COVER PAGE

Study Title: Postpartum Smoking Relapse Prevention by Breastfeeding Promotion

NCT number: NCT04670822

Document Date: March 15, 2017

RESEARCH STRATEGY

1. Significance

Both opportunities and challenges exist for smoking cessation during pregnancy and postpartum. High motivation to quit during pregnancy is often followed by high chance of relapse during postpartum. About 23% of U.S. women smoke before pregnancy, and 54% of them report quitting smoking during pregnancy.[1] But most of women who quit smoking during pregnancy return to smoking ("relapse") after delivery, e.g., 45%, 70%, and 81% relapse by 6, 9, and 12 months postpartum, respectively.[2,3] Postpartum smoking relapse not only limits the benefits of smoking abstinence to the mother, but also has serious negative consequences for child health and development via tobacco metabolites in breast milk and ambient air.[4-6] Child risks include fast infancy weight gain[7] and childhood overweight[5] as shown in our studies, infant respiratory illness such as asthma,[8] and sudden infant death syndrome.[9] Preventing postpartum smoking relapse remains a challenge for research and clinical practice. According to a recent review of 32 relevant studies, few existing pharmacological, behavioral, and contingent incentive-based interventions have been proven to be effective on preventing smoking relapse in long-term postpartum period (e.g., 9 months or longer),[10] although several incentive-based studies were still effective up to 6 months postpartum (longer assessment unavailable).[11-14] Indeed, in a large trial published recently, the intervention focusing on ex-smoking mothers' concerns about mood, stress, and weight did not improve smoking abstinence at 12 months postpartum.[15] Novel and effective interventions are urgently needed to address the large public health issue of postpartum smoking relapse.

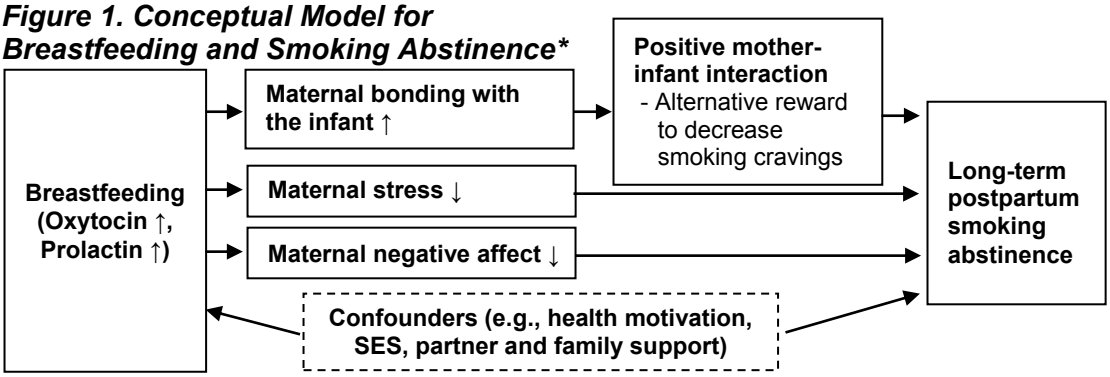
Numerous stressors in postpartum life and negative affect such as depression, anxiety, anger, and fear are reported as common triggers for postpartum smoking relapse. Interestingly, breastfeeding mothers tend to have lower perceived stress[16-18] and less negative affect including depression[19-21] and anger,[19] than formula-feeding mothers. While the directions of these associations are difficult to determine from observational studies, in a within-subject design study, maternal negative affect decreased after a breastfeeding episode in the same mothers, whereas maternal positive affect decreased after a formula-feeding episode.[17] In addition, early termination of breastfeeding is associated with increased depression and anxiety.[18,22] These breastfeeding-related emotional benefits may be attributed to unique neuroendocrine changes to maintain breastfeeding, especially higher oxytocin and prolactin levels.[23-25] These neuroendocrine and psychological mechanisms may explain our and others' observation that smoking relapse is much less common (30-60% lower risk) among breastfeeding mothers than formula-feeding mothers.[2,26,27] Therefore, it is reasonable to hypothesize that a breastfeeding promotion intervention can reduce the risk of postpartum smoking relapse through increased oxytocin and prolactin while decreased maternal stress and negative affect. Our application is the first randomized controlled trial to test this promising hypothesis.

In a pilot survey (Dr. Wen as PI), we found poor breastfeeding knowledge among 21 pregnant and 18 postpartum women who quit smoking during pregnancy. Only 28.6% agreed "Babies should be exclusively breastfed for the first 6 months (current clinical guidelines[28,29])", 52.4% agreed "The best way to feed a baby is breastfeeding", and 38.1% agreed "If a baby is breastfed he or she will be less likely to get diarrhea". Their intention to breastfeed was also relatively low: only 42.9% planned to breastfeed exclusively in the first weeks after delivery, and only 9.5% planned to exclusively breastfeed for 6 months. Their actual breastfeeding practice was even lower: only 22.2% and 0% breastfed exclusively in the first week and 6 months after delivery, respectively. Quitters tend to wean their infants sooner than nonsmokers, and their breastfeeding rate at 4 months postpartum is similar to that of continuous smokers'.[30] These consistent data suggest an urgent need to promote breastfeeding among women who quit smoking during pregnancy. One of our key team members, Dr. Yukiko Washio (Co-I), has developed, tested, and published a highly effective intervention funded by NIH to increase breastfeeding duration among low income mothers. For instance, the breastfeeding rate at 6 months postpartum was 72% in the intervention group, compared to 0% in the usual care group.[31] We plan to modify her intervention to promote breastfeeding among ex-smoking mothers in the proposed project.

Conceptual model (**Figure 1**). Through suckling stimuli and intimate mother-infant contact (e.g., infant hand massage and skin-to-skin contact, breastfeeding promotes the release of two key lactation hormones from the pituitary gland: prolactin (hormone for milk synthesis) and oxytocin (hormone for milk ejection).[23,32-35] Both oxytocin and prolactin can reduce maternal stress response through dampening hypothalamic-pituitary-adrenal (HPA) activity, which can lead to lower and/or fewer surges of adrenocorticotropic hormone (ACTH) and cortisol.[36,37] Oxytocin and possibly prolactin also decrease maternal negative affect[19-21,38] through reducing amygdala activation.[39,40] In addition, breastfeeding helps to enhance mother-infant bonding,[41-43] possibly through the activities of oxytocin in brain to decrease aggressive responses toward offspring.[38,44-46] Intervention targeting mother-infant bonding can decrease postpartum smoking relapse up to 8 weeks postpartum,[47] possibly because a strong mother-infant bond can foster positive mother-infant interaction.[48] The positive

mother-infant interactions may serve as an alternative reward pathway that may decrease frequency and intensity of cravings for cigarettes. It could potentially shift the focus of ex-smoking breastfeeding mothers' dopamine reward system from smoking to caring for the child.[48-50] As positive mother-infant interactions increase, the mother's investment (e.g., time, attention, money) in her child may increase, while her cravings for smoking are expected to diminish.[48,51,52] The mother's process of replacing cravings for smoking with increasing investment in the child and building a positive caregiving relationship may be gradual but may also be sustainable over time,[48] which is indirectly supported by previous research in which parenting intervention is associated with substance use reduction[53-55] and pup suckling is more rewarding than cocaine in the immediate postpartum period.[56] This natural alternative reward system (positive mother-infant interaction) along with low maternal stress and negative affect may help breastfeeding mothers to achieve long-term postpartum smoking abstinence.

Figure 1. Conceptual Model for Breastfeeding and Smoking Abstinence*



* Other hormonal mechanisms, such as progesterone, estrogen, ACTH, cortisol, and tetraiodothyronine (T4), will be included in the larger R01 grant.

2. Innovation Our proposed project is innovative in several ways. First, it is the first randomized controlled trial to assess the effect of breastfeeding promotion on postpartum smoking relapse. It can help to yield causal evidence by overcoming self-selection bias and confounding (e.g., health motivation, socio-economic status [SES], partner and family support) in previous observational research. Secondly, we will collect pilot data to examine neuroendocrine (oxytocin and prolactin), behavioral (mother-infant interaction), and psychological (mother-infant bonding, stress, and negative affect) mechanisms through which breastfeeding may reduce risk of postpartum smoking relapse. Thirdly, this is a pioneering study to combine contingent incentives with other intervention strategies such as education, counseling, support, and early limited formula milk via syringe to further improve breastfeeding practice among disadvantaged mothers.

3. Approach

3.1 Research Design. 3.1.1 Overview. This is a randomized controlled trial to prevent postpartum smoking relapse by breastfeeding promotion. The intervention group (N=30) will receive multicomponent breastfeeding intervention from late pregnancy to 6 months postpartum, while the usual care control group (N=30) will only receive minimum breastfeeding education. The key outcome is the timing of postpartum smoking relapse.
3.1.2 Recruitment. We will recruit 60 pregnant ex-smokers from Erie County and Niagara County, NY. Recruitment flyers will be distributed around the communities (e.g., churches, bus/subway stops, laundromats, grocery stores, thrift stores, community centers) and organizations providing basic services (e.g., social services, WIC, job training) to low income pregnant women.[57] Recruitment flyers will also be displayed in 4 large city OB/GYN clinics. Health professionals at these clinics will pass our recruitment materials to ex-smoking patients. We will also send an invitation letter to potentially eligible pregnant women in these clinics.[58-60] The PI has successful experience in recruiting 50 pregnant smokers for his NIH funded study on smoking cessation during pregnancy over 6 months from 2 obstetric clinics (see **Letters of Support** from clinics).[61,62] Social media such as Facebook will also be used. Assuming a rate of 23% maternal smoking at conception and 54% smoking cessation rate during pregnancy,[1] we estimate 1,490 out of about 12,000 newborns[63] will be born to mothers who quit smoking during pregnancy within 1 year in the two counties. We assume 60% of these mothers (N=894) will be exposed to recruitment materials, and then 30% (N=268) are interested in this project. Therefore, it is feasible to recruit 72 (60 + 20% attrition) eligible participants within 1 year.
3.1.3 Screening. Interested participants will sign consent forms and complete a survey on socio-demographics, pregnancy, medical history, mental health, history and status of cigarette smoking, use of other tobacco or nicotine products, alcohol and other substance use, and infant feeding plan. Those who had quit smoking cigarettes within 3 months before or during this pregnancy will be invited for lab screening to verify their current non-smoking status by urine cotinine (metabolite of nicotine). Collateral reports (e.g., from employer, partner, friend, or relative) or medical records will be used to verify their previous smoking history.[64] Eligible participants

must be at 28 weeks of pregnancy or earlier to ensure adequate time for prenatal breastfeeding intervention. Exclusion criteria are teen pregnancy (<18 y); current use of other tobacco or nicotine products; medical conditions contraindicating breastfeeding such as HIV infection, active tuberculosis, and breast removal;[28] current heavy drinking (more than 2 drinks a day); current use of other psychoactive substances;[65] being strongly against breastfeeding; and non-English speakers. To increase the possibility to obtain a representative sample of the generable population, the study will be advertised as a program to promote healthy infant feeding among pregnant women without requiring breastfeeding intention or mentioning smoking relapse prevention. We will not exclude women without current intention to breastfeed (28.6% in our pilot survey), unless they are strongly against breastfeeding (4.8%). Similarly, we will not exclude women who plan to relapse after delivery (0% in our pilot survey), as our breastfeeding promotion may motivate them to maintain abstinent for a longer period especially if they know tobacco metabolites are concentrated in breast milk.[4]

3.1.4 Pre-test visit. During this visit, we will measure the participant's urine cotinine, height, and body weight. Participants will complete a survey on smoking temptations, nicotine dependence before quitting, smoking cessation history, self-efficacy to maintain smoking abstinence, smoking environment including partner smoking behaviors, breast health, breastfeeding knowledge, breastfeeding history, relationship with partner and other family members, social network, stress, negative affect, physical activity, sleep, and diet.

3.1.5 Randomization. At 28 weeks of pregnancy, eligible participants will be randomized into either the intervention group (N=30) or the control group (N=30) using a sequence of random numbers in blocks of 2, which can ensure equal or close numbers of participants between the two groups throughout the project.

3.1.6 Intervention. *a) Intervention group.* The goal of our breastfeeding intervention is consistent with AAP[28] and ACOG[29] guidelines: exclusive breastfeeding for the first 6 months of life, with continued breastfeeding as complementary foods are introduced through the infant's first year of life, or longer as mutually desired by the mother and her infant. Participant will complete 13 one-hour intervention visits: 5 during pregnancy and 8 in postpartum. Prenatal breastfeeding education will be weekly for 5 weeks at the PI's lab. After delivery, we will meet the participant at her home in 1 week, 2 weeks, and 1 month; and then monthly at the PI's lab until 6 months postpartum. Participants will receive $15/visit compensation. Our team has rich experience in running behavioral interventions for the target population, for instance, most participants (73.9%) in the PI's smoking cessation study have completed 35 one-hour prenatal intervention visits and 12 monthly 1.5-hour postpartum follow-up visits with similar compensation ($15/hour).[61,62] The breastfeeding intervention components will include prenatal breastfeeding education, postnatal lactation counseling, family/peer/employer support, contingent financial incentives, and early limited formula milk via syringe (optional for at-risk infants).

Component #1 - Prenatal breastfeeding education: To maximize the effect, breastfeeding education will start in late pregnancy (28+ weeks) when the mother and family seriously plan on infant feeding.[66] The breastfeeding plan will cover breastfeeding on demand, pain management, skin-to-skin contact, breastfeeding tips after cesarean section, lactation support, breastfeeding away from home, breast pump, maternity leave, and back-to-work scheduling. Educational approaches will include readings with quizzes ($2 reward if passed), discussion, video, demonstration and practice with a baby simulator. Prenatal breastfeeding education will be delivered in a group with 6 pairs of participants and their family supporters per group. Physical examination of breasts and nipples will be conducted by a certified lactation counselor (CLC) to address potential issues including infectious conditions, dermatitis, breast masses, breast surgery, and flat or inverted nipples.

Component# 2 - Postnatal lactation counseling: A part-time CLC will be hired to provide timely and individualized postnatal lactation counseling. She will monitor each participant's breastfeeding progress, help them initiate breastfeeding immediately after delivery, correct myths and flaws, answer questions, and offer suggestions and tips to solve potential problems. Besides face-to-face meetings, text messages and phone calls will also be used to provide timely assistance of lactation practice and routine checking of feeding status.

Component #3 - Family/Peer/Employer support: The participant will nominate a family supporter for her breastfeeding. The family supporter will receive our training for skills in supporting the participant's decision and effort to breastfeed. Other family members will also receive a breastfeeding support booklet. The PI has successful experience of training 16 family supporters for our Pregnancy and Smoking Cessation Study.[61] In addition, we will encourage participants to attend Buffalo's Baby Cafés (6 sites), a breastfeeding drop-in support group, to seek further support from lactation counselors and other breastfeeding mothers (peers).[67,68] Particularly, the PI has been collaborating with one of these sites, the Durham's Central City Baby Café[69] (see **Letter of Support**). This Baby Café has been helping 214 of 221 mothers (96.8%) to breastfeed successfully since 2013. A booklet on benefits of breastfeeding for business, how to support a nursing mother in workplace, laws and policies of promoting breastfeeding will be available to the participant's employer.[70]

Component #4 - Contingent financial incentives: Participants will receive incentives for breastfeeding if they are able to nurse or to provide milk by pumping in the presence of a female research staff. In the Co-I Dr. Washio's effective intervention (e.g., 72% breastfeeding rate at 6 months postpartum vs. 0% usual care),[31] contingent incentives start at $20 at the end of first month and increase by $10 every month until the end of 6 months postpartum. We will adapt this incentive schedule for the proposed project by adding two home visits in week 1 and week 2 postpartum for breastfeeding consultation with $20 incentive for breastfeeding, given that the first month postpartum is critical to address high risk of breastfeeding discontinuation and smoking relapse.[71,72] The family supporter will also receive $10 incentive/month if the participant is still breastfeeding.

Component #5 - Early limited formula milk (ELF) via syringe: This component is optional and is only reserved for at-risk infants with 5% or more birth weight loss within the first 2 days of life when the mother decides to add formula.[73] The goal of ELF is to ensure supplemental use of formula to help the at-risk infant grow but will not interrupt the success of breastfeeding. Specifically, the mother should always breastfeed the infant first. If needed, a limited amount (10.0 mL or less) of formula milk is then complemented. The formula milk is administered via syringe rather than baby bottles, which can prevent nipple confusion. ELF will be discontinued once breast milk can meet the infant's need, usually within 2 weeks. ELF has been shown to increase exclusive breastfeeding rate (79% vs. 42% usual care) at 3 months postpartum for at-risk infants.[73]

b) Control Group. Participants in the control group will receive a brief pamphlet on the benefits and skills of breastfeeding. They will complete the same frequency of study visits as the intervention group to monitor their feeding plan/practice with a fixed compensation ($15/visit), regardless of feeding practice.

c) Fidelity and quality control. Step-by-step instructions will be used for each visit. The PI will train all research staff. Evaluation will be conducted via exams and trial runs before their interacting with participants. All visits will be audio-recorded for quality assurance and 20% of audio files will be reviewed by the PI.

Comments. We decided not to include any education or intervention directly targeting on smoking relapse. This will allow us to test the independent effect of breastfeeding promotion on smoking relapse.

3.1.7 Follow-up. To evaluate sustainability of the effects of breastfeeding promotion on smoking relapse, we will follow participants and infants in 9 months postpartum (i.e.,3 months after breastfeeding intervention ends).

3.1.8 Outcome measures. The key outcome measures include timing of postpartum smoking relapse (primary) and breastfeeding duration (secondary) derived from data repeatedly collected throughout the study (**Table 1**). Outcomes will be assessed by graduate research assistants who are blinded to group assignments.

Table 1. Schedule for outcome and mediator measures

	Pregnancy	**Postpartum (W-week, M-month)**								
Measure	**Enrollment (pre-test)**	**W1**	**W2**	**M1**	**M2**	**M3**	**M4**	**M5**	**M6 (post-test)**	**M9 (follow-up)**
Outcomes										
Smoking status	X	X	X	X	X	X	X	X	X	X
Breastfeeding (BF) status	BF intention	X	X	X	X	X	X	X	X	X
Mediators										
Oxytocin and prolactin				X		X		X		
Mother-infant bonding		X	X	X	X	X	X	X	X	X
Mother-infant interaction				X		X		X		
Maternal stress	X	X	X	X	X	X	X	X	X	X
Maternal negative affect	X	X	X	X	X	X	X	X	X	X

Timing of maternal postpartum smoking relapse. The participant will use a calendar to record the daily number of cigarettes smoked, if any, throughout the study period. Based on SRNT guidelines,[74] the date of postpartum relapse will be defined as the first day after delivery on which an ex-smoker has smoked for 7 consecutive days, or has smoked at least once each week over 2 consecutive weeks. Similar definitions were used in previous studies on postpartum smoking relapse.[15,52,75,76] The self-reported smoking abstinence will be biochemically confirmed by urine cotinine tests with HPLC-MS/MS assay performed by an outside laboratory (Kaleida Health Lab, Buffalo, NY). As recommended by SRNT,[77] a cut-off point of 50 ng/mL urine cotinine will be used to define current smoking. There is a possibility that some participants may relapse during pregnancy (*prenatal* smoking relapse). If the relapse happens before randomization, they will be removed from this study. Otherwise, they will continue to receive breastfeeding intervention and be included in intent-to-treat analysis.

Breastfeeding duration. We will measure the initiation, duration, and exclusivity of breastfeeding by interviewing the mother at each postpartum visit using a questionnaire modified from 2 US national birth cohorts.[78,79] Postpartum mothers will report their current feeding method, frequency, and duration of breastfeeding and/or formula use. Breastfeeding status will be visually verified by a female research staff,

looking for one of the following indicators of successful breastfeeding in the infant – audible swallowing, a regular suck/swallow/breath pattern, or visible milk in the infant's mouth after they are not latched anymore.[31] For a mother who pumps milk, staff will observe pumping combined with the resulting milk being fed to the infant. This visual verification of breastfeeding/pumping was acceptable to participants in Dr. Washio's study.[31]

3.1.9 Mediator measures. *Maternal plasma oxytocin and prolactin*. The release of oxytocin and prolactin is often pulsatile and stimulated by suckling or pumping, and also varies across postpartum periods.[80-82] To capture hormonal changes, we will measure the baseline and peak levels of these two hormones during a typical feeding session ($40 extra compensation/session) in 1, 3, and 5 months postpartum (**Table 1**). The hormonal response to feeding will be calculated as the difference between the baseline and the peak during feeding. Based on a published protocol (co-authored by Dr. Grewen, Consultant),[80] the mother will bring her infant to the PI's lab at 1PM for an observed feeding session in a quiet dedicated room administrated by the PI and a phlebotomist. A 20 gauge Venflon cannula will be inserted into the mother's forearm vein to enable multiple blood draws. To reduce the impact of experimental manipulation (e.g., needle stick induced stress) on measured hormonal levels, a 10-minute habituation period and another 10-minute baseline rest will be used before a 5.0-mL venous blood sample is obtained to measure baseline oxytocin and prolactin. The mother will then feed her infant as usual, e.g., breastfeeding, formula feeding, or mixed. Based on previous studies with 6 or 7 repeated hormonal measures around a feeding episode, we expect the typical peaks to occur at about 3 minutes for oxytocin[80] and 30 minutes for prolactin[81,83] after the onset of breastfeeding or pumping. Thus we will obtain 5.0-mL blood samples repeatedly at 3 and 30 minutes after the onset of feeding or pumping. Plasma oxytocin and prolactin will be assayed at Dr. Grewen's lab in UNC at Chapel Hill (see **Letter of Support**). The level of oxytocin will be measured by enzyme immunoassay with extraction (Enzo Life Sciences).[84] The level of detection for oxytocin is 1.1 pg/mL and intra-assay and inter-assay coefficient of variation (CV) are 4.6% and 8.7%, respectively.[80] Prolactin will be measured with commercial radioimmunoassay (MP Biomedicals) with level of detection being 1.0 ng/mL and intra-assay and inter-assay CV being 5% and 9%, respectively.[85]

Comments. We recognize the timings for peak oxytocin and prolactin vary among individuals. Using the average peak times (3 and 30 minutes, respectively) is a cost-efficient way to capture hormonal responses.

Mother-infant bonding. Mother-infant bonding will be measured with the Postpartum Bonding Questionnaire (PBQ) at all postpartum visits.[86] PBQ is a 25-item scale reflecting a mother's feelings or attitudes towards her baby, such as ''I feel close to my baby' and ''I regret having this baby''. Cronbach's alpha was 0.76 for the total PBQ scale, and test-retest reliability was 0.95, 0.95, 0.93, and 0.77 for the 4 subscales (impaired bonding, rejection and anger, anxiety about care, and risk of abuse), respectively. PBQ is a valid screening tool for bonding disorders[86] and is well correlated with Kennerley Blues Scale.[87]

Mother-infant interaction. At 1, 3, and 5 months postpartum, maternal and infant behaviors will be assessed during observations of mother-infant interactions during free play in the PI's lab. The observation room will be set up with toys on top of a colorful blanket placed on the floor. Mothers will be asked to spend some time with their infants as they normally would at home. These interactions will be videotaped for 10 minutes and independently coded by two coders blinded to group assignment or other information about mothers and infants. These observations will be coded using 5-point rating scales originally developed by Clark[88] and used extensively in our prior studies led by Dr. Eiden (Co-I).[89-91] Three composite maternal scales will be derived: maternal sensitivity, negative affect, and positive engagement. Three composite infant behavior scales will be computed: infant responsiveness, positive affect, and negative affect. These scales are reliable and valid across different samples of substance using women, including pregnant smokers.[89]

Maternal stress. We will measure maternal stress with Perceived Stress Scale (PSS) at enrollment and all postpartum visits.[92] The 14-item PSS measures subjective stress or the degree to which life situations are appraised as stressful. Participants will report how often they have felt upset, nervous, angered, in control, or that they have coped effectively in the past month. Cronbach's alpha of PSS was 0.85 and test-retest reliability was 0.85. It was correlated with life-events, depressive and physical symptomatology, and social anxiety.[92]

Maternal negative affect. Maternal negative affect will be measured at enrollment and all postpartum visits using the Positive and Negative Affect Schedule (PANAS).[93,94] The 10 items on negative affect include being distressed, upset, guilty, ashamed, hostile, irritable, nervous, jittery, scared, and afraid. Participants will rate their experience of each particular emotion in the past few weeks. With this time frame, its Cronbach's alpha was 0.87 and test-retest reliability was 0.48.[93] In addition, we will measure maternal anxiety with the State-Trait Anxiety Inventory[95] and postpartum depression with the Edinburgh Postnatal Depression Scale.[96]

3.1.10 Covariates. We consider partner smoking, maternal secondhand smoke exposure, education, household income, health motivation,[97] perceived social support,[98] as covariates. They may confound our results and will be controlled in data analysis if not comparable between intervention and control groups.

3.1.11 Economic costs. We will collect information on intervention (not research and development) costs such as personnel, recruitment, materials, compensation, incentives, and transportation. We will include outcome costs for formula, medical care of NICU stay and sick child visits, and parental absenteeism for infant illness.[99]
3.1.12 Statistical Analysis. *Specific Aim 1 (Breastfeeding promotion and postpartum smoking relapse)*. The timing of postpartum smoking relapse (outcome) will be analyzed with Cox regression model by including group assignment (intervention vs control), smoking status (1=smoking, 0=non-smoking), the observed time, and significant covariates. The proportional hazard ratio of postpartum smoking relapse between two groups will be calculated.[15] We will estimate Kaplan-Meier survival curves to visually compare smoking trajectories. Intent-to-treat analysis will be used with all randomized participants being included in their originally assigned groups. Dropouts, losses to follow-up, fetal deaths, and miscarriages will be coded as missing data on smoking status. If missing data on smoking status is more than 20%, we will run pattern mixture models and sensitivity analyses.[13,74,100] A supplemental analysis will be run to examine *prenatal* smoking relapse during pregnancy.

Specific Aim 2 (Mediation analyses on mechanisms). We will use dynamic path analysis[101,102] to examine the mechanisms through which breastfeeding reduces smoking relapse. The dynamic path analysis includes simultaneous fitting of the Aalen additive hazards model (the R package 'timereg') and ordinary path analysis (PROC TCALIS in SAS). It fits our survival data with internal time-dependent mediators (e.g., oxytocin or prolactin) and a counting process as the outcome (i.e., smoking relapse). Hormonal baseline level and response to infant feeding (oxytocin or prolactin) will be included into the model as independent mediators. We will estimate the direct, indirect (through one or multiple mediators), and total effects of breastfeeding on smoking relapse (**Figure 1**). The PI has sufficient experience in mediation analysis.[103,104]
3.1.13 Sample size. According to the literature,[65,66] we estimate 45% rate of smoking relapse by 6 months postpartum in the control group. Assuming 45% protection effect of breastfeeding against postpartum smoking relapse,[2,26,27] we estimate 25% (45%*[1-45%]) rate of smoking relapse by 6 months postpartum in the intervention group. The corresponding sample size is 98 per group with type 1 error α of 0.05 and power 1-β of 0.8 to detect significant difference in smoking relapse between two groups in the larger R01 project. If 9-month postpartum smoking relapse rates (70% control vs 39% intervention) are used, the corresponding sample size is 46 per group. Therefore, 30 participants per group for this pilot study should provide sufficient sample size to test feasibility and efficacy as well as provide preliminary data regarding mediators and cost-effectiveness.

3.2 Potential Problems & Alternative Strategies. A potential challenge for the proposed project is to recruit a sufficient number of pregnant ex-smokers. If significant delay is observed, we will expand recruitment areas, use direct mailing, and advertise in local media. Another potential problem is attrition during intervention and follow-up periods. We expect 20% attrition rate due to dropouts, losses to follow-up, miscarriages, and fetal deaths. If we lose contact, we will reach out to the 3 people that have close relationship with the participant indicated at enrollment. We will recruit more participants to ensure sufficient statistical power if attrition is high.

3.3 Timeline of Study Process

	MONTH											
TASKS	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**22**	**24**
IRB approval (before start)												
Hiring and training staff	X											
Testing study procedures	X											
Surveys and intervention materials	X											
Participant recruitment	X	X	X	X	X	X	X					
Prenatal breastfeeding intervention		X	X	X	X	X	X					
Postnatal breastfeeding intervention			X	X	X	X	X	X	X			
Follow-up (9-month postpartum)						X	X	X	X	X	X	X
Data analysis					X	X	X	X	X	X	X	X
Manuscripts & reports											X	X
R01 proposal & submission											X	X

3.4 Future Directions. The preliminary data from this pilot study will support our application of a R01 grant, which will extend breastfeeding intervention to 1 year and follow-up to 2 years postpartum. In the larger R01, we hope to have the opportunity for multiple measures of oxytocin and prolactin around a feeding episode to calculate area under the curve,[80,81,85] and testing mediation of other hormones such as progesterone and cortisol. We will add cost-effectiveness analysis to evaluate economic impact of intervention. It is also possible to scale up our intervention to communities through paraprofessionals (e.g., community health workers[105]).