity (mL or ounces) expressed if known, whether they collected and stored it for their infant, and whether they fed it to their infant.

6. After delivery hospitalization discharge 2 weeks postpartum, all subjects will be administered the Perception of Inadequate Milk Supply (PIMS) (40) survey to assess their perception of their milk supply. In addition, the EQ-5D♥™ and EQ-TIPS♥™ surveys will be administered.

7. After delivery hospitalization discharge, between 4 to 12 weeks postpartum (ideally 6 to 8 weeks postpartum), all subjects will be contacted by text message, telephone, or by email (based on subject preference) to evaluate if they experienced any unanticipated outpatient or inpatient visits for them or their infants since delivery hospitalization discharge. Subjects will also be asked if they ever attempted to breastfeed postnatally.

8. Among subjects who state they attempted to breastfeed at least once postnatally; these subjects will be administered the Maternal Breastfeeding Evaluation Scale (MBES) (33) between 4 to 12 weeks postpartum (ideally 6 to 8 weeks postpartum) to assess their breastfeeding success. In addition, the EQ-5D♥™ and EQ-TIPS♥™ surveys will be administered.

9. After delivery hospitalization discharge, at 6 months postpartum, all subjects will be sent an electronic survey to assess infant feeding method. In addition, the EQ-5D♥™ and EQ-TIPS♥™ surveys will be administered. Subjects may be contacted by telephone or by email (based on subject preference) to follow-up any incomplete surveys.

10. For spontaneous labor control patients, time/duration since contractions began, receipt of outpatient prostaglandins such as misoprostol, outpatient nipple stimulation, and time of

last membrane stripping will be collected by a combination of patient recall and medical record review.

11. Oxytocin concentration will be measured by mass spectrometry. Cytokines will be determined by enzyme-linked immunosorbent assay.

12. Plasma samples will be analyzed for proteomic profile by the Keck MS & Proteomic Resource and for microRNAs by the Yale Center for Genome Analysis. De-identified urine samples will be sent to an external core facility with expertise in prostaglandin measurement (Vanderbilt Eicosanoid Laboratory).

13. Milk samples will be analyzed at the Golan Lab (Cornell University, Ithaca). Sodium concentration and conductivity in milk will be measured using the LAQUATwin electrodes (Horiba, TX, USA). Milk lactose, fat and total protein will be measured in samples where a larger milk volume (>1ml) is available using the SpectraStar™ XT-R - High-Performance Extended Range NIR Analyzer (KPM, Analytics, MA, USA).

6.2.1 Efficacy

6.2.2 Safety/Pregnancy-related policy

The study protocol has been designed to minimize risks to subjects and their neonates including the requirement for continuous fetal monitoring during receipt of either study intervention, as well as detailed steps outlined to ensure the desired contraction frequency pattern to minimize risk of abnormal fetal heart rate patterns. All subjects who are undergoing continuous fetal monitoring are observed by labor nurses, and fetal monitoring is continuously reviewed and recorded.

6.2.2.1 Adverse Events Definition and Reporting

Adverse study events are expected to be rare. However, Reportable Events (which are events that are serious or life-threatening and unanticipated (or anticipated but occurring with a greater frequency than expected) and possibly, probably, or definitely related to study procedures or Unanticipated Problems Involving Risks to Subjects or Others (UPIRSO) that may require a temporary or permanent interruption of study activities, as determined by the Principal Investigator or sub-site PI(s) will be reported to the Yale IRB. Events that may require a temporary or permanent interruption of study activities by the Principal Investigator to avoid potential harm to subjects will be reported to the IRB **immediately** (if possible), followed by a written report to the IRB using the UPIRSO Reporting Form (710 FR 4) **no more than 5 calendar days** after the Yale Principal Investigator (M.S.) becomes aware of the event**.**

Detailed information concerning adverse events will be collected and evaluated throughout the conduct of the protocol. Any adverse events deemed serious unexpected and definitely, possibly or probably related, will be immediately (within twenty-four hours of notification)

forwarded by the Yale Principal Investigator (M.S.) to the DSMC Chair and any other DSMC member who requests notification. If a death is reported, a copy of the patient's medical record will be made.

Adverse events which do not qualify under the below definition will be collected and sent to the Chair, and any other requesting DSMC member on a monthly basis. The Chair decides whether the adverse event reports should be disseminated to the rest of the committee, and whether a follow-up call or meeting is required. All adverse events will be considered along with other interim safety data in the DSMC deliberations.

The following events may represent UPIRSOs that should be promptly reported:

- Adverse device effects that are unanticipated
- Adverse events or injuries that are serious, unexpected, *and* related
- Breaches of confidentiality involving risks
- Revisions to safety information, such as Investigational New Drug (IND) Safety Reports and MedWatch Reports, that meet the definition of a UPIRSO
- New information indicating an unexpected increase in risks or decrease in potential benefits (e.g., literature/scientific reports or other published findings)
- Protocol deviations, violations, or other accidental or unintentional changes to the protocol or procedures involving risks or with the potential to recur
- Unapproved changes made to the research to eliminate an apparent immediate hazard to a subject
- Other problem or finding (e.g., loss of study data or forms) that an investigator or research staff member believes could influence the safe conduct of the research

6.2.3 Pharmacokinetics (if applicable)

Oxytocin concentration will be measured by enzyme-linked immunosorbent assay (ELISA) using a commercially available kit using internal standards and controls to assess intra-assay and inter-assay coefficients of variance. All samples will be run in duplicate. The primary outcome is the difference (delta) in oxytocin concentration between baseline and when the patient first achieves adequate contraction pattern in patients undergoing nipple stimulation compared to patients receiving exogenous oxytocin.

6.2.4 Biomarkers (if applicable)

Unbiased screening approaches will be applied to plasma samples from patients in the two arms of the study. Protein profiling will be performed by the Keck MS & Proteomics Resources. microRNA screen will be conducted at the Yale Center for Genome Analysis. Prostaglandin measurements will be conducted by an external core facility with expertise in prostaglandin measurement (Vanderbilt Eicosanoid Laboratory). Proteomic, small molecule

(prostaglandin), or microRNA changes may be identified that represent novel biomarkers for labor.

Milk RNA extraction of de-identifiable milk samples will be performed at the Golan Lab at Cornell University. RNA sequence will be performed at the Genomic Core at Cornell University or sent to NovoGene for sequencing. Data analysis will be performed at the Golan Lab.

6.3 Study Procedures

Main Trial Study Procedures:

1. Recruitment

- A letter will be sent to all practicing obstetricians and midwives at each site to provide information about this study and to ask them if their patients may be approached for potential recruitment once they are admitted for labor induction.
- Trial information will be available on clinicaltrials.gov.
- All nulliparous women with singleton gestations at or greater than 36 weeks of gestation undergoing labor induction or augmentation are potentially eligible for screening.
- The research team at Yale may also utilize the Joint Data Analytics Team (JDAT) in two ways:
    - 1. Direct-to-patient recruitment method. This is a behind-the-scenes electronic medical record search run by JDAT for the Yale New Haven Health System (YNHHS), based on study-specific inclusion/exclusion criteria. This search will not identify potential participants to the researchers. Once JDAT identifies eligible patients, these potential participants will be sent a message via MyChart providing information about the research study and information on how to contact study personnel if they are interested in participating. Potential subjects who do not use MyChart may receive a paper mailing with the same information.
    - 2. We will request a report of patients who are potentially eligible and their treating obstetrician or midwife from JDAT through the Induction of Labor scheduling tool. We will request permission from the OB doctor/midwife to contact their patient directly to inform them of this research study. Given the number of potentially eligible patients and the time-sensitive nature of recruitment (must occur after induction is planned and before actual induction, which could be less than 1 and up to 7 days), it is not feasible to ask the treating provider to contact the patients first, and this would also place a significant burden on the treating provider. A member of the research team

will send the following message to the patient through MyChart or call the patient if MyChart is not available:

- Dear [Patient Name],

  My name is [Name] and I am a [insert job title] at Yale University and part of the research team for the STIM Study. I spoke with your doctor/midwife, and we believe you may be eligible to participate in this free and confidential research study investigating two different methods of labor induction. One method involves nipple stimulation using a breast pump and the other uses intravenous oxytocin infusion according to standard of care and hospital protocol. If you enroll, you will also be asked to complete brief surveys at 5 different times. You will be compensated [$X] each time you do a survey, for a total of [$X]. If you're interested in learning more, please reply to this message or call [insert phone]. We will also be available to answer any questions while you are at Labor & Birth at Yale New Haven Hospital. If you choose not to participate, you will continue with your usual care from your doctor/midwife. Thank you for your time.

  Sincerely,

  [Name]

- Inclusion/exclusion criteria will be reviewed with the patient's chart and/or discussion with the patient's responsible physician or midwife prior to approaching the patient to ensure that she meets eligibility criteria.
- If a patient appears to meet criteria for the trial, she will be told about the study and asked for written informed consent to participate in the trial. Consent will be obtained from a trained research team member.

2. Baseline Procedures

In addition to information collected for eligibility, the following information will be obtained at randomization from a subject interview followed by a review of her chart:

- Indication for labor induction
- Demographic information
- Medical history
- Obstetrical history including outcome(s) of any prior pregnancies (e.g., ectopic pregnancy, spontaneous or elective abortion prior to 20 weeks of gestation)
- Social history
- Current pregnancy complications (e.g., hypertensive disorders of pregnancy, gestational diabetes, etc.)

- Estimated fetal weight based on ultrasound (if within 2 weeks of randomization) or Leopold maneuvers
- Group B Streptococcus carrier status

3. Study Interventions

Women randomized to nipple stimulation will undergo the following procedures (intervention arm):

- Nipple stimulation will be done in the hospital after subjects receive proper training, and under the direction of the study and nursing teams.
- Subjects will be encouraged to stimulate with the aid of an electric breast pump, but they will also have the option to perform nipple stimulation by hand if desired.
- Subjects will undergo a 5-minute tutorial on the use of the electronic breast pump and hand stimulation technique by the labor nurse or certified research staff. Information handouts will be given to the subject.

Women randomized to exogenous oxytocin infusion (control arm) will undergo the following procedures as current standard of care:

- Continuous fetal monitoring will be maintained as per hospital protocol.
- Oxytocin will be administered using only premixed oxytocin intravenous solutions prepared by the pharmacy **as per current standard of care.**
- Oxytocin will be initiated via infusion smart pump at a starting rate of 2 mU/min.
- Dosage may be increased every 15 minutes for 2-hours, then every 30 minutes thereafter.
- Rate changes for oxytocin administration are based on the assessment of all three of the following: fetal status, contraction status, and maternal coping
- Decreases of the infusion rate by 1 mU/min require reassessment after 15 minutes.
- Oxytocin maximum dosage should not exceed 20 mU/min per institutional policy.
- The oxytocin will be stopped, and the provider will be notified for the following reasons:
- If oxytocin has been discontinued, it may be restarted after a minimum of 15 minutes
    - If oxytocin had been discontinued for <40 minutes and the fetal heart rate tracing is reassuring and uterine activity has returned to normal, oxytocin may be restarted at no more than one half of the rate that resulted in the non-reassuring FHR and/or uterine tachysystole and then increased by 1 or 2 mU/min per original provider order

- If oxytocin had been discontinued for >=40 minutes and the fetal heart rate tracing is reassuring and uterine activity has returned to normal, oxytocin may be restarted at 1 or 2 mU/min and increased by 1 or 2 mU/min per original provider order.

- The oxytocin line will be disconnected from the main IV line if it has been determined that a cesarean section will be performed.

4. Other Interventions

The use of amniotomy (if the subject has intact membranes) and other labor procedures will be at the discretion of each subject's responsible maternity care provider (pragmatic study) and considered standard of care. If standard of care is altered based on clinical decision making by the subject's responsible treating physician or midwife, the subject will still remain in the study as long as the subject does not withdraw her consent to continue in the study.

5. Subject Management and Follow-Up

All subjects will be monitored for progression of labor and their fetus' response to labor by their responsible treating obstetrician or midwife. Each subject will undergo individualized assessment by their treating obstetrician or midwife, and the clinical decision will be made by the treating obstetrician or midwife if or when subjects in the nipple stimulation arm will be switched to the standard of care with exogenous oxytocin administration. There can be no absolute cut-offs for time or contraction responses since standard of care dictates individualized assessments for labor progression and fetal response; each birth experience is unique. Subjects in both arms of the trial should be allowed adequate time to labor before considering the induction "failed" and proceeding to cesarean section. An induction will be considered "failed" after at least 24 hours of exogenous oxytocin administration has failed to generate regular (e.g., every 3 minutes) contractions and cervical change, as is considered standard of care (36). Additionally, exogenous oxytocin generally should be administered for at least 12 hours after membrane rupture before considering the induction to have failed as is considered standard of care. (37). The time devoted to cervical ripening and/or nipple stimulation will not be included when calculating the length of induction for diagnosing failed induction.

It is expected that the fetal heart rate and uterine activity will be monitored continuously during labor and that subjects will stay in the hospital until delivery once the induction is started as is considered standard of care. Aside from the interventions described above, labor and delivery management decisions will be left to the discretion of the subject's responsible maternity care provider as is considered standard of care.

Subjects will be administered study questionnaires at multiple timepoints, as outlined below. Questionnaires will be administered using the REDCap Survey tool, and electronic QR codes will be provided to the subjects via email or text message (whichever the subject prefers). Paper versions will also be available if requested.

After randomization, subjects will be asked to complete four questionnaires to establish baseline: the visual analog pain scale (28), the Edinburgh Postnatal Depression Scale (EPDS) (32), the Infant Feeding Intention Scale (IFI) (39), and the EQ-5D survey (41). Two hours after initiation of study intervention, subjects will again be asked to complete the visual analog pain scale (28) to assess their pain after intervention start.

During their hospital postpartum stay prior to discharge, subjects will be asked to complete three questionnaires: the visual analog pain scale (28) to rate their pain experienced during childbirth; the Labour Agentry scale (29) to assess their feelings of control during childbirth; and the Birth Satisfaction Scale-Revised (BSS-R) (30) (31) to rate their satisfaction with the childbirth process. They will also be asked about whether they plan to attempt to breastfeed their infant. They will also be asked if they had any intrapartum expression of colostrum or breast milk, the quantity (mL or ounces) expressed if known, whether they collected and stored it for their baby, and whether they fed it to their baby.

After hospital discharge (preferably two weeks postpartum, but up to three weeks postpartum will be acceptable), all subjects, regardless of study group, will be asked to complete one questionnaire: the Perception of Milk Supply (40). The unique QR code or link to this electronic survey will be emailed or texted to the subject based on their preference. Follow-up phone call will be made if needed.

After hospital discharge (preferably six to eight weeks postpartum, but anytime within the window of four to twelve weeks postpartum will be acceptable), all subjects, regardless of study group, will be contacted to ask about any unanticipated outpatient or inpatient visits for them or their infants since discharge. Subjects will also be asked to complete two questionnaires: the Maternal Breastfeeding Evaluation Scale (33) and the EPDS (32).

After hospital discharge (6 months postpartum), all subjects will be asked to complete one questionnaire to assess infant feeding method.

Biospecimen Procedures:

Specimen collection will be conducted as described in the previously published methodology (38).

For patients in non-spontaneous labor, blood and urine will be collected at 1) prior to cervical ripening, 2) after cervical ripening, and 3) after 2 hours of treatment (IV oxytocin or nipple stimulation). This is a maximum of 3 blood samples per subject. For control patients in spontaneous labor, a blood and urine specimen will be obtained at enrollment. Blood may be drawn in several ways depending on what is clinically appropriate: (1) venipuncture; (2) IV catheter placed as part of standard of care (SOC); and/or (3) IV catheter placed for research purposes. The research IV may be placed in addition to the SOC IV and may be used instead of research venipuncture. That is, research venipuncture and research IV will not both occur at the same time (only one or the other).

Urine samples (amount variable by patient) will be collected mid-stream in a sterile screw-top container 1) prior to cervical ripening, 2) after cervical ripening, and 3) after 2 hours of treatment (IV oxytocin or nipple stimulation). If a patient has an indwelling foley catheter, urine from the tubing will be collected at the time of adequate contractions. If a patient is being intermittently catheterized for urine, a urine sample will be collected as able, but the patient will not undergo additional bladder catheterization for the purpose of this study.

Blood and urine samples may not be collected at one or more of the specified time points due to logistical issues (eg. patient enrolled after cervical ripening, labor floor acuity prevents nurse from drawing blood, patient delivers before time point, etc.) and/or clinical care taking precedence.

Proteomic profiling will be conducted by mass spectrometry at the Keck MS & Proteomics Resource. Prostaglandin measurements will be performed by an external core facility with expertise in prostaglandin mass spectrometry methods (Vanderbilt Eicosanoid Facility). microRNA profiling will be conducted via bulk RNA sequencing by the Yale Center for Genome Analysis/Keck Core.

Milk samples will be collected up to twice a day. Antepartum milk samples (10 drops to up to ½ teaspoon) will be collected from individuals in the nipple stimulation group during the intrapartum phase. Samples will be collected every 12 hours until labor. Volume will be determined by the mothers based on the amount of milk produced and how much they want to use to feed the infant postpartum.

Postpartum milk samples will be collected from IV oxytocin and nipple stimulation treatments participants at approximately 12, 24, and 48 hours postpartum. At 12, 24 and 72 hours 6-10 drops of milk (or more if participant allows) will be collected to a sterile tube using a pump or manually expressed from the breast. At ~48 hours postpartum, we will aim to collect 0.5-2.5ml of milk to perform all the sodium and milk conductivity assays mentioned below. If mothers are still hospitalized at 72 hours, additional samples will be collected every 12 hours up to twice per day (6-10 drops).

0.5ml of milk is needed for analysis of Sodium and milk conductivity, 0.5ml of milk for RNA analysis and 1ml of milk for macronutrients analysis.

Biological samples will be collected and stored at Yale and Weill Cornell Medicine (WCM) under secure, temperature-controlled conditions before shipment to Dr. Golan’s lab in Ithaca. At collection time, all samples will be de-identified and labeled with unique study ID numbers and time since delivery, which contain no personally identifiable information. Yale and WCM sites will use REDCap to code and manage the associated clinical data and sample collection records. A secure, access-restricted linkage key connecting study IDs to patient identifiers will be maintained solely by the local research teams. Dr. Golan’s lab will only receive de-identified samples and coded data labeled with the study ID, and will have no access to patient identifiers. Results generated by Dr. Golan’s lab will be uploaded to the WCM REDCap database using the matched study IDs provided with the samples, ensuring

all data remain de-identified and participant confidentiality is maintained throughout the study.

6.3.1 Study Schedule

There will be five expected study visits or encounters:

Study Encounter #1	● Screening and consent ● Enrollment and randomization ● Assess subject's baseline pain level using visual analog scale ● Administer Edinburgh Postnatal Depression Scale to establish baseline ● Administer Infant Feeding Intention Survey to establish baseline ● Administer EQ-5D questionnaire to establish baseline ● Initiation of assigned intervention ● Assess subject's pain level two hours after intervention start using visual analog scale ● Plasma and urine samples will be collected at Yale site ● Milk samples will be collected	During hospitalization for induction of labor
Study Encounter #2	● Assess subject's pain experienced during childbirth with visual analog scale ● Administer Labour Agentry questionnaire ● Administer Birth Satisfaction Scale-Revised questionnaire ● Administer EQ-5D♥™ questionnaire ● Administer EQ-TIPS♥™ questionnaire ● Milk samples will be collected up to twice a day (if applicable) postpartum	During hospital postpartum stay prior to discharge
Study Encounter #3	● Administer PIMS questionnaire ● Administer EQ-5D♥™ questionnaire ● Administer EQ-TIPS♥™ questionnaire	Contacted by email or text message 2 weeks postpartum

Study Encounter #4	• Administer Maternal Breastfeeding Evaluation Scale (MBES) questionnaire to all subjects who report at least one attempt at breastfeeding during the postpartum period. • Administer Edinburgh Postnatal Depression Scale questionnaire • Administer EQ-5D♥™ questionnaire • Administer EQ-TIPS♥™ questionnaire	Contacted by email or text message 4-12 weeks postpartum
Study Encounter #5	• Administer short questionnaire to assess infant feeding method • Administer EQ-5D♥™ questionnaire • Administer EQ-TIPS♥™ questionnaire	Contacted by email or text message 6 months postpartum

6.3.2 Informed Consent

Informed consent will be obtained by trained study personnel who are qualified to obtain informed consent. Informed consent will be obtained in a private room, free of distraction, on the Labor and Birth Units at study sites. A full explanation of study procedures will be provided to the subject and all subjects will be given the opportunity to ask questions. All questions will be answered to the best of the ability of study personnel, and if queries are made that cannot be addressed at that time, the site investigators will be asked to respond. The subject will be notified of the IRB contact should she have any questions. The subject's level of comprehension will be assessed by study personnel, and if not sufficient to assure that consent is truly informed, additional information will be provided. Patients will be informed that study participation is voluntary to minimize undue influence or coercion and they will be offered sufficient time to review the study prior to obtaining their informed consent. Women will be given ample time to review the consent, ask questions, and discuss with their partners (if applicable) before deciding to enroll in the study. Women ages 18+ will be enrolled, so parental permission is not required. Women will only be included if they are English or Spanish speaking. We do have study consent personnel who are fluent in Spanish. If there is no available research member who is fluent in Spanish, we will use a hospital phone interpreter to review the consent form and study information with the patient. If another person is with the woman during this process, she will be asked if she would like to discuss the study on her own or together with the accompanying person or people.

Informed consent will be documented with the subject's signature and date. Subjects will be provided with a copy of the informed consent form. The form will be placed in the subject's coded chart, which will be maintained in a location separate from any clinical care

documents and will be coded with an alphanumeric code. This code will be the subject's unique identifier that will carry through the entire study.

6.3.3 Screening

All patients who are scheduled for an induction of labor or are admitted to the hospital's labor and birth unit to undergo induction of labor will be screened for study eligibility by a trained member of the investigative team. With the assent of their primary obstetric physician or midwife, patients meeting eligibility criteria will be approached for potential recruitment, The study will be explained in detail and all questions will be answered prior to signing written informed consent to participate in the study. Screening will occur before planned exogenous oxytocin intravenous infusion has been initiated.

6.3.4 Recruitment and Enrollment Procedures

A certified member of the investigative team will review the list of patients admitted to the labor and delivery unit for labor induction and assess their eligibility using the inclusion and exclusion criteria. A partial waiver of consent will be obtained to allow review of their medical chart to ensure their eligibility prior to approaching patients. A certified member of the team will enroll a consented subject into the study. The subject will be randomized to a study arm with RedCap database, and the assigned intervention will be initiated. All study data will be entered into the RedCap database.

6.3.5 On Study Visits

Study Encounter #1	● Screening and consent ● Enrollment and randomization ● Assess subject's baseline pain level using visual analog scale ● Administer Edinburgh Postnatal Depression Scale to establish baseline ● Administer IFI Survey to establish baseline ● Administer EQ-5D questionnaire to establish baseline ● Initiation of assigned intervention ● Assess subject's pain level two hours after intervention start using visual analog scale ● Milk samples will be collected	During hospitalization for induction of labor	40 minutes

Study Encounter #2	● Assess subject's pain experienced during childbirth with visual analog scale ● Administer Labour Agentry questionnaire ● Administer Birth Satisfaction Scale-Revised questionnaire ● Administer EQ-5D♥™ questionnaire ● Administer EQ-TIPS♥™ questionnaire ● Milk samples will be collected up to twice a day (if applicable) postpartum	During hospital postpartum stay prior to discharge	40 minutes
Study Encounter #3	● Administer PIMS questionnaire ● Administer EQ-5D♥™ questionnaire ● Administer EQ-TIPS♥™ questionnaire	Contacted by email or text message 2 weeks postpartum	15 minutes
Study Encounter #4	● Administer Maternal Breastfeeding Evaluation Scale (MBES) questionnaire to all subjects who report at least one attempt at breastfeeding during the postpartum period ● Administer Edinburgh Postnatal Depression Scale questionnaire ● Administer EQ-5D♥™ questionnaire ● Administer EQ-TIPS♥™ questionnaire	Contacted by email or text message 4-12 weeks postpartum	30 minutes
Study Encounter #5	● Administer short questionnaire to assess infant feeding method ● Administer EQ-5D♥™ questionnaire ● Administer EQ-TIPS♥™ questionnaire	Contacted by email or text message 6 months postpartum	10 minutes

6.3.6 End of Study and Follow-up

The study will be concluded when the planned sample size of women has been enrolled, randomized, have delivered, and the last subject to deliver is past the 6-month postpartum timepoint, and all planned data have been collected.

If a subject withdraws from the interventional portion of the study but agrees to continued follow-up of associated clinical outcome information (as described in the original informed consent form), these data will be collected from the medical record. If a subject withdraws

from the interventional portion of the study and does not consent to continued follow-up of associated clinical outcome information, we will not access for purposes related to the study, the subject's medical record or other confidential records requiring the subject's consent.

6.3.7 Removal of subjects

Subjects will be informed that they may withdraw from the study at any time for any reason, without prejudice to their medical care. The investigators also have the right to withdraw subjects from the study for the following reasons: non-adherence to the protocol requirements (both in the intrapartum nipple stimulation and exogenous oxytocin infusion arms) by the subject or the subject's primary maternity care provider, or the subject no longer meets the protocol eligibility criteria. If a subject withdraws from the study, the primary reason for a subject's withdrawal will be recorded in the data collection. Subject who withdraw or are withdrawn from the study will not be replaced. If immediate deviation from the protocol is required to eliminate an immediate hazard(s) to subjects, the investigators will contact the IRB, if circumstances permit, to discuss the planned course of action. Any departures from the protocol must be fully documented. Unplanned protocol deviations will be reported to the IRB per institutional requirements. If the opinion of the study Principal Investigator (M.S.) is that there is sufficient reasonable cause for premature study termination, written notification documenting the reason(s) for study termination will be provided to the IRB. If a subject withdraws from the study, the data collected on the subject to the point of withdrawal will remain part of the study database and will not be removed. Removal of already collected data would undermine the scientific, and therefore the ethical, integrity of the research. Incomplete data could potentially put enrolled study subjects and future study subjects at unreasonable risk as a result of inaccurate data/conclusions.

If a spontaneous labor control patient develops labor complications including prolonged or arrested labor requiring IV oxytocin or cesarean delivery, vaginal bleeding, or fetal heart rate decelerations requiring resuscitative efforts by the primary obstetric provider, biospecimens will not be collected at the time of entering active labor. The initial sample will still be used and medical records will still be reviewed unless the patient opts to withdraw from participation.

If a subject randomized to nipple stimulation withdraws from her intervention, her maternity care provider will be informed so the subject can be transitioned to routine obstetric care (e.g. initiation of exogenous oxytocin infusion).

If a subject withdraws, biological specimens that have not yet been analyzed will be destroyed and not analyzed further.

6.4 Statistical Method

6.4.1 Statistical Design

Data analyses will adhere closely to the CONSORT guidelines. (27) Analyses will follow the intention-to-treat principle in which subjects will be analyzed in the group to which they were randomized, regardless of whether or not they received the assigned intervention.

6.4.2 Sample Size Considerations

1) Total sample size for the trial: The sample size for the trial is based on the primary outcome of spontaneous vaginal delivery. Therefore, the sample size of n=988 is selected for this proposed study to detect a 10% absolute increase while accounting for an expected 5% attrition rate. We then estimate, on the basis of the sample size for the primary outcome, the power we will have to detect clinically significant differences in the secondary outcomes.

Sample size estimation for primary outcome:

Detectable absolute difference	Anticipated with nipple stimulation therapy	Total Sample size Across 2 Study Groups for 90% power
15%	79%	426
14%	78%	492
13%	77%	574
12%	76%	676
11%	75%	812
10%	**74%**	**988**
9%	73%	1,222

All sample size and power estimates are based on two-tailed tests. *This is important because we will be powered to detect both increases and decreases in outcomes with intrapartum nipple stimulation therapy (intervention) versus immediate synthetic oxytocin infusion without nipple stimulation (comparator).* The expected rate of spontaneous vaginal delivery in the setting of immediate synthetic oxytocin infusion without nipple stimulation used for the sample size estimation is based on averaged institutional data from the study sites. We estimate that a total of **988** (494 nipple stimulation therapy and 494 immediate synthetic oxytocin infusion without nipple stimulation) will be sufficient to detect a **10% absolute difference** (estimated 74% versus 64%) in spontaneous vaginal delivery with 90% power (alpha of 0.05).

Justification: At first glance, the anticipated 10% or greater absolute increase in the spontaneous vaginal delivery rate in our proposed trial appears modest. On the contrary, because the potential public health impact is large, this effect size is significant. It is estimated that 40% of the *one million* women who undergo inpatient labor induction each year in the U.S. are nulliparas. Therefore, a 10% increase in spontaneous vaginal delivery translates to 40,000 more spontaneous vaginal deliveries and *40,000 fewer operative deliveries each year*. Avoiding 40,000 operative deliveries each year will have substantial impact on overall morbidity and healthcare resource utilization in the U.S. and beyond.

2) The sample size of 988 for the primary outcome will be sufficient to detect a 9.4% or 10.8% absolute difference in breastfeeding as the sole source of nutrition (BSSN) at the time of delivery hospitalization postpartum discharge with 80% power and 90% power, respectively (two-sided test, alpha=0.05). This represents the difference between an expected BSSN rate of 47% in the setting of synthetic oxytocin infusion without nipple stimulation (average rate at the study sites based on institutional data) and 56.4% (with 80% power) or 57.8% (with 90% power) with nipple stimulation therapy.

3) Assuming a 15% attrition rate at 2 weeks postpartum (the time frame for our primary cost-effectiveness analysis in Aim 3), the sample size of 988 will have 90% power for detecting a standardized effect size of 0.22 for the between-group difference in cost and difference in quality adjusted life years (QALY) (two-sided test, alpha=0.05). This is considered a small effect size based on conventional criteria, hence if we observe an even larger effect size, we should be sufficiently powered.

The sample size for this biospecimen trial is based on the primary comparison, specifically the difference in primary biomarkers from baseline to after 2 hours of treatment between patients performing nipple stimulation therapy and those receiving IV oxytocin. Sample size calculations are based on differences between non-laboring and laboring patients from prior reports (Table). Sample size was determined to achieve 80% power to detect a difference with a two-tailed alpha level of 0.05. The final sample size of 48 per exposure group is based on the largest sample size required (IL1beta). Therefore, there is more than adequate power for the remainder of the biomarkers of interest.

To detect a 10 mM difference in sodium concentration between the intrapartum nipple stimulation therapy group and the immediate synthetic oxytocin infusion group at 48 hours postpartum, with a standard deviation of 20 mM, 80% power, and a significance level of 0.05, a sample size of 32 participants per group is required. Accounting for an expected 30% dropout rate, we plan to recruit 46 participants per group.

To preserve the overall 5% false positive error rate, we first defined primary, secondary, and exploratory hypotheses with respect to the comparison groups. Second, we defined our hypotheses with respect to the each biomarker of interest (Table) as either disjunctive, conjunctive or individual.[64] When hypotheses are disjunctive, a rejection of one of them would yield a rejection of the overarching (joint) null hypothesis, which requires an adjustment of the alpha level for multiplicity of tests. Conceptually, since hypotheses are divided into primary, secondary, and exploratory, and individual biomarker targets identified, we created individual overarching null hypotheses where alpha-level adjustments are not required. Additionally, if we set them as conjunctive hypotheses where all the individual null hypotheses must be rejected to reject the overarching (joint) null hypothesis, the alpha level adjustment would also not be needed.

Sample Size Estimation for Biospecimen Sub-Study:

Biomarker	Reported Group Difference	Standard deviation	Sample size (per group) [a]	Estimated Effect Size [b]	Detectable Difference for n=48 [c]
Primary: Oxytocin	24.25%[d]	40.8	45	528 pg/mL	23.6%
Cytokines					
Primary: IL1b	1.56 pg/mL	2.19	48	1.56 pg/mL	1.56 pg/mL
IL6	3.34 pg/mL	2.69	23	2.5 pg/mL	1.77 pg/mL
Acute phase reactants					
Primary: PAI-1	2.27 [e]	2.655	23	23.2 ng/mL	1.76 [e]
Plasminogen	1.32 [e]	1.32	16	-3.5 mg/L	1.17 [e]
C reactive protein	1.91 [e]	1.19	21	-2.3 mg/L	1.11 [e]
Immune mediator					
Primary: ICOS Ligand	3.07 [e]	2.73	13	n/a	1.79 [e]
SFTPD	3.76 [e]	3.39	14	n/a	2.02 [e]
Metabolic					
Primary: Apolipoprotein A4	-0.79 [e]	1.19	9	n/a	-1.11 [e]
IGFBP1	-0.57 [e]	1.97	23	n/a	-1.48 [e]
Apolipoprotein E	-27.5 mg/L	23	16	-27.5 mg/L	-13.3 mg/L
Apoptosis					
ERP29	1.54 [e]	1.51	15	n/a	1.27 [e]
Prostaglandins					
PGE2	10ng/mL	4.2	3	10ng/mL	2.4 ng/mL
PGF2a	3.7 ng/mL	1	8	3.7ng/mL	0.58 ng/mL

[a] Sample size (per group) that would provide 80% power to detect the reported group difference with the reported standard deviation using two-tailed tests.

[b] For percent change in oxytocin, effect size was estimated based on average values in two treatment groups at the time of adequate contractions in our preliminary data. For MS data, effect size was estimated based on group differences expressed in log scale ratios converted to typical laboratory units. n/a indicates concentration in maternal circulation is unknown for estimation. Effect size refers to effect size if the study was only powered for that biomarker.

[c] Detectable difference is calculated as what difference we anticipate being able to identify given the final sample size of 48. For MS counts,

[d] Based on our preliminary data

[e] MS counts

6.4.3 Planned Analyses

6.4.3.1 Primary Analyses

1) Overview: Data analyses will adhere closely to the CONSORT guidelines. Analyses will follow the intention-to-treat principle in which subjects will be analyzed in the group to which they were randomized, regardless of whether or not they received the assigned intervention.

2) Primary Analyses:

Descriptive statistics will characterize the group of individuals recruited and investigate comparability of the two study groups at baseline. Formal statistical testing will be limited to select baseline characteristics considered to be prognostic factors for the primary outcome including hospital admission body mass index (BMI), primary indication for labor induction, Bishop score at randomization, and birth weight. Categorical variables will be compared between trial groups by using the Chi-squared or Fisher's exact tests as appropriate, and continuous variables will be compared using Student's t-test or Wilcoxon Rank Sum Test, as appropriate. Distributions of continuous variables will be assessed by visual inspection of histograms.

The primary outcome (spontaneous vaginal delivery) and other categorical secondary outcomes will be compared between trial groups using Chi-square or Fisher's exact tests as appropriate. The estimates of the relative risk and 95% confidence intervals (95% CI) associated with the primary and secondary outcomes will be calculated using Agresti and Coull method. The time to event regression analyses for labor length (regardless of delivery mode) and labor length censored for cesarean will be evaluated by Kaplan-Meier estimates and plots, and tested with the log-rank test, whereas Cox proportional hazards analysis will be used in adjusted analyses accounting for Bishop scores at study entry (proportionality assumption will be checked graphically with ln(-lnSurvival) plots), and results summarized using hazard ratios (HR) and 95% CI). A sensitivity analysis will be performed using the induction method that the patient actually received (per-protocol analysis) to determine whether crossovers influenced the results. The distribution of maximal percent newborn weight loss is not expected to follow a normal distribution in the population, so we plan to use Wilcoxon Rank Sum Tests to compare the distribution of the maximal percent weight loss between treatment groups, and summarize the results as medians (25^{th} and 75^{th} percentiles) and bootstrapped 95% CIs. Similarly, the distributions of the PIMS survey scores and MBFES survey scores are not expected to follow normal distributions in the population, so we will use Wilcoxon Rank Sum Tests to compare the distribution of the MBFES scores between treatment groups and summarize the results using the group medians (25^{th} and 75^{th} percentiles) and bootstrapped 95% CIs.

6.4.3.2 Secondary Objectives Analyses

Adjusted Analyses and Planned Subgroup Analyses

We will perform other analyses as needed aimed at obtaining adjusted assessments of treatment effectiveness, adjusting for baseline patient characteristics (covariates). The objectives of these analyses are to estimate the influence of covariates on the outcome and to use covariates to improve the estimated difference between treatment groups. The Poisson regression model (link=log) with robust standard errors stratified by study site will be used to identify and estimate the effect of multiple prognostic factors on the probability of spontaneous vaginal delivery and other categorical outcomes, with results summarized as adjusted risk ratios. For continuous secondary outcomes such as maximal newborn weight loss and PIMS and MBFES survey scores, quantile regression, e.g., modeling 50^{th} percentile, will be considered to adjust for prognostic factors. Interaction tests will be used to determine whether the effectiveness of nipple stimulation therapy significantly differs across these subgroups. These analyses will be considered exploratory in nature and will not be viewed as providing confirmatory tests of hypotheses.

The following pre-specified subgroup analyses will be conducted:

1. Study site
2. Amniotic membrane status (intact versus ruptured) at enrollment
3. Presence versus absence of maternal diabetes (inclusive of pre-gestational and gestational diabetes)
4. Insurance type (commercial versus public insurance)
5. Maternal race (Black versus White versus Other)
6. Obesity (obese versus non-obese)

In separate models, each of these sub-group variables will be included as an additional covariate in the models for outcomes of interest, plus their interaction with treatment variable; followed by stratified analyses (by each of the above variables) of the effect of treatment on each outcome of interest.

Data Analysis Plan for the Biospecimen Sub-Study:

The primary comparison for Aim 1 is the difference in biomarker concentration or MS counts from baseline to 2 hours of treatment between patients performing nipple stimulation therapy and those receiving IV oxytocin. Secondary comparisons evaluate the change with labor induction versus spontaneous labor. This unadjusted between-group comparison of the within-group change will be based on a Student's t-test, with a supportive analysis using Analysis of Covariance (ANCOVA) where the baseline biomarker level will be a covariate in a mean response profile model (MRP), which allows for different variances and covariances across time. The primary exposure variable will be treatment group with either compound symmetry (CS) covariance or heterogenous compound symmetry covariance (HCS) to account for the within-patient correlation in biomarker values across the time points of data collection; and the choice between the two covariance matrices will be made using a likelihood ratio test (LRT)

as these are nested models. Results will be summarized as estimated means of fold change at adequate contractions for each group and the between-group mean differences, with surrounding 95% Confidence Intervals. By specifying the model as an MRP, we can also conduct any comparisons of means (within/between groups across time), by using linear contrasts and the multivariate Wald Test. In the adjusted analyses, we will include in the MRP as covariates the between-group differences identified in the between-group comparisons. In a sensitivity analysis, since we will know the timing of each measurement relative to the initiation of treatment, we will use the actual time (compare linear, polynomial or spline effect of time) instead of the categorical time point in a linear mixed effects model (LME) with a random intercept for each woman and a possible random slope for each woman (also tested using an LRT). All biomarkers listed in the Table will be analyzed in the same manner, as all will be treated as continuous variables. For the exploratory hypothesis using the time to active labor, we will use a joint modeling approach: time to active labor modeled jointly with the mean trajectory of change over time in biomarker values; this model will allow us to estimate the between-group differences in the time to active labor, as it is also influenced by the biomarker values within each group.

Data Analysis Plan for the Milk Biospecimen Sub-Study:

The primary comparison will be looking at milk composition before and after birth for participants in the nipple stimulation group. We will use Student's t-test to compare the paired milk samples from the same individuals.
Secondary comparison will be looking into milk composition between patients performing nipple stimulation therapy and those receiving IV oxytocin versus spontaneous labor at 12, 24, 48 and 72 hours postpartum. We will account for multiple measurements for each participant (if multiple samples will be collected) and those will be used for a mixed effect model. Structural equation model will be used for modeling the effect of additional variables (delivery mode, intentions to breastfeed, feeding frequency, maternal intention to feed etc.) on milk biomarkers and breastfeeding outcomes.

6.4.3.3 Safety/Pregnancy-related policy

The study PD/PI (M.S.) will be informed of and review any potential Adverse Events. The DSMB will review all Adverse Event Reports and other interim safety data and will provide a report to the local IRB in compliance with local standards. If a participant develops a serious adverse event, the safety of continuing the intervention will be ascertained by the participant's obstetric care provider in collaboration with the site PI.

Maternal and perinatal morbidity outcomes are pre-specified secondary outcomes and will be statistically compared between intervention groups to assess for any signal of safety concerns.

6.4.3.4 Analysis of Subject Characteristics

Descriptive statistics including frequencies, percentages, means (+/- standard deviations) and medians (interquartile ranges) will be obtained. Baseline univariate and bivariate analyses will be performed using Fisher's exact tests and Chi-squared tests for categorical

variables and Student t-tests and Mann-Whitney U tests as appropriate based on the normality of the data.

6.4.3.5 Interim Analysis (if applicable)

Not applicable

6.4.3.6 Health economic evaluation

To estimate difference in cost and difference in quality adjusted life years (QALY) between the intervention and comparator groups, we will use a generalized linear model (GLM) with log link and gamma distribution for cost (given skewness in cost data) and identity link and normal distribution for QALY. Explanatory variable will be an indicator for intervention (vs. comparator) group. All analyses will follow the intention to treat principle. As randomization will be stratified by study site and amniotic membrane status, we will test if they are associated with cost or QALY and decide whether to include them as covariates in analysis. Cost-effectiveness will be evaluated by calculating the incremental cost effectiveness ratio (ICER), defined as $\Delta C/\Delta E$ where ΔC denotes the estimated difference in cost between the intervention and comparator groups and ΔE reflects the estimated difference in QALY between the two groups. ICER informs the additional cost associated with the intervention for each additional QALY gained. We will use non-parametric bootstrap resampling to estimate 95% CI of ICER and produce a cost-effectiveness plane and cost-effectiveness acceptability curve. In special situations where the intervention leads to significantly lower cost and higher QALY, the intervention is considered the dominant strategy. In situations where there is no significant difference in QALY between the two groups, a comparison of cost will inform the cost-effectiveness of the intervention; and vice versa. Our primary cost-effectiveness analysis will examine costs and health effects up to 2 weeks postpartum. We will also conduct two secondary analyses by: 1) extending the time period to 6 months postpartum, and 2) calculating QALY using participants' EuroQol five dimensions (EQ-5D) visual analogue scale (VAS) rating as a utility score (with linear transformation to a 0 to 1 scale).

6.4.3.7 Other

None

6.4.4 Subsets and Covariates

Adjusted Analyses and Planned Subgroup Analyses

We will perform other analyses as needed aimed at obtaining adjusted assessments of treatment effectiveness, adjusting for baseline patient characteristics (covariates). The objectives of these analyses are to estimate the influence of covariates on the outcome and to use covariates to improve the estimated difference between treatment groups. The Poisson regression model (link=log) with robust standard errors stratified by study site will be used to identify and estimate the effect of multiple prognostic factors on the probability of spontaneous vaginal delivery and other categorical outcomes, with results summarized as adjusted risk ratios. For continuous secondary outcomes such as maximal newborn weight

loss and PIMS and MBFES survey scores, quantile regression, e.g., modeling 50th percentile, will be considered to adjust for prognostic factors. Interaction tests will be used to determine whether the effectiveness of nipple stimulation therapy significantly differs across these subgroups. These analyses will be considered exploratory in nature and will not be viewed as providing confirmatory tests of hypotheses.

The following pre-specified subgroup analyses will be conducted:

1. Study site
2. Amniotic membrane status (intact versus ruptured) at enrollment
3. Presence versus absence of maternal diabetes (inclusive of pre-gestational and gestational diabetes)
4. Insurance type (commercial versus public insurance)
5. Maternal race (Black versus White versus Other)
6. Obesity (obese versus non-obese)

6.4.5 Handling of Missing Data

Data quality reports will be generated monthly and include volume of missing data, edits, time to edit resolution and number of overdue forms. Remedial measures, including re-abstraction of data and retraining of staff, will be used as needed to minimize missing data.

At the time of statistical analysis, if there is significant missing data, multiple imputation methods will be employed as necessary.

7 - Trial Administration

7.1 Ethical Considerations: Informed Consent/Assent and HIPAA Authorization

Women will be assumed to have capacity to provide informed consent for research unless there is indication to the contrary. These indications include known cognitive impairment, altered mental status, difficulties in communication observed during research staff interaction, psychotic symptoms, bizarre behavior. Women with these indications will not be included in the research study. If any of these symptoms or behaviors are new, their medical provider will be informed immediately for further assessment.

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki.

7.2 Institutional Review Board (IRB) Review

The protocol will be submitted to the IRB for review and approval. Approval of the protocol must be obtained before initiating any research activity. Any change to the protocol or study team will require an approved IRB amendment before implementation. The IRB will determine whether informed consent and HIPAA authorization are required.

The IRB will conduct continuing review at intervals appropriate to the degree of risk, but not less than once per year.

A study closure report will be submitted to the IRB after all research activities have been completed.

Other study events (e.g., data breaches, protocol deviations) will be submitted per Yale's IRB's policies.

7.3 Subject Confidentiality

There will be multiple procedures in place to minimize the risk of subject confidentiality and will be outlined in the study protocol. Subject confidentiality will be held in strict trust by all members of the research teams. Subject medical record review will be limited to just the elements needed to complete the study. Only authorized HIPAA and GCP trained study team members will be allowed to extract research data from medical records and enter it into the *REDCap* database.

At each study site, subject records are maintained in the Epic System® EMR and all research data will be collected in and maintained in *REDCap*, an established, secure, web-based capture and management tool developed at Vanderbilt University. Any paper records (e.g. signed consent forms) will be kept in a locked cabinet within a locked office that is secured always and located on the main campus within the departmental research administrative office space at each study site to ensure confidentiality of subject data.

The IRB may inspect study records at any given time. In addition, all scientific publications will refer to the subjects by study identifiers only. At no time will any of the subjects in the study be identified. Identifiable study information will be maintained for up to ten years after the research is complete. After that time, it will be destroyed or de-identified. The principal investigator will keep a link that identifies subjects to coded information, but this link will be kept secure and available only to the PIs or selected members of the research team.

This study will comply with the National Institutes of Health (NIH) Data Sharing Policy. Data obtained from biospecimens will be sent to an NIH-supported Scientific Data Repository. All samples and related data will be coded and de-identified, and the NIH will not identify or make any attempt to identify information as coming from a specific participant. NIH will share the collected information with researchers who submit applications to NIH to do research with information from the NIH-supported Scientific Data Repository. Special data sharing committees within the NIH will review those applications and decide whether or not to share the information with the researcher.

7.4 Deviations/Unanticipated Problems

If the study team becomes aware of an anticipated problem (e.g., data breach, protocol deviation), the event will be reported to the IRB office within five (5) working days of becoming aware of the incident/issue via a Reportable New Information (RNI) mechanism in IRES IRB.

7.5 Data Collection

Inclusion criteria and randomization data will be entered in real time, screening information entered weekly, data extraction every other week, and chart completion monthly.

The medical record of each subject's hospital stay will be reviewed and used to collect data. The Data Collection Form (Appendix) will be configured into the REDCap (Research Electronic Data Capture) database and will be utilized by the study investigators to extract and enter data from each subject's medical record. All study questionnaires will be completed by subjects using REDCap survey tools and data will be stored and analyzed using this tool. As stewards of the research data, the Study Principal Investigator and site PI’s will be responsible and accountable for research data as it is collected, processed, stored, analyzed, and reported. Every effort will be made to maintain high quality data that will remain protected and kept strictly confidential in accordance with local, state, and federal law; as such, institutional resources REDCap will be used for data entry and monitoring. Once all data collection forms and medical charts have been reviewed, all identifiers will be removed, and subject data will be stored and archived in a de-identified manner. The identifiable data will be permanently discarded by permanently deleting the password protected encrypted database with subject identifiers at the earliest opportunity.

7.6 Data Quality Assurance

The data form will be configured into the REDCap (Research Electronic Data Capture) database and utilized by the study investigators to extract data from each subject's medical

record. All subjects will complete study questionnaires using the Yale Qualtrics Survey tool, and data will be stored and analyzed using this tool. Every effort will be made to maintain high quality data that will remain protected and kept strictly confidential in accordance with local, state, and federal law; as such, institutional resources REDCap and Qualtrics will be used for data entry and monitoring.

7.7 Study Records

The study protocol, consent forms, subject medical records, and surveys will all be considered study records.

7.8 Access to Source

The source documents will include the consent forms, subject medical records, and completed surveys. Only certified members of the investigative team will have access to these documents. All data will be entered into the REDCap database, and de-identified at the earliest opportunity to do so.

7.9 Data or Specimen Storage/Security

Every effort will be made to maintain high quality data that will remain protected and kept strictly confidential in accordance with local, state, and federal law; as such, institutional resources REDCap (Research Electronic Data Capture) will be used for data entry and monitoring. This database is on a server on a secure, encrypted hospital-issued computer. Once all data collection forms and medical charts have been reviewed, all identifiers will be removed, and subject data will be stored and archived in a de-identified manner with the use of unique study identification numbers. The identifiable data will be permanently discarded by permanently deleting the password protected encrypted database with subject identifiers at the earliest opportunity. The paper records (e.g., signed written informed consent forms) will be stored in a locked cabinet in the Principal Investigator (M.S.)'s private academic office that is locked and only accessible by key and university badge access.

Data use agreements will be obtained and approved across all three institutions: Weill Cornell as the primary grant institution where the PD resides, Yale University (grant sub-award institution), and Northwestern University (grant sub-award institution).

7.10 Retention of Records

The study records will be retained for a minimum of seven (7) years after the conclusion of the study investigation. If permission is needed to move or destroy the records, the Principal Investigator (M.S.) will be contacted.

7.11 Study Monitoring

Plan for Monitoring trial performance to ensure protocol adherence and data fidelity

The Data Management Team will create electronic data capture forms. Conference calls will be held at least monthly with all site investigators as well as a separate call monthly for study coordinators, or more frequently as needed. Data will be available in REDCap regarding recruitment, retention and protocol adherence by site with specific details regarding

subgroups deemed clinically important such as race/ethnicity, body mass index, and maternal age. This will be reviewed on a monthly basis by the Principal Investigator and sub-site PI(s), to ensure that appropriate milestones are met. REDCap® is the secure web platform for building and managing online databases and surveys that will be employed at study sites. The software allows for data quality checks, secure file storage and sharing, custom reporting, and data-based triggers and alerts. In addition to the above-mentioned mechanisms in place to ensure fidelity and data integrity, all research staff will be trained in good clinical practice and will follow GCP regulations and guidelines. The PD/PI and site PI's will meet on a monthly basis, or more frequently as needed, with their respective site-specific staff to discuss recruitment, enrolled participants, regulatory updates, safety issues, data entry and any other pertinent topics. Detailed meeting minutes will be kept, ensuring proper documentation of such oversight. Any adverse event, near miss or protocol deviation will be reported to the site PIs as soon as it is discovered, and the site PIs will then take the appropriate actions discussed above. These items will be discussed collectively during bi-monthly conference calls, with the exception of protocol deviations, adverse events and any other issues that would require immediate reporting.

7.12 Data Safety Monitoring Plan

The interventions compared in this trial are exogenous oxytocin infusion (current standard of care) and nipple stimulation with an electric breast pump or manual stimulation. Both interventions require continuous fetal heart rate and contraction frequency monitoring, with clear recommendations for titration of both interventions to ensure frequency of contractions within the recommended goal range. Therefore, no serious or life-threatening adverse events are expected. However, the risks associated with the current study are deemed greater than minimal for the following reasons:

1. We do not view the risks associated with nipple stimulation as minimal risks since the data surrounding its use remain limited.
2. However, given the limited reassuring data and our experience with breast pump stimulation from our pilot study (IRB# 2000029909, Clinicaltrials.gov NCT04756089) and the fact that exogenous oxytocin use is current standard of care and widely used in obstetric practice, we do not view the proposed study as high risk.

Although we have assessed the proposed study as one of greater than minimal risk, the potential exists for anticipated and/or unanticipated adverse events, serious or otherwise, to occur since it is not possible to predict with certainty the absolute risk in any given individual or in advance of first-hand experience with the proposed study methods. Therefore, we provide a plan for monitoring the data and safety of the proposed study as follows:

<u>The data safety monitoring plan is as follows</u>:

1. Personnel responsible for the safety review and its frequency:

The principal investigator will be responsible for monitoring the data, assuring protocol compliance, and conducting safety reviews at the study completion of each subject. During the review process, the principal investigator (monitor) will evaluate whether the study should continue unchanged, require modification/amendment, or close to enrollment. Either the principal investigator or the IRB have the authority to stop or suspend the study or require modifications.

2. Independent Data and Safety Monitoring Board (DSMB):

We will establish an independent study-specific Data Safety and Monitoring Board (DSMB) to oversee the trial. Members of the DSMB will be at least four physicians who represent the appropriate expertise (perinatology, epidemiology, biostatistics, neonatology, and clinical trials) and will provide appropriate oversight to assure that the trial accrues at a sufficient rate, and that the safety and privacy of all study participants is assured. Members will not be involved in any aspect of the trial operation. The DSMB will meet regularly (at least quarterly) to review study progress and to monitor adverse events. The DSMB will be briefed before each meeting regarding study progress (recruitments and drop-outs), and study outcomes. The DSMB will issue a written report to the investigators after each meeting outlining any study problems or needed actions

3. Adverse event reporting: Detailed information concerning adverse events will be collected and evaluated throughout the conduct of the protocol (Appendix). The PI and DSMB will determine whether the adverse event meets the criteria for a Serious Adverse Event (SAE). If a participant develops a serious adverse event, the safety of continuing the intervention will be ascertained by the subject's obstetric care provider in collaboration with the PI. The Principal Investigator will conduct a review of all adverse events upon completion of every study subject. The DSMB will review all Adverse Event Reports and other interim safety data and will provide a report to the local IRB in compliance with local standards. The PI and the DSMB will evaluate the frequency and severity of the adverse events and determine if modifications to the protocol or consent form are required.

4. Attribution of Adverse Events:

Adverse events will be monitored for each subject participating in the study and attributed to the study procedures/design by the Principal Investigator and the DSMB according to the following categories:

a.) Definite: Adverse event is clearly related to investigational procedures(s)/agent(s).

b.) Probable: Adverse event is likely related to investigational procedures(s)/agent(s).

c.) Possible: Adverse event may be related to investigational procedures(s)/agent(s).

d.) Unlikely: Adverse event is likely not to be related to the investigational procedures(s)/agent(s).

e.) Unrelated: Adverse event is clearly not related to investigational procedures(s)/agent(s).

5. Plan for reporting UPIRSOs (including Adverse Events) to the IRB

The principal investigator will report the following types of events to the IRB:

Any incident, experience or outcome that meets ALL 3 of the following criteria:

1. Is unexpected (in terms of nature, specificity, severity, or frequency) given (a) the research procedures described in the protocol-related documents, such as the IRB-approved protocol and informed consent document and (b) the characteristics of the subject population being studied; AND
2. Is related or possibly related to participation in the research (*possibly related* means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); AND
3. Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, legal, or social harm) than was previously known or recognized.

Unanticipated Problems Involving Risks to Subjects or Others (UPIRSOs) may be medical or non-medical in nature, and include — but are not limited to — *serious, unexpected, and related adverse events* and *unanticipated adverse device effects*. ***Please note*** that adverse events are reportable to the IRB as UPIRSOs **only** if they meet all 3 criteria listed above.

These UPIRSOs/SAEs will be reported to the IRB in accordance with IRB Policy 710, using the appropriate forms found on the website. All related events involving risk but not meeting the *prompt* reporting requirements described in IRB Policy 710 should be reported to the IRB in summary form at the time of continuing review. If appropriate, such summary may be a simple brief statement that events have occurred at the expected frequency and level of severity as previously documented***.*** In lieu of a summary of external events, a current DSMB report can be submitted for research studies that are subject to oversight by a DSMB (or other monitoring entity that is monitoring the study on behalf of an industry sponsor).

7.13 Study Modification

All modifications to the study will be submitted through IRES IRB. The research will continue to be conducted without inclusion of the modification(s) until IRB approval is received, except where necessary to eliminate apparent immediate hazards to human subjects.

7.14 Study Discontinuation

Reportable Events (which are events that are serious or life-threatening and unanticipated (or anticipated but occurring with a greater frequency than expected) and possibly, probably, or definitely related to study procedures or Unanticipated Problems Involving Risks to Subjects or Others (UPIRSO) that may require a temporary or permanent interruption of study activities. Such events will be reported to the IRB to determine whether the study needs to be discontinued based upon review.

7.15 Study Completion

The study is expected to complete within 7 years of initiation. The IRB will be notified when the study is completed.

7.16 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the trial. The study leadership in conjunction with the appropriate conflict of interest review committee has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

All investigators will follow the applicable conflict of interest policies.

7.17 Funding Source

This trial is funded in part by the Albert McKern Scholar Award and the National Institutes of Health. The materials for biospecimen collection and analysis are funded by a Trainee Grant from the Perinatal Research Consortium. The funders of the study will have no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; or decision to submit the manuscript for publication.

7.18 Publication Plan

Every attempt will be made to publish the results of this trial after they are completed, all data are collected and analyzed. The Principal Investigator (M.S) will hold primary responsibility for publishing the study results. The funders of the study will have no role in the decision to submit the manuscript for publication.

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