Vitamin C to Decrease the Effects of Smoking in Pregnancy on Infant Lung Function (VCSIP): A Randomized Trial

Protocol

Exemption for IND obtained from FDA

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1. Introduction

1.1 Study Abstract

Despite strong anti-smoking efforts, at least 12% of American women do not/ can not quit smoking when pregnant, resulting in more than 450,000 smoke-exposed infants born yearly (1). Smoking during pregnancy is the largest preventable cause of low birth weight, prematurity and perinatal mortality (2). Maternal smoking during pregnancy is also the largest preventable cause of childhood respiratory illness (3-5). Recent studies have shown a protective effect of vitamin C supplementation on the lung function of offspring exposed to in-utero smoke in both a non human primate model (6) and a pilot trial in humans (7). The purpose of this randomized, controlled intervention trial is to evaluate the pulmonary function at 3 months of age in infants delivered to smoking mothers who are randomized to 500 mg/day of supplemental vitamin C versus placebo during pregnancy.

1.2 Primary Hypothesis

Vitamin C supplementation (500 mg/day) during pregnancy will block the adverse effects of maternal smoking on infant pulmonary function measured at 3 months of age (forced expiratory flows) in infants born to smoking mothers.

1.3 Purpose of the Study Protocol

This protocol serves as a road map for the execution of the Vitamin C to Decrease the Effects of Smoking in Pregnancy on Infant Lung Function Study (VCSIP). It describes the background, rationale, design and organization of this randomized, double-blind, placebo-controlled dietary intervention trial, and may be considered as a written agreement among the study centers and investigators. It will be reviewed by the Study Steering Committee, the Study Data Coordinating Center (DCC), the institutional IRBs as well as the Data and Safety Monitoring Board (DSMB) appointed by the NHLBI. Any significant changes to the protocol during the study require the approval of the Steering Committee, the NHLBI, the DSMB.

A manual of operating procedures (MOP) will supplement the protocol and includes detailed information on the execution of the study and all procedures used.

2. Background

2.1 Introduction and Significance

Smoking during pregnancy is the largest preventable cause of low birth weight, prematurity and perinatal mortality (2). It is also the largest preventable cause of childhood respiratory illness, and children whose mothers smoked during pregnancy show lifetime decreases in pulmonary function and increased respiratory illnesses and asthma (2, 4, 5). Maternal smoking is estimated to cause 10% of direct medical expenditures in the first year of life (8), and Stoddard and Gray (9) estimated that

approximately 20% of expenditures for childhood respiratory illness are caused by maternal smoking amounting to $660 million annually in 1997 dollars. Several studies have now clearly linked maternal smoking during pregnancy to increased childhood respiratory illness (5).

2.2 Background Studies

2.2.1 General Background

One of the initial reports indicating a connection between maternal smoking and children's respiratory function was from Tager et al, (10) who reported decreases of 7-10% in the forced expiratory volume in one second (FEV_1) in children 1-5 years of age with smoking mothers. Hanrahan et al, (11) examined pulmonary function of infants shortly after birth (~4.2 weeks) as a function of maternal smoking during pregnancy and found a significant decrease in maximal expiratory flow at functional residual capacity (VmaxFRC). Similarly Hoo et al, (12) found a significantly decreased time to peak tidal expiratory flow to expiratory time ratio ($T_{PTEF}:T_E$) in premature infants studied at a corrected gestational age of 36 weeks whose mothers smoked during pregnancy. Milner et al, (13) studied 100 infants delivered to non-smoking women and 189 born to smokers. They showed a significantly decreased respiratory system compliance (Crs) in infants born to smoking mothers. In a follow-up study, Tager et al, (14) showed that the decreased VmaxFRC seen with prenatal smoke exposure correlated directly with increased lower respiratory illnesses (LRI). Cunningham et al, (15) performed pulmonary function test (PFT)s on 8800 non smoking school children aged 8-12 and similarly found reduced forced expiratory flows in children whose mothers smoked during pregnancy, suggesting that deficits continued at least until adolescence. A recent prospective study with a 21 year follow up has now extended the decreases in FEV_1 and FEF_{25-75} (forced expiratory flows between 25% and 75% of FVC [forced vital capacity]) to 21 years of age in males (4). In multiple other studies; Brown et al,(16); Stick et al, (17); Lodrup et al, (18); and Li et al, (19) have all found decreased expiratory flows in infants and children born to smoking mothers. Therefore evidence indicates that the influence of smoking in pregnancy persist through early adult life.

Several studies have shown that decreased pulmonary function in infants correlate with increased rates of respiratory illness (14, 20-25). Measurements of $T_{PTEF}:T_E$, Crs and forced expiratory flows (VmaxFRC, FEF_{25-75},) early in infancy are important predictors of later respiratory outcomes. One of the largest trials was a prospective birth cohort study of 802 healthy babies in Norway (23) with follow-up from the newborn period through 10 years of age. This study demonstrated that infants with measurements of $T_{PTEF}:T_E$ at or below the median shortly after birth were significantly more likely at 10 years of age to have a history of asthma (24.3% vs 16.2%, p=0.01; OR of 1.58); have current asthma (14.6% vs 7.5%, p=0.005; OR of 2.10); and to have severe bronchial hyperresponsiveness (9.1% vs 4.9%, p=0.05) (23). From this same cohort, infants whose Crs was at or below the median shortly after birth were significantly more likely to have a history of asthma (27.4% vs 14.8%,p=0.001; OR of 2.18) and current asthma (15.0% vs 7.7%, p=0.009; OR of 2.01). An increased risk of wheezing in the first years

of life has been reported in children with a decreased ratio of T_{PTEF}:T_E measured in the first week of life (24) or at 3 months of age (21, 24), and in those with reduced VmaxFRC at 1, 3, and 6 months of age (14, 21, 25, 26). Another population based longitudinal study demonstrated that infants less than six months of age with a decreased VmaxFRC developed wheezing lower respiratory tract illnesses in the first year of life (14).

Consistent with this, multiple studies have shown increased lower respiratory illness in infants born of mothers who smoke (27-30). Taylor and Wadsworth (29) studied 12,743 children and found significantly increased bronchitis and hospital admissions for lower respiratory illness in children from smoking mothers.Tager et al. (14) similarly found increased lower respiratory illness in infants with prenatal smoke exposure but not with postnatal. Other studies have also demonstrated increased wheezing and asthma in children born of mothers who smoked during pregnancy (16, 31-33). Thus maternal smoking during pregnancy clearly leads to altered lung development manifested by impaired pulmonary function and reflected in turn by increased respiratory illness.

Recent studies have also indicated the key role of genotype relative to the development of asthma, sensitivity to maternal smoking and difficulty in quitting smoking. Notably several common polymorphisms are linked in terms of sensitivity of offspring to maternal smoking. In particular common deletions or structural polymorphisms in the glutathione transferase (GST) genes increase both the risk of asthma and sensitivity of the fetus to maternal smoking (34-38). The GST genes play a key role in antioxidant defense. Similarly, the common structural polymorphism of the α5 nicotinic receptor in which residue 398 is mutated from an Asp to an Asn (rs16969968) increases nicotine addiction, makes quitting more difficult, and increases risk of lung cancer and COPD (39).

Given the above, the obvious question is: why can't pregnant women stop smoking and if they don't, why should we invest limited research dollars? The reality is that smoking is a unique morbidity in that it is addictive, heavily advertised (40-42) and certain genotypes significantly increase the likelihood of nicotine addiction / failure to quit (43-45). Teen pregnancy, low income, low education, and living with a smoker are important factors increasing the odds of smoking during pregnancy (46-49). It is particularly tragic that infants are victimized through no fault of their own by the addiction of their mothers caused in part by socioeconomic class, advertising, and genetics. Given nicotine's addictive nature, the low socio-economics of this population, and the constant advertising by tobacco companies, smoking during pregnancy will continue to adversely affect millions of babies worldwide. Recent CDC statistics show that decreases in smoking rates have plateaued and smoking rates have even increased in recent years for teenagers (50). This unfortunate reality makes finding ways to lessen the effect of smoking during pregnancy of vital importance.

2.2.2 Pilot Vitamin C Studies

Our underlying hypothesis is that the effect of *in utero* smoke exposure on lung development is mediated by oxidant mechanisms and therefore can be prevented by maternal vitamin C supplementation. A primary mediator of smoking-induced oxidant injury is nicotine (51-54). Consistent with this as shown in Figure 1, prenatal nicotine exposure in pregnant rhesus monkeys leads to decreased pulmonary function of offspring similar to that caused by maternal smoking, and the effects were decreased by maternal vitamin C supplementation. Vitamin C also prevented the effects of nicotine on lung surfactant and elastin expression. These results were published in the *Am J Respir Crit Care Med* (6).

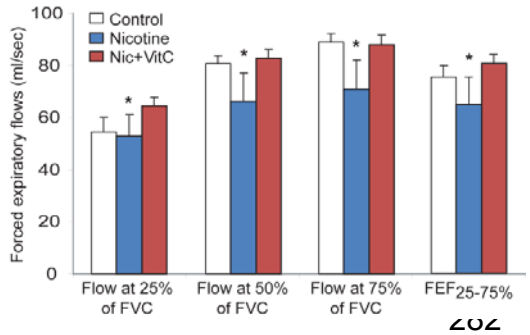


Figure 1. *Forced expiratory flows in animals treated as shown (Mean ± SEM). *p<.05 for overall comparison of Nicotine-treated group to control and Nicotine + vitamin C-treated groups by MANOVA. $FEF_{25\%-75\%}$ = the average flow between 25% and 75% of forced expired volume. N= 20 total animals* (6).

Based on the results shown in Figure1, Dr. McEvoy performed a trial randomizing pregnant smokers in a double blind design to 500 mg vitamin C /day or placebo; a reference group of non smokers was also included. We chose a 500 mg vitamin C dose as that is the lowest dose that will saturate the vitamin C plasma receptors and it is well within the dosage range of vitamin C generally considered safe (55). Patients were monitored for medication compliance by fasting plasma ascorbic acid levels and pill counts; urine cotinine levels were monitored for nicotine exposure. At 24-48 hours of age, pulmonary function testing was performed to assess tidal breathing (including the ratio of TPTEF:TE and passive respiratory system compliance (Crs). As shown in Table 1, in analysis of 235 infants (159 born to randomized smokers and 76 born to non smokers), maternal smoking during pregnancy caused the expected decrease in Crs; and the downward trend was reversed by vitamin C. In addition, this dose of supplemental vitamin C (500 mg) blocked the maternal smoking-induced changes in the newborn's tidal breathing as measured by TPTEF:TE (p<.01).

Table 1. Newborn Pulmonary Function Test Results

	Newborns of non smokers (n=76)	Newborns of placebo treated smokers (n=83)	Newborns of vitamin C treated smokers (n=76)	P value for treatment groups	95% CI for difference in means in treated groups
Respiratory rate (bpm)	53 ± 12	57 ± 12	57± 12	0.944	(-3.98, 3.71)
TPTEF:TE	0.399 ± 0.077	0.345 ± 0.078	0.383 ± 0.084*	<0.01	(0.01, 0.06)
Crs/kg (mL/cmH2O/kg)	1.36 ± 0.30	1.20 ± 0.24	1.32 ± 0.30*	<0.01	(0.03, 0.21)

Mean ± SD; p values and 95% confidence intervals adjusted for gestational age at randomization (>16 weeks vs ≤ 16 weeks)

Infant follow-up showed that through one year of age, vitamin C supplementation also decreased the incidence of wheezing seen in the offspring of smokers (p=0.065). See Figure 2. These latter data are preliminary, as only about half of our randomized offspring had reached one year of age at the time of this analysis.

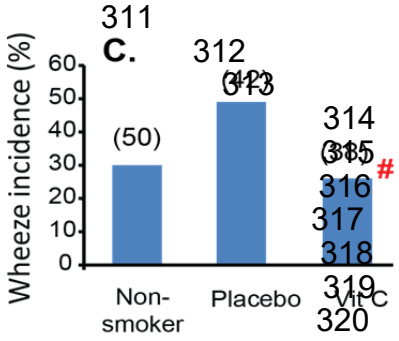


Figure 2. *The increase in incidence of wheeze in the first year of life associated with maternal smoking was blocked by vitamin C supplementation. # p = .065 comparing Vit C to placebo based on binomial regression with two randomization groups adjusting for stratification variable. Data is percent of infants in the group who have had one or more incidents of wheezing in the first year of life. Number in parentheses shows the number of infants in each group who have reached 1 year of age.*

Smoking during pregnancy decreases available ascorbic acid to the fetus. As shown in Figure 3 from our pilot trial, levels of plasma ascorbate in pregnant smokers were significantly decreased compared to non smokers and 500 mg vitamin C supplementation restored levels to normal. We have also observed similar data in monkeys exposed to just nicotine, in which prenatal nicotine exposure also decreased ascorbate levels in both cord blood and amniotic fluid. These levels were restored by vitamin C supplementation. This data shows that the 500 mg vitamin C dose is ±sufficient to restore vitamin C levels in the pregnant smoker to levels of the pregnant non smoker, but importantly does not significantly increase vitamin C levels over levels seen in pregnant non smokers.

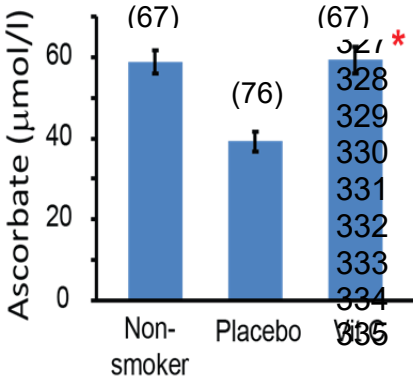


Figure 3. *Maternal plasma vitamin C levels (third trimester). Values are Mean ± SEM with sample number in parentheses. *p <0 .05 for vitamin C treated group compared to placebo.*

2.3 Safety of Vitamin C Therapy

2.3.1 Maternal and Fetal/Neonatal

Vitamin C is a common anti-oxidant and the Institute of Medicine states that daily doses of up to 2000 mg are likely to pose no risks of adverse effect (56). The median total intake in a study of 2200 pregnant women (in North Carolina with 62% at <185% of the poverty level) was 250 mg (57).

Concerns over the potentially harmful effect of supplemental vitamin C during pregnancy on prematurity have been described. A 2005 Cochrane review of vitamin C

supplementation in pregnancy concluded that women supplemented with vitamin C were at an increased risk of preterm birth (RR 1.38, 95% CI 1.04 to 1.82, 3 trials, 583 women) (58). This meta-analysis appears unduly influenced by one of the trials (59) which was the only trial of the three to show increased preterm deliveries in the treated group. Of note, in this study 43% of the randomized patients were smokers, and there was no significant difference in the rate of prematurity within the subgroups of smokers by treatment group. A recent trial of 10,154 women randomized to vitamins C and E versus placebo did not show any significant difference in preterm births (60). Dr. McEvoy included this large recent trial (outcome data available on 9,969 patients) into a meta-analysis of existing studies totaling 10,552 patients and the RR for preterm delivery <37 weeks was 1.1, 95% CI of 0.98 to 1.11; p= 0.12 (done with Comprehensive Meta-analysis).

Dr. McEvoy's pilot study also lends support for the safe dosing of 500 mg of vitamin C daily as there was no significant difference in maternal, fetal, or neonatal adverse outcomes between the groups of randomized smokers. In the pilot study, there were no significant differences in prematurity or gestational age between babies born to smoking mothers randomized to vitamin C versus placebo.

2.4 Rationale for a Randomized Clinical Trial

Although our pilot study showing significantly improved pulmonary function tests in newborns whose mothers received supplemental vitamin C is very encouraging, one of the key effects of maternal smoking is a decrease in infant forced expiratory flows which was not measured in the pilot project. This new randomized trial also offers us the opportunity to robustly examine clinical outcomes, specifically the incidence of wheezing, in the first year of life of infants born to randomized smokers. The measurement of forced expiratory flows is thought to be a more sensitive measure of peripheral airway function and will be measured in the current study at the two participating locations of Portland, Oregon Metropolitan area and Indianapolis, Indiana. This will reconfirm the benefit of vitamin C supplementation on pulmonary function and more precisely define its benefit on the infant's peripheral airways.

3. Study Design

3.1 Primary Research Aim

The primary aim of this study is to demonstrate improved pulmonary function, as measured by forced expiratory flows and specifically FEF_{75} (forced expiratory flows at 75% of FVC), in infants delivered to smoking mothers who are randomized to 500 mg/day of supplemental vitamin C versus placebo during pregnancy. We hypothesize that vitamin C supplementation during pregnancy will block the adverse effects of maternal smoking on infant pulmonary function measured at 3 months of age (forced expiratory flows) in infants born to smoking mothers.

3.2 Secondary Research Aims

A secondary aim of this study is to demonstrate a decreased incidence of wheezing through 12 months of age, in infants delivered to smoking mothers who are randomized to 500 mg/day of supplemental vitamin C versus placebo during pregnancy. We hypothesize that vitamin C supplementation during pregnancy will decrease the incidence of wheezing in the first 12 months of life in offspring of smokers.

An additional secondary aim is to demonstrate improved pulmonary function at 12 months of age in infants delivered to smoking mothers who are randomized to 500 mg/day of supplemental vitamin C versus placebo during pregnancy. We hypothesize that vitamin C supplementation during pregnancy will block the adverse effects of maternal smoking on infant pulmonary function measured at 12 months of age (forced expiratory flows) in infants born to smoking mothers.

3.3 Study Design Summary

The study is a placebo-controlled, double-blind randomized trial conducted at two clinical locations: Portland, OR Metropolitan Area (clinics delivering at Oregon Health & Science University and clinics delivering at PeaceHealth Southwest Medical Center) and Indianapolis, IN (recruiting at Indiana University and Wishard Hospital). Two hundred fifty-two pregnant smokers less than or equal to 22 weeks gestation will be randomized to one of two groups:

1. Vitamin C (500 mg)

2. Matching placebo

The Oregon Health & Science University (OHSU) research pharmacy will dispense the study medication for patients for each recruitment site. Vitamin C and placebo medications will be compounded in identical tablets at an outside site through the OHSU Research pharmacy. Each vitamin C tablet will contain 500 mg of ascorbic acid powder; the placebo tablet will contain microcrystalline cellulose and 100 mg of citric acid to mimic the taste of Vitamin C. The tablets will be otherwise identical in appearance, size and shape. The medications will be dispensed in 21 and 100 tablet quantities (21 count during the compliance period and 100 count during the treatment period). The OHSU research pharmacy will dispense the study medication for patients for each recruitment site to assure consistency.

3.4 Eligibility Criteria

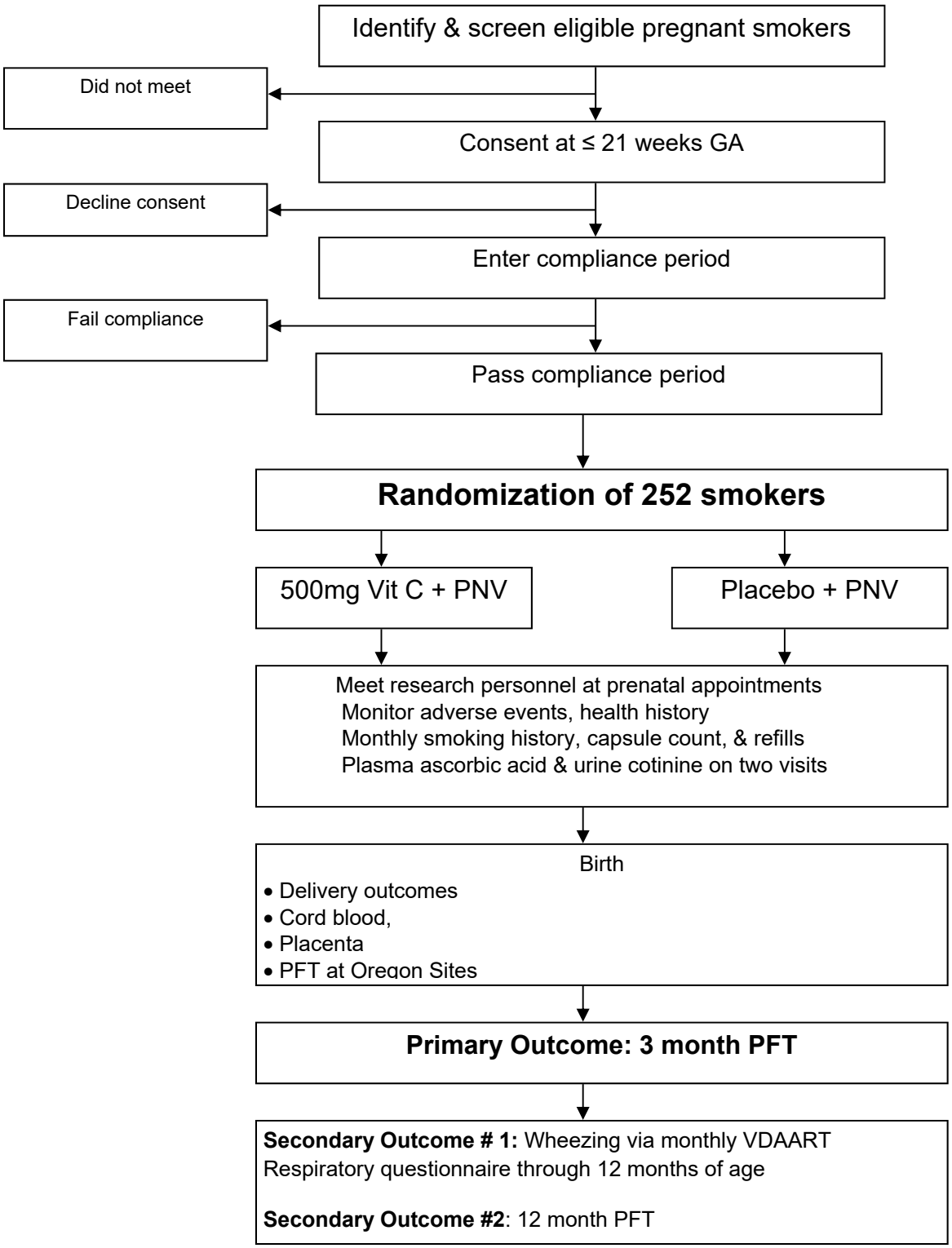
3.4.1 Inclusion Criteria at Randomization

1. Singleton gestation
2. $\geq$15 years old
3. Gestational age between $13^0/_7$ and $22^6/_7$ weeks based on clinical information and ultrasound as described below in “gestational age determination” (run in period can be up to 21 days).
4. Receiving prenatal care at clinics delivering at OHSU, PeaceHealth Southwest Hospital, Wishard, or Indiana University (IU) Hospital or

surrounding clinics

5. Current smoking
6. English speaking

Figure 4. Study Overview



3.4.1.1 Gestational Age Determination

For purposes of enrollment in the run-in, gestational age will first be estimated in the pregnant woman by reviewing the results of the first available ultrasound examination. Women whose obstetrical ultrasound indicates a gestational age too advanced for the run-in (>21 weeks) will be excluded for further consideration. In cases where an ultrasound examination has not been performed, gestational age should be estimated using whatever information is available and their best judgment i.e. they may use the first day of the last menstrual period (LMP) or uterine size to assess whether gestational age is appropriate.

All women not otherwise excluded will be interviewed for LMP and sent for an ultrasound examination, if one has not already been performed. Gestational age will be based solely on the earliest obstetrical ultrasound estimate as determined at each clinical center. Women whose gestational age at the time of randomization has been determined to be ≤ 22 weeks by ultrasound will be eligible to enroll in the study, pending complete review of inclusion and exclusion criteria and results of the run-in.

3.4.2 Exclusion Criteria at Randomization

1. Gestational age ≥ 23 $^{0}/_{7}$ weeks
2. Multiple gestation
3. Documented major fetal congenital anomalies
4. Current use of illicit drugs
5. Current alcohol abuse as defined by questionnaire (≥ 3 drinks on ≥ 5 days per week during this pregnancy or any hospitalization for alcohol abuse or complications of alcohol abuse or outpatient visit for acute alcohol intoxication during the past year)
6. Use of vitamin C (≥ 500 mg/day) > 3 days per week since LMP
7. Refusal to abstain from vitamin or supplements containing significant vitamin C other than those provided through the study or approved by study staff
8. History of kidney stone in patient
9. Insulin dependent diabetes
10. Complex maternal medical conditions
11. Participation in other conflicting research projects
12. Unable to demonstrate stable method of communication or incarcerated
13. Pregnancy by in-vitro fertilization
14. Plan to terminate pregnancy
15. Failure of medication compliance trial
16. Failure to return in designated period during placebo run-in
17. BMI > 50 at screening

3.5 Informed Consent

Written informed consent will be obtained from each subject at entry into the study. Informed consent is obtained by the following process:

1. Study will be introduced to the subject by research staff.
2. Research staff will review the consent with the subject, confirm understanding, and answer any questions.
3. Once understanding is confirmed, the consent is signed. Individuals authorized to obtain written consent are the principal investigator (PI), co-investigators, and assigned staff specifically designated by the PI to work on this project.

3.6 Randomization Method and Masking

Randomization: Women will be randomly allocated to vitamin C or placebo using permuted block randomization stratified by gestational age at time of randomization (less than or equal to 18 weeks versus greater than 18 weeks gestation) and clinical site (Oregon, Washington and Indiana). Stratifying by gestation and site in this way will generate approximately equal numbers of individuals in each of the two treatment groups for each combination of site and gestational age. Gestational age was selected for stratification as this is a potentially critical covariate because of the effect of duration and total dose of vitamin C on the developing infant. Weeks of gestation and study site at randomization will also be incorporated in the analysis as covariates. In setting up the blocks for randomization we will randomly choose between two block sizes in order to minimize the chance that study personnel can predict treatment assignment.

Blinding: Vitamin C and placebo medications (using vitamin C powder or cellulose) will be compounded in identical tablets at an outside site through the OHSU Research Pharmacy. The cellulose will contain 100 mg of citric acid (degradable to inert products) to ensure identical organoleptic properties. A randomization schedule will be given to the research pharmacy for preparation of the medication sequence to dispense to research subjects. The study medication will be labeled with study identification and a consecutive study code for the patient, aligned with the randomization scheme.

After a patient has given consent, inclusion and exclusion criteria will be confirmed for each patient centrally at the DCC. She will be assigned an identification code and dispensed a three week supply of placebo to be taken as a check on compliance; subsequently, if the woman successfully completes the compliance period, she will be assigned to the next sequential allocation for the appropriate site and gestational age. Similarly, the research pharmacy will log the ID of the patient.

As a check on the integrity of the medication preparation, potency testing of the vitamin C tablets will be done prior to the initiation of the study. As a check on the integrity of the randomization scheme, returned medication will be stored; a random 10% sample of returned medication will be assessed to ensure that the correct medication has been delivered. At the end of the study as a check on the effectiveness of blinding, participants will be asked which treatment group they believe they were assigned. The expectation is that each cohort (vitamin C versus placebo) will not be more than 50% correct in assessing their allocation. Maintaining integrity of randomization allocation

and blinding is the most important asset of the study and will be maintained as a primary goal of the DCC.

4. Study Procedures

4.1 Identification / Screening for Eligibility

Recruitment will be maximized by assigning a specific research coordinator in Portland and in Indiana to screen and consent eligible patients. Pregnant women will be recruited by referrals from providers and by self-referral via advertisements. Research staff will query the hospitals' electronic medical records to identify obstetrical patients who fit initial eligibility criteria. All obstetrical questionnaires inquire about smoking status. If the response is positive and the patient agrees, the patients will be referred to study research staff who will be available at the patient's next routine prenatal appointment. The providers at each of these clinics have approved this approach.

4.2 Baseline Procedures/ Counseling and Enrollment

At the prenatal visit, the patient will be approached about the study and once again smoking cessation will be encouraged. At a minimum, the **5 A's** for smoking intervention will be assessed: **Ask** about tobacco use; **Advise** every smoking patient to quit; **Assess** willingness to make a quit attempt; **Assist** in quit attempt; **Arrange** for follow-up, future support(61). Pregnancy-specific smoking cessation pamphlets will be given. We will strongly encourage the patient to participate in a smoking cessation program, therefore this population will actually receive greater encouragement to quit smoking than the general population. Only women truly unable to quit smoking will be involved in the study. The research team will be certified in smoking cessation training. Dr. Gonzales of the OHSU Smoking Cessation Center is a co-investigator on the study (62, 63) and advises the research teams about cessation techniques. Study personnel will document that cessation counseling was offered. A substantial proportion of pregnant smokers will stop spontaneously before they begin prenatal care and those who are active smokers at their first prenatal visit are less likely to change their smoking habits (64-66). Upon randomization, a detailed smoking questionnaire geared towards pregnancy (61) will be administered. Smoking cessation counseling will continue throughout the study. **Participation in this study does not give the pregnant smoking woman license to continue smoking, as smoking cessation and education of the negative effects of smoking on the newborn are done at each prenatal visit. In our pilot study we had an additional cessation rate of 10% in our patients and their families.** If a patient quits smoking after randomization, this will be noted and they will be instructed to continue to take the study medication.

To exclude highly noncompliant subjects, consented patients will enter a medication compliance trial of 14 ± 7 days. The compliance medication is dispensed by research pharmacy and is a tablet filled with cellulose that is slightly acidic. This is the same tablet that the patient will receive if randomized to the placebo arm of the study. Patients

will be excluded if they fail to return for a post compliance visit within 14 ± 7 days of the screening visit or if they took < 75% of the required tablets.

The second study visit will include assessment of the compliance trial. If the patient is compliant (≥ 75% tablets consumed and returns within 14 ± 7 days of first screening visit) the detailed smoking questionnaire will be administered, blood, urine, and hair samples will be collected, an exhaled carbon monoxide test will be done, and the patient will be randomized into the study. Body weight, body mass index, and blood pressure will be recorded from the patient's chart.

The following table includes all information collected during the visits prior to and at randomization.

Table 2. Summary of Screening and Baseline Procedures

Screening Visit 1	Run –In / Randomization
Health & medical history Smoking questionnaire Demographic/contact information Informed consent Distribute compliance drug Pre-pregnancy weight Height, weight, blood pressure	Evaluate compliance trial Smoking questionnaire Hair sample for nicotine Plasma vitamin C (fasting) Urine cotinine Exhaled carbon monoxide Distribute study drug Distribute prenatal vitamin Health history change Weight, blood pressure Reimbursement check

4.3 Patient Management and Follow-up

Interval study visits will be coordinated with routine prenatal visits by the research assistant assigned to each specific study site. Frequent, consistent, and personal contact is critical to retain this vulnerable population in a research protocol. Interval phone calls or text messages or Emails to patients will be done approximately monthly, and investigators will request multiple contact phone numbers and addresses (including those of relatives and friends), methods to contact patient confidentially through the social media, and have small travel reimbursements to maximize the retention of the study cohort. Patients will receive standard prenatal care as defined by their institution. Patients will be instructed to take one study tablet and one prenatal vitamin (provided by the study containing the minimum daily requirement for vitamin C) daily, preferably with meals, and to bring medication bottles to each study visit.

4.4 Prenatal Study Visit Procedures

Study patients will be seen every four weeks at standard prenatal visits through delivery. At each post-randomization study visit, the following procedures will be performed:

- Interval smoking questionnaire
- Pill counts
- Assess adverse events
- Assess open label use
- Changes in health & medical history
- Review of contact information, documentation of changes in work or home status, and best times and method of contact
- Document clinic measurement of weight and blood pressure

At baseline, 26 ± 2, and 32 ± 2 weeks, urine and fasting blood will be collected for cotinine, oxidant, anti-oxidant status, and compliance testing. Any extra urine or blood may be stored for future use. An exhaled carbon monoxide level will also be done.

4.4.1 Administration of Validated Smoking and Respiratory Questionnaire

4.4.1.1 Maternal Questionnaire

Maternal smoking and respiratory history will be documented through the administration of a modified validated respiratory questionnaire published by the American Thoracic Society (67).

4.4.2 Urine Cotinine Testing

Urine samples will be collected for assessment of cotinine and other biomarkers such as 8-isoprostanes at study visits at baseline, 26 ± 2, and 32 ± 2 weeks of gestation. Cotinine analysis (68) will be performed by HPLC-mass spectrometry in Eliot Spindel's laboratory.

4.4.3 Plasma Ascorbic acid / Vitamin C Testing

Fasting ascorbic acid (about 1.5 mL of blood) will be done at baseline, 26 ± 2, and 32 ± 2 weeks of gestation. Plasma ascorbic acid measurements will be performed using HPLC with coulometric electrochemical detection by Dr. Balz Frei's (69) laboratory at the Linus Pauling Institute, Oregon State University.

4.4.4 Hair Nicotine Testing

Small samples of the mother's hair will be taken at baseline and near delivery for the assessment of hair nicotine. In addition, mother and infant hair will be collected at 3 and 12 months of age for the same analysis. Hair nicotine will be determined by a modification of the methods of Hegstad et al (70) by tandem mass spectrometry (LC-MS/MS) using deuterated internal standards.

4.4.5 Carbon Monoxide Measurement

Non-invasive measurements of carbon monoxide (CO) will be performed by mother at baseline, 26 ± 2, and 32 ± 2 weeks of gestation, and at 3 and 12 month PFT (Smokerlyzer, Bedfont Scientific, London, UK) (71).

4.4.6 Study Medication Compliance

At each study visit the patient will bring her study medication bottle. Pills will be counted and compliance assessed. Enrolled patients will be asked to return all unused tablets at every study visit and when hospitalized for delivery, and also will receive interval phone calls regarding medication intake. Compliance will be computed by dividing the number of tablets taken by the total number of prescribed tablets and will be recorded in the study database.

Samples for plasma ascorbic acid will be collected prior to randomization, at 26 ± 2, and 32 ± 2 weeks of gestation, and from the umbilical cord at delivery for analysis of vitamin C as a measure of compliance. Near the time of delivery, women are customarily not allowed to eat, and study personnel will request that they do not drink any liquid high in vitamin C in the hours prior to delivery.

4.4.7 Side Effects

Patients will be assessed for potential medication side effects at each study visit.

4.4.8 Measurement of Placental Blood Flow

Preliminary data in nonhuman primates has recently demonstrated decreased placental blood flow in nicotine-exposed fetal monkeys and has demonstrated that supplemental vitamin C blocks this effect. Based on this data, extra ultrasound views will be done pending funding, timed as often as possible during a routinely ordered prenatal ultrasound to specifically measure umbilical blood flow using color and pulsed/wave Doppler, uterine artery and placental volume blood flow (72). These extra views will be done at 34 ± 2 weeks of gestation. . Extra ultrasound views done specifically for the vitamin C study will be paid for at the established research rate and will not be charged to the patient. Patients in the Pacific Northwest will be given the option of having testing done at OHSU or Peace Health/ Southwest to offer greater scheduling options.

4.4.9 Genotyping

If the mother consents, she and her infant will be genotyped for the α5 nAChR structural polymorphism (rs16969968), the null alleles for GSTM1 and GSTT1 and the decrease of function allele for GSTP1, and remaining DNA will be stored for further genetic analyses of smoking-induced changes. Blood will be collected in 3 mL EDTA tubes and stored at -80º C. Genomic DNA will be isolated from 0.35 mL of blood using the Machery-Nagel nucleospin-8 kit. Polymorphisms will be determined by quantitative PCR (qPCR). Maternal blood for genotyping will be collected at the same time as another blood draw. Infant blood for genotyping will be collected from the umbilical cord or at the

3 or 12 month PFT. In addition, buccal swabs will be taken from mothers and offspring at birth, 3 months of age, 12 months of age (73). Buccal swabs will be taken using Epicentre Catch-All soft foam swabs which are suitable for use with infants. Swabs are sterile and individually wrapped with collection tubes. Swabs are performed by removing the swab from its packaging, then rolling the Epicentre Catch-All sample collection swab on the inside of the cheek, approximately 10-20 times on each side, making certain to roll the brush over the entire cheek. After collection the swab is air dried for 10-15 minutes at room temperature then the swab is placed back in the collection tube and stored at –20°C. To increase yield of cells a separate swab may be used for each cheek surface. For older subjects, the mouth should be rinsed with water prior to beginning sample collection. These genetic analyses will be done under a separate consent

4.5 Procedures at Labor and Delivery

Identification of a study patient upon admission for delivery will be done via an automated flagging of the patient's electronic medical record (EMR), when possible, to alert on call research personnel. Patients will also be encouraged to call or text research personnel on the way to delivery. Umbilical cord blood and placenta samples will be collected.

4.5.1 Maternal Data Collection at Delivery

Careful attention will be made to record mother's timing of last study medication intake as well as dietary intake (requesting if possible no juices with vitamin C) prior to delivery as this will affect the ascorbic acid level from the umbilical cord sample. At delivery, a sample of the cord blood will be collected for ascorbic acid levels and genotyping. Placenta samples will be obtained and will be banked pending further funding using standard procedures. Maternal hospital records will be reviewed by research staff and contact information including that of family members reviewed.

4.5.2 Neonatal Data Collection at Delivery

As above, umbilical cord blood will be collected at delivery for ascorbic acid levels and genotyping. Infant hospital records through discharge will be reviewed by research staff and birth weight, length and head circumference will be obtained. At OHSU and SWW pending further funding, a pulmonary function test will be done after delivery. This test will include the measurement of flow volume loops, passive respiratory compliance and functional residual capacity (74-76). No sedation will be required. When possible a small sample of baby hair will be gently collected for nicotine analysis.

4.6 Infant Follow-Up and Procedures

4.6.1 Infant Respiratory Questionnaire

After delivery, a validated respiratory questionnaire (77-79) will be administered at least quarterly to the infant's primary caregiver to compare the incidence of wheezing (80) between the infants of smoking mothers randomized to vitamin C and placebo. This is the format and questionnaire used in the ongoing Vitamin D Antenatal Asthma Reduction Trial (VDAART) study. No education or training will be given to the infant's primary care taker with regards to the recognition of wheeze so that the results will be generalizable to previously published studies. This is the same approach that the VDAART trial is using. Caregivers are asked about new episodes of wheezing since the last questionnaire , as well as administration of prescribed medications, other illnesses, trips to the emergency room, and hospitalizations. If an infant has wheezing associated with an illness lasting up to seven days, this will be counted as one episode. These questionnaires will be administered in person at the 3 and 12 month PFT visits (see below) while the remainder of the monthly questionnaires will be administered by phone. A small time reimbursement will be available for those patients after each questionnaire is completed.

4.6.2 Infant Pulmonary Function Tests With Sedation

Infant pulmonary function testing will be performed at 3 months (range of 12 - 18 weeks of post term age) and 12 months (range of 10-14 months of age). No testing will be done within 3 weeks of a respiratory illness; every attempt will be made to obtain testing within this timeframe. A test may be repeated if the quality was not acceptable. This testing will include the measurement of forced expiratory flows following the guidelines of the American Thoracic Society and European Respiratory Society which were co-written by Dr. Tepper (81-82). The pulmonary function testing equipment and operational procedures will be rigorously calibrated across study sites according to the manufacturer's directions and outlined explicitly in the operations manual. The respective hospital pediatric sedation protocol will be followed for the three and 12 month PFT using oral chloral hydrate (82). The infant will be given 50 to 100 milligrams per kilogram of chloral hydrate by mouth, with a maximum dose of 1 gram. The parents will be counseled about possible side effects including amnesia, confusion, hyperactivity, nausea, respiratory depression, and hypotension and will be asked to sign a separate consent for sedation, and also be given the patient education-discharge instructions. Adverse events will be reported as outlined below. The parents will also be told whether the results of the 3 and 12 month pulmonary function test were within normal limits or outside of normal limits, and this report will be faxed to the patient's physician.

4.7 Adverse Event Reporting

4.7.1 Adverse Event Collection, Review, and Grading

Adverse events (AEs) in this study will be identified by interviewing the subject, review of the subject's electronic medical record (EMR), and by physical exam / observations (including vital signs monitoring during sedation). AEs will be graded as to their

expectedness and attribution (unrelated, possibly, probably or definitely related to the protocol). AE's will be reviewed in real-time by the physicians at each location.

4.7.2 Definitions

Definitions

Adverse Event (AE): Any untoward or undesirable, although not necessarily unexpected, event experienced by a human subject that may be a result of:

- The interventions and interactions use in the research
- The collection of identifiable private information in the research
- An underlying disease, disorder, or condition of the subject
- Other circumstances unrelated to the research or any underlying disease, disorder, or condition of the subject

Serious Adverse Event (SAE): Any AE that:

- Is fatal
- Is life-threatening
- Is persistent or significantly disabling or incapacitating
- Results in inpatient hospitalization or prolongation of hospitalization
- Results in psychological or emotional harm requiring treatment
- Creates a persistent or significant disability
- Causes a congenital anomaly or birth defect
- Results in a significant medical incident (considered to be a serious study related event because, based upon appropriate medical judgment, it may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition)

Unanticipated problems (UP): Events that are not expected given the nature of the research procedures and the subject population being studied and suggest that the research places subjects or others at a greater risk of harm or discomfort related to the research than was previously known or recognized. Harm to a subject need not occur for an event to be an unanticipated problem.

4.7.3 Reporting of Adverse Events

All AEs, SAEs, and UPs should be documented on the appropriate adverse event logs/forms, entered into the REDCap database, and reported to the DCC as outlined below.

- All SAEs and UPs should be reported by the clinical site to the DCC within 1 business day.
- All other AEs should be entered into REDCap within 7 days.

The DCC will evaluate each event and will determine reporting requirements. The DCC will report events to the DSMB according to the following timeframes:

- All SAEs and UPs that require expedited reporting (SAEs that are deemed related and unexpected, all UPs) will be reported to the DSMB within 7 business days of the time the PI learns of the event.
- All other SAEs will be reported within 30 business days of the time the PI learns of the event.
- All other AEs will be reported to the DSMB quarterly.

Additionally, the following events will also require expedited reporting:

a. Maternal death through delivery
b. Fetal loss, neonatal or infant death through 1 year of age
c. Any sedation related event requiring significant resuscitation and/or hospitalization

4.7.3.2 Periodic Summary Reporting

All other AE's (that did not require expedited reporting as above) will be summarized quarterly for the DSMB. Summary AE and SAE reports will also be submitted to the IRB at least annually.

4.7.3.3 Expected Risks

As detailed in the consent form, expected risks during the course of the study may include:

- Possible stomach upset with medications
- Restlessness during PFTs
- Pain during blood draws
- Embarrassing questions
- Sedation may include coughing, nausea, mild desaturations, obstruction of airway, apnea
- Breach of confidentiality

These risks are considered moderate and are addressed in the consent form.

The following are expected to require termination of study medication:

- Side effect presumed to be from study medication

- Termination of pregnancy
- Urolithiasis
- Serious complication of pregnancy where continuation of study medication is deemed inappropriate or impossible

4.8 Study Outcome Measures

4.8.1 Primary Outcome: Pulmonary Function Testing at Three Months

The primary outcome of this study is the measurement of infant pulmonary function tests (PFT)s, specifically, the measurement of forced expiratory flow at 75% of the expired volume (FEF_{75}) using the raised volume rapid thoracic compression RVRTC technique. Forced expiratory flow measurements are the parameters of airway function shown to be most sensitive to the effects of maternal smoking in infants and most predictive of increased risk of future pulmonary disease (14,21,22). Forced expiratory flows cannot be routinely performed in the immediate newborn period due to the instability of the newborn's lung volumes in the first weeks of life, sedation needs, and separation from the family. Thus, PFTs will be performed at three months of age to minimize cohort loss and exposure to passive smoke, while minimizing any possible side effects from the required chloral hydrate sedation (84-86). Since mothers who smoked during pregnancy are very likely to continue after pregnancy and live with other smokers, these details will be carefully recorded in the at least quarterly questionnaires, though postnatal smoke exposure has relatively little effect on forced expiratory flows. Hair nicotine (87, 88) level is a valid and reliable measure of longer term tobacco exposure and an infant and maternal hair sample will be collected at the 3 and 12 month pulmonary function test to document postnatal exposure.

All pulmonary function tests will be performed in the controlled infant pulmonary function testing laboratory located at OHSU/Doernbecher Children's Hospital (PFT laboratory in existence since 2000), the James Whitcomb Riley Hospital for Children (PFT laboratory in existence since 1983), or the PFT laboratory at Peace Health/ Southwest Medical Center (existence since 2005). Patients in the Pacific Northwest will be given the option of having testing done at OHSU or Peace Health/ Southwest to offer greater scheduling options. These laboratories are the few across the country equipped to perform this testing in infants and have a history of successful studies in the area of infant pulmonary function testing. All labs are staffed with skilled technicians who will be performing the pulmonary function tests under physician supervision (Diane Schilling, RRT ,Christina Jo Tiller, RRT, Keith Jackson, RRT) and skilled personnel, nurses or physicians performing sedation. Since growth/length is an important determinant of the forced expiratory flows (FEF)s, the pulmonary function test will be performed as close as possible to 12 weeks of corrected age. However, since testing cannot be done within 3 weeks of a respiratory illness, we will allow the upper limit of testing to be 18 weeks of age to minimize cohort loss. Testing will be performed following the American Thoracic Society/ European Respiratory Society criteria for performance and acceptance (81) co-written by Dr. Tepper and measurements reported as absolute values as well as corresponding z scores (82).

4.8.2 Secondary Outcome # 1: Incidence of Wheezing through 12 months

A standardized infant respiratory questionnaire (77-79) will be administered at least quarterly by phone and at the 3 and 12 month in-person visits when the PFTs are performed. This detailed questionnaire documents the incidence and prevalence of wheezing in infants through one year of age as well as a number of other respiratory symptoms and medications of interest. This will be administered to the infant's primary caretaker. Multiple phone numbers, preferred mode of communication and day and time of communication will also be noted to maximize the successful completion of these questionnaires. Incremental time reimbursement will be given to the mother for successful completion of the study through 12 months of age.

4.8.3 Secondary Outcome # 2: Pulmonary Function Testing at 12 months

Infant pulmonary function testing at 12 ± 2 months of age (but not within 3 weeks of a respiratory illness) will be performed as outlined above in the primary outcome PFT at 3 months of age. In addition, correlation with clinical symptoms of wheezing and with PFT at 3 months of age will be performed.

4.8.4 Other Fetal and Neonatal Outcomes

1. Fetal and neonatal death
2. Gestational age at delivery
3. Incidence of prematurity < 37 weeks and < 32 weeks of gestation
4. Mode of delivery
5. Birth weight, birth length, and head circumference.
6. Small for gestational age as measured by birth weight: a) <3rd percentile and b) < 10th percentile for gestational age, assessed specifically by sex and race of the infant and expressed in appropriate z scores
7. Apgar score at 1 and 5 minutes
8. Admission to neonatal intensive care Unit (NICU) and hospital days
9. Respiratory distress syndrome (RDS): requires oxygen or respiratory support (including continuous positive airway pressure) for ≥ 6 of the first 24 hours of life and has consistent clinical features with premature lung disease
10. Intraventricular hemorrhage (IVH)
11. Retinopathy of prematurity (ROP)
12. Necrotizing enterocolitis (NEC)
13. Bronchopulmonary dysplasia (BPD)
14. Patent ductous arteriosus (PDA)
15. Sepsis defined as a positive blood, urine, or cerebrospinal fluid culture
16. Placental weight, dimensions, appearance, and histology
17. Growth as documented by research staff at 3 and 12 month PFT procedures including weight, height (length) and head circumference.
18. Incidence of Sudden Infant Death Syndrome
19. Duration of breast feeding

4.8.5 Other Maternal/Pregnancy Outcomes

1. Clinical diagnosis of preeclampsia or eclampsia
2. Placental abruption
3. Placenta previa
4. Stillbirth
5. Gestational diabetes
6. Premature rupture of the membranes (PROM) and preterm ROM
7. Clinical diagnosis of pregnancy associated Gestational hypertension: Defined as mild or severe pregnancy-associated hypertension in the absence of proteinuria or other diagnostic criteria qualifying for preeclampsia, as defined above
8. HELLP Syndrome: Defined as pregnancy-associated hypertension (mild or severe) with both of the following:
   a. Thrombocytopenia: platelet count < 100,000/ mm^3
   b. SGOT (AST) ≥ 100 U/L
9. Days of maternal hospitalization

5. Statistical Considerations

5.1 Sample Size and Power Considerations

We hypothesize that infants of pregnant smokers randomly allocated to vitamin C versus placebo will show significant increases in PFTs, specifically FEF_{75} measured at 3 months of age, using the raised volume rapid thoracic compression technique (RVRTC). Data from our pilot study indicate that as compared to placebo, vitamin C prevents some of the maternal smoking-induced changes in pulmonary function in the offspring, particularly in the time to peak tidal expiratory flow to expiratory time (T_{PTEF}:TE). This will be tested by more sensitive measures in this proposed study.

We have obtained our estimates of sample size and power using our experience in the pilot study and on measures of variability as published by Dr. Tepper, a co-investigator of proposed study. For the primary outcome, FEF_{75} , we use Tepper's reference data (82) for estimating needed sample sizes with the RVRTC technique; forced expiratory flows were measured in 155 healthy infants born to both nonsmoking and to smoking women. The estimated standard deviation from a regression model comparing infants of non-smoking to smoking women was 0.28, using the natural logarithm of FEF_{75} as the outcome. The estimated increase in FEF_{75} for infants of nonsmoking women was 17.6% greater than that for infants born to smoking women. For power calculations, we use this value along with increases of 15.0% and12.5%.

Consideration of Effect Size. Our pilot PFT data show a significant difference of 11% ($p<0.01$) in T_{PTEF}:TE and a difference in respiratory compliance (Crs) between babies born to smoking pregnant women randomized to vitamin C versus placebo. Both are clinically significant, particularly the change in T_{PTEF}:TE , for a number of reasons: 1) several studies have shown this flow ratio to be decreased in babies delivered to smoking versus nonsmoking pregnant women (12, 21, 23, 24); 2) a longitudinal study

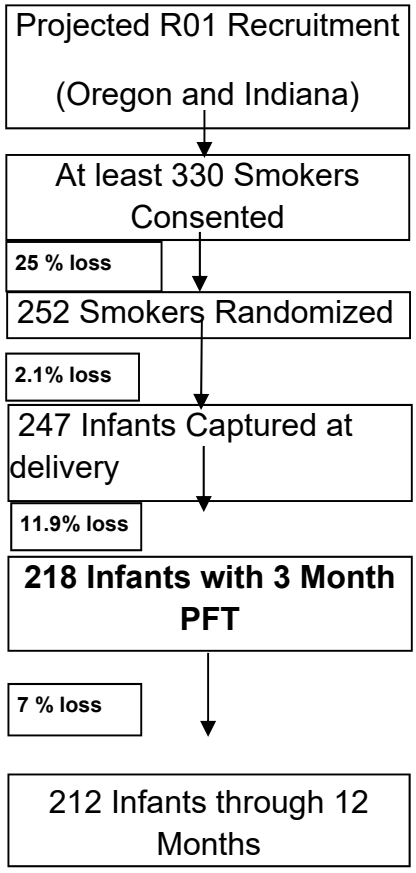
(23) has shown that a decrease of 8% in this flow ratio of T_{PTEF}:TE measured at birth was associated with an increased risk of asthma (24.3% vs 16.2%, p=0.01; OR, 1.58); or current asthma (14.6% vs 7.5%, p=0.005; OR, 2.10) at 10 years of age However, this ratio is complex because it reflects the degree to which expiratory flow and timing are modulated to slow lung emptying during expiration. It may be affected by neural alterations in the control of breathing (which could also be affected by smoking in pregnancy) as well as changes in respiratory mechanics. Based on Spindel's nicotine exposed primate data (6) which show decreased collagen deposition after vitamin C supplementation and our pilot data which demonstrate decreased wheezing, we believe that the changes we have shown in T_{PTEF}:TE are likely due to changes in respiratory mechanics/airway function. The measurement of forced expiratory flows will more precisely define the beneficial effect of the vitamin C on peripheral airway function.

Hoo reported both forced expiratory flows and T_{PTEF}:TE to be decreased in infants delivered to smoking mothers (12), but there is no data to directly translate changes in T_{PTEF}:TE into expected changes in FEF_{75}. Measurements of T_{PTEF}:TE tend to have increased variability than FEF_{75} due to patient state and spontaneous breathing. We now propose to use RVRTC, a more sensitive pulmonary function technique for measuring the peripheral airway function in infants. The measurements of FEFs with the RVRTC are more reproducible with decreased variability as the patient is sedated and the flows are measured repetitively from the same known lung volume. With this in mind, we powered our study to show a 15% difference in FEFs between randomized patients. We chose this as clinically significant since the NHLBI asthma guidelines (89) defines a positive response to a bronchodilator as an increase in FEV1 ≥ 12%.
Below are sample sizes needed for 80% and 90% power at significance level 0.05 using the estimated standard deviation from Jones et al.(82) as well as a 90% upper confidence bound for the standard deviation, 0.30 using the chi-squared distribution of the estimated SD. Calculations are based on two-sided tests.

Table 3. Sample sizes per group for FEF_{75} with 90 (80%) power using a two-sided test at level 0.05.

Mean difference in ln(FEF_{75}) between infants in the vitamin C and the placebo groups.	Ratio of FEF_{75} for infants in mothers allocated to vitamin C relative to placebo.	Sample size per group needed for 90% (80%) power using the estimated SD of 0.28.	Sample size per group for 90% (80%) power using estimated 90% upper confidence bound for SD (0.30).	Sample size per group for 90% (80%) power using estimated 90% upper confidence bound for SD: 0.30 and assuming 4% non-adherence/crossover
0.162	17.6%	64 (48)	75 (56)	82 (61)
0.1398	15.0%	86 (64)	**100** (75)	**109** (82)
0.1178	12.5%	120 (90)	140 (105)	152 (114)

Figure 5. Original Projected Study Recruitment with Projected Cohort Losses Based on Pilot Data



Conservatively, using the 90% upper confidence bound for the standard deviation, with a sample size of 100 mothers per group, we would have 90% power at level 0.05 to detect an increase of 15% in FEF_{75} for infants randomized to Vitamin C compared to those randomized to placebo and measured at 3 months of age. Adherence data from the pilot study demonstrates that only 4% of the patients were non-compliant as defined as taking < 50% of their medications. **Adjusting for this non-adherence, we increased our sample size to 109 patients per group studied at the 3 month PFT.** Figure 5 shows the original projected study recruitment to achieve desired sample size based on recruitment and cohort loss data from pilot study.

With regard to secondary outcome #1 aim 2, our data from the pilot study on 80 infants from mothers allocated to vitamin C versus placebo have suggested a difference in episodes of wheezing in their infants at 1 year of age: placebo 48%, vitamin C 26% (p=0.06). The incidence in infants of the placebo group is similar to that reported by Dezateux (22) (45%), in 101 infants of mothers who smoked in pregnancy evaluated at 1 year. The incidence in infants of non-smoking mothers was 14%. Based on the pilot study results and those in Dezateux (22), Table 3 shows the sample sizes needed to detect various hazard ratios with 80% and 90% power with a two-sided significance level of 0.05. Calculations are based on the log-rank test.

Table 4. Sample sizes per group to detect wheezing incidence with 90% (80%) power

Proportion wheeze in placebo group within first year	Proportion wheeze in vitamin C group within first year	Hazard Ratio vitamin C group relative to placebo	Sample size per group **needed at delivery** for 90% (80%) power with drop-out of 0.5% per month	Sample size per group **needed at delivery** for 90% (80%) power with drop-out of 1% per month
0.48	0.14	0.23	35 (27)	36 (28)
0.48	0.20	0.34	57(43)	59 (45)
0.48	0.26	0.46	99 (74)	101 (77)
0.45	0.14	0.25	41 (32)	42 (33)
0.45	0.20	0.37	71 (54)	73 (55)
0.45	0.26	0.50	131 (99)	135 (102)
0.40	0.14	0.30	54 (44)	58 (45)
0.40	0.20	0.44	108 (81)	111 (84)

Our targeted sample size of 113 per group at delivery (109 per group obtaining successful FEF_{75} measures at 3 months; see Figure 5 above) allows for 80%-90% power for detection of hazard ratios of 0.45-0.50 with 1% drop-out per month. (Based on data from our pilot study, we expect a total of 5% drop-out over 12 months or about 0.5% per month.)

5.2 Interim Analysis

We will use the group sequential spending function approach to set guidelines for early stopping for effectiveness based on the primary outcome FEF_{75}**.** In particular, we plan one interim and one final analysis using the O'Brien Fleming type spending function as described by Proschan et al (90) with an overall type I error rate of 0.05. Specifically we plan analyses after 102, and 218 patients complete the 3-month pulmonary function tests. However, the spending function approach allows for unforeseen changes in the number and/or timing of analyses. For example, if the DSMB requests an additional interim analysis after the first one is complete, this is accommodated by changing the nominal significance levels for the second analysis and for the final analysis. The overall type I error rate will be maintained. The O'Brien Fleming-type spending function is conservative in early analyses, requiring strong

evidence for early stopping, but the critical value for the final analysis is close to what it would have been without any interim analyses. The above are guidelines only for the DSMB's consideration.

5.3 Analysis Plan (For additional detail for section 5.3 please see separate document "Statistical Analysis Plan"(SAP))

Aim 1/Primary Outcome: Comparison of pulmonary function tests between infants delivered to smoking mothers randomized to vitamin C versus placebo.

5.3.1 Analysis of Primary Outcome (forced expiratory flow at 75% of expired volume, FEF_{75})

The primary analysis of the pulmonary function outcome (FEF_{75}) will compare the FEF_{75} of infants born to mothers randomized to Vitamin C versus placebo, using mixed model analysis of covariance (ANCOVA) regression analysis adjusting for the infant's sex, maternal race as a binary variable i.e. white/non-white), age and length at the time of FEF_{75} measurement, and the stratification variables (site and gestational age at randomization i.e. ≤18 weeks versus > 18 weeks). Although the infant's length is not a baseline variable, forced expiratory flows are highly correlated with body length and are a standard component in the interpretation of FEF_{75} and similar measures (81, 82). Since FEF_{75} is not normally distributed, we will use for the primary analysis the natural logarithmic transformation of FEF_{75} to reduce expected skewness (91). Assessment of outliers and influential points will be conducted and reported.

5.3.2 Supplementary Analyses

5.3.2.1 **Missing Data** – For Further Detail see SAP, section 7

The primary analysis described above provides valid inference if the missing response data is missing at random (MAR). Given that there is one measurement of the primary outcome at 3 months, there is no opportunity to use prior measures. However, in the case that the data are not missing at random (NMAR), then the proposed analyses can be biased. By missing data, we refer to the primary outcome of FEF_{75} as we expect to have virtually complete data on the covariates for the primary model (maternal race as a binary variable i.e. white/non-white), infant age, length, sex, site, and gestational age at randomization).

We will undertake several analyses to assess the impact of the missing response data on our conclusions. See SAP section 7 for detailed plan for handling missing data.

5.3.2.2 Pre-defined Subgroup Analyses for Comparison of FEF_{75}

Additional comparisons of FEF_{75} between treatment groups will be conducted for defined sub-groups with significance values adjusted using the Holm multiple comparison procedure (92).

1. By medication adherence
2. Infants by gestational age at birth <37 weeks versus ≥ 37 weeks;
3. Infants with and without maternal family history of asthma
4. Infants of mothers that had low versus high urine cotinine levels. We will use the median cotinine level for smokers as the cutpoint for the low and high cotinine level categories
5. Infants of mothers by genotype of alpha 5 nicotinic receptor (ASP/ASP, ASP/ASN, ASN/ASN)
6. Infants of mothers with a pregravid BMI ≤ 35 versus > 35
7. By infant gender
8. By infant gestational age at randomization

5.3.2.3 Additional Pulmonary Function Tests- See SAP for further detail

Although the primary endpoint is FEF_{75} , similar analyses will be repeated for other forced expiratory flow measures: FEF_{25-75}, FEF_{50},. Forced expiratory volumes will also be evaluated including forced vital capacity (FVC), forced expired volume in the initial 0.4 sec (FEV0.4) and 0.5 sec (FEV0.5) and the ratio of FEV0.5/FVC.

5.3.2.4 Futility Analyses

The study does not include plans for a formal futility analysis, although the DSMB can recommend early termination of the study at any time. We will continue to collect data through one year after delivery both to obtain 12 month pulmonary function tests and to assess clinical outcomes to estimate the clinical benefit of therapy. This will be critical information regardless of whether there is a difference in pulmonary function tests at three months post-delivery. Although the pilot data support a change in pulmonary function in the group randomized to vitamin C treatment, it is conceivable that the benefit of vitamin C supplementation may be through a different mechanism other than pulmonary function, such as immunologic or neurologic (78, 93) this would warrant continued follow-up of patients to determine clinical outcome at 1 year. Additionally, at the conclusion of the study, confidence intervals can be computed for all outcome measures within each group and for the comparisons between groups. Allowing for completion of the study in the presence of insignificant differences in primary pulmonary function outcomes at interim analyses will yield greater precision for estimation of pulmonary function and clinical outcomes.

5.3.2.5 Primary Analysis of Wheezing

To compare the incidence of wheezing between the infants of mothers allocated to vitamin C and placebo, we will utilize data obtained from a standardized respiratory questionnaire (78) using self-report from parents asked about new episodes of wheezing asked at least quarterly.. If an infant has wheezing associated with an illness

lasting several days, this will be counted as one episode. The incidence of wheeze will be compared between the two groups using a generalized linear mixed model ANCOVA. The randomization variables and covariates will also be included in the analysis.For further detail see separate Statistical Analysis Plan.

5.3.2.6 Additional Clinical Outcomes

We will report the incident bronchodilator use and healthcare provider diagnosis of wheeze between the two groups.

5.3.2.7 Infant Pulmonary Function Testing at 12 Months of Age

This additional PFT at 12 months will allow us to track the effect on PFTs of the vitamin C versus placebo allocation over time, to associate these PFTs with the incidence of wheezing, and to potentially differentiate the impact of in-utero versus postnatal smoke on infant PFTs. For further detail on this analysis see separate SAP.

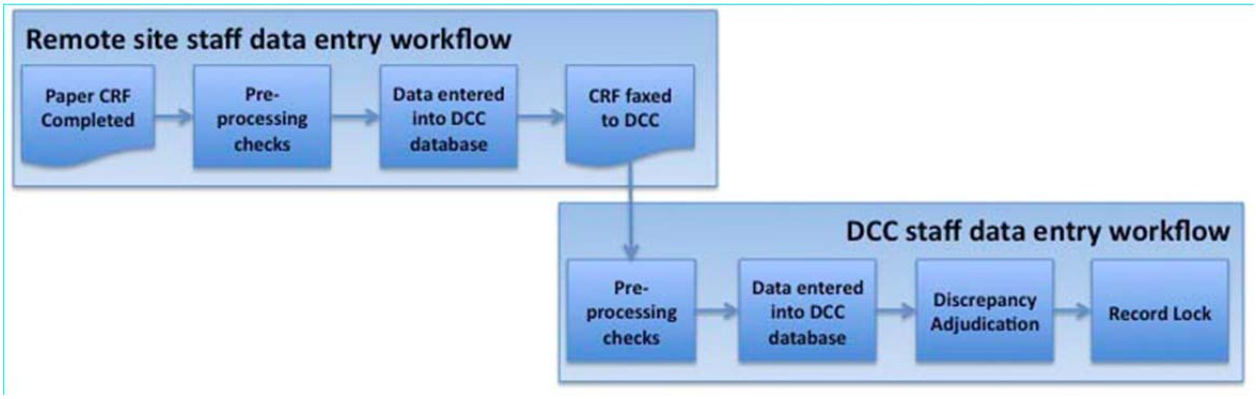
6. Data Collection

6.1 Data Collection Forms

Data will be collected on standardized forms on which nearly all responses have been precoded.

6.2 Data Entry

The data collection protocol for the VCSIP study will utilize paper case report forms and independent double data entry into a REDCap database. The data entry workflow and validation scheme are illustrated in the following Figure 6:



The Data Coordinating Center will create all paper and electronic case report forms and the manual of operations. Once the paper forms have been tested and validated, they will be distributed to study sites. OHSU and Indiana site personnel will complete the paper-based case report forms, perform pre-processing checks for completeness and consistency of key data fields, and independently enter data into the study database via secure web-based forms. The paper forms will be faxed or mailed to the DCC where the data will be entered a second time and adjudicated against the original record entered.

DCC staff will reconcile any discrepancies in coordination with clinical site staff and record the disposition of any discrepancies found.

6.3 Data Management

Data will be collected within each study site by a combination of study-specific forms that capture clinical information not routinely recorded in patient charts, by abstraction of standard clinical information and outcomes from the records of patients, and by patient self-reports. The case report forms will also serve as source documentation. We will monitor for timely entry of data and faxing of case report forms by maintaining a study calendar using the database's study and participant calendar feature and reviewing expected visits, forms, and specimens on a weekly basis.

The discrepancy management process includes a variety of important data quality tasks. These include running production edit checks, reviewing data discrepancies produced by edit checks, generating queries, resolving discrepancies, and updating any relevant data in the database. In addition to the computerized checks, the data management staff will conduct manual reviews of the data throughout the study and these may generate additional discrepancies. All discrepancies will be reviewed by the DCC staff who then either resolve the discrepancies or forward queries to each site for clarification or resolution on data clarification forms. The sites will return the data clarification forms after resolution. The data team will then modify the data with a full audit trail generated for each modification.

6.4 Performance Monitoring

The DCC will present regular reports to the VCSIP Steering Committee and the Data and Safety Monitoring Board. These include:

- Monthly recruitment reports: reports of the number of women screened and enrolled by month and by clinical center will be provided weekly and provided monthly to the VCSIP Steering Committee.
- Quarterly Steering Committee reports: a report detailing recruitment, baseline patient characteristics, data quality, incidence of missing data and adherence to study protocol by clinical center, will be provided quarterly to the VCSIP Steerring Committee.
- Data and Safety Monitoring Board reports: for every meeting of the DSMB, a report is prepared which includes adverse events, patient recruitment, retention, drug adherence data, baseline patient characteristics, center performance information with respect to data quality, timeliness of data submission and protocol adherence (in addition to safety and efficacy data). Data will be reported by site. In addition, a data report will be sent to the VITEL DSMB after 30-50 participants have been randomized into the study with key demographic information included to evaluate for significant confounders by site.

7. Study Administration

7.1 Organization and Funding

The study is funded jointly by the National Institute of Health's Heart, Lung and Blood Institute (NHLBI) and the Office of Dietary Supplements. The study is conducted at two clinical centers (Portland, Oregon and Indianapolis, Indiana) and the Data Coordinating Center at OHSU.

7.1.1 Participating Clinical Centers

The Principal Investigators of the clinical centers have agreed to abide by the study protocol, to have comparable staff, facilities and equipment and to ensure the proper conduct of the study at each of their centers including: recruitment and treatment of patients as specified in the protocol, accurate data collection and the transmission of information to the Steering Committee.

7.1.2 Data Coordinating Center

The DCC is responsible for all aspects of biostatistical design, data management, interim and final statistical analyses. The Principal Investigator of the DCC reports to the Steering Committee and the Data and Safety Monitoring Committee.

7.2 Committees

7.2.1 Steering Committee

The VCSIP Steering Committee will be co-chaired by Drs McEvoy and Morris to further preserve DCC independence. Drs. McEvoy and Tepper will contact Dr Morris in the event there are any concerns regarding data analysis or data management. The Steering committee will consist of Drs. McEvoy, Morris, Tepper, Spindel, Peters and Robert Schuff.

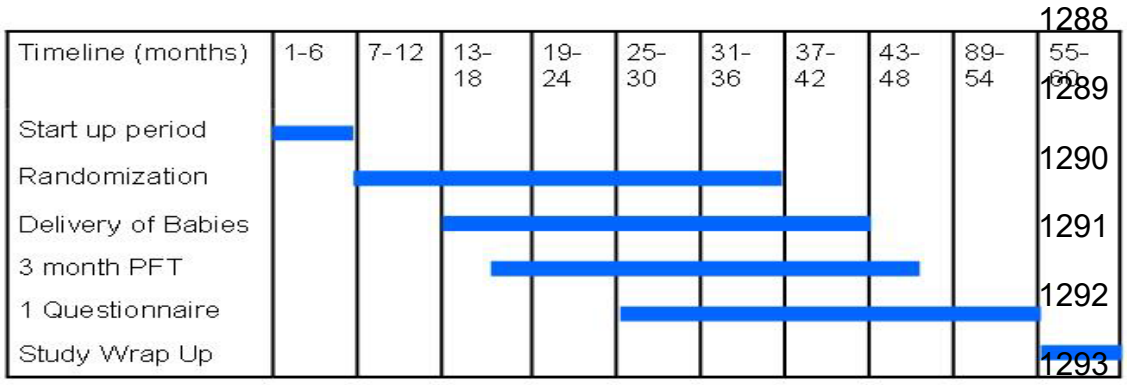
7.2.2 Data and Safety Monitoring Board

The DSMB will be appointed by the NHLBI and will be a group of individuals not affiliated with any of the institutions. Before the trial can begin, the protocol must be approved by the committee. During the conduct of the study, the committee is charged with monitoring the emerging results for efficacy and safety, in addition to center performance and protocol adherence. Recommendations by the committee can include protocol modification, early termination for efficacy, or for unexpected safety problems.

Recommendations are made to the NHLBI and disseminated to the Steering Committee.

8. Study Timeline

Figure 7. Study Timeline



8.1 Training and Certification

Prior to the beginning of the study, research coordinators and study staff will undergo training on all aspects of data collection and procedures at either OHSU in Portland, OR or UI in Indianapolis. Both participating centers must be certified in all study procedures before patient recruitment can begin.

8.2 Recruitment and Data Collection Period

The proposed grant period is for 5 years. There were 9,660 deliveries last year from the practices this study will recruit from which offers a robust patient population (See Table below). The rate of smoking during pregnancy varies from 12 to 35% in these practices. This population will allow us to meet our desired sample size of at least 330 patients consented over 30 months (see Figure 5 for projected recruitment). The study will have a 6 month start-up period. Patient recruitment and follow-up will occur over 30 months (month 7 through 37). The delivery of the infants of women recruited and randomized are projected to occur in months 12 to 42 with 3-month PFTs performed from month 15 through 45. Serial questionnaires culminating in the one year questionnaire will be completed in months 24 through 54 with the final 6 months to complete final data analysis.

Table 5. Potential Recruitments for Study

Hospital	Deliveries/ year	Smokers (%)	Smokers available/year	Smokers available/month	Needed#/month for sample size
OHSU	2362	12	283	23	2-3
Southwest	3500	12	420	35	2-3
Wishard	2500-3000	35	875-1050	73 - 88	4-5
IU Hospital	800	35	280	23	1-2

8.3 Final Analysis

After a two month period for completion of data entry for the trial, the data set will be locked and available for analysis. Approximately six months will be required to complete the final report to the VCSIP Steering Committee and to submit the study's primary report for publication.

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APPENDIX A: Study Design

Vitamin C to Decrease the Effects of Smoking in Pregnancy on Infant Lung Function. A Randomized Trial			
OBJECTIVE: To determine the effect of vitamin C supplementation initiated ≤ 22 weeks gestation on pulmonary function in infants born to smoking women.			
Clinical Centers:	Oregon Health & Science University and Indiana University School of Medicine		
DESIGN:	Double-masked randomized clinical trial	**SCHEDULED STUDY VISITS AND DATA COLLECTION, CONTINUED**	
Major Eligibility Criteria:	Gestational age $13^{0/7}$ – $22^{6/7}$ Current cigarette smoker Singleton gestation Informed consent	Delivery	Cord blood Placenta Hair Smoking questionnaires Chart review for diagnostic criteria Review of contact information
Groups:	Experimental: 500 mg vitamin C Control: Placebo	Post-Delivery	PFT at 48 hours (Pacific Northwest only), 3 months and 12 months; respiratory questionnaire at least quarterly
Random Allocation	Permuted block design	**OUTCOME MEASURES**	
Stratification	Clinical center and gestational age (≤ 18 weeks ; >18 weeks)	Primary Outcome Secondary outcomes	PFT at 3 months PFT at 12 months; Incidence and prevalence of wheezing through 12 months
Sample Size	252	**TIMELINE**	
Statistical Assumptions	90% Power to show 15% difference between groups	Enrollment	Dec 2012 - June 2015 (months 7 – 37) *Average* recruitment should be 5 patients per institution per month after ramp-up
Interim analysis	Group sequential spending method	Delivery	May 2013 - Nov 2015 (months 12-42)
SCHEDULED STUDY VISITS AND DATA COLLECTION		PFTs @ 3 months	Aug 2013 – Feb 2016 (months 15–45)
Pre-randomization	Health & Med History	PFTs @ 12 months	May 2014 – Nov 2016 (months 25-54)
	Dating ultrasound required	Closeout/Final Analysis	Dec 2016 – May 2017 (months 55-60)
	Demographics/Contact info		
	Smoking questionnaire		
	Distribute compliance meds		
	Urine, blood and hair samples		
	Exhaled CO		
	Conversant consent		
Post-randomization	Evaluate q 4 weeks for compliance, side effects and AEs; smoking questionnaire; collect maternal hair sample at visit prior to delivery; blood, urine and exhaled CO at 26 and 32 wks; monitor weight and BP from chart; genetics consent prior to delivery		

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APPENDIX B: Study Visit Flow Sheet

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STUDY VISITS									
	V1	**V2**	**V3**	**V4**	**V5**	**V6**	**V7 (delivery)**	**V8**	**V9**
Weeks gestation (estimated)	12≤ 21	13≤22	17≤26	21≤30	25≤34	29≤38	39≤42	Month 3	Month 12
Screening	X								
Health/Med History	X								
Demographic/Contact Info	X								
Consent/HIPPA	X								
Height, weight, BP from chart	X	X	X	X	X	X	X		
Smoking questionnaire	X	X	X	X	X	X	X		
Dispense compliance meds	X								
Ultrasound confirmed	X	X							
Schedule FU appointment	X	X	X	X	X	X	X	X	
Between visit phone call	X	X	X	X	X	X	X	X	X
Assess run-in compliance		X							
RANDOMIZATION		X							
Dispense study meds		X			X				
Fasting blood draw Vit C		X		X		X			
Urine for cotinine, isoprostanes		X		X		X			
Additional blood/urine aliquots		X		X		X			
Exhaled Carbon Monoxide*		X		X		X		X	X
Hair nicotine (mom)*		X					X	X	X
Health/Med hx change		X	X	X	X	X	X		
Contact Info change		X	X	X	X	X	X	X	
Pill counts			X	X	X	X	X		
Consent for genetics testing (on or before this date)						X			
Cord blood sample							X		
Placenta collection and processing samples							X		
Pulmonary function tests								X	X
Hair nicotine (baby)*							X	X	X
Buccal swab from mom and baby							x	x	x
Respiratory Questionnaire (at least quarterly beginning 1 month post partum)								X	X

Ages & Stages Questionnaire									X
Patient reimbursement		X		X		X		X	X

APPENDIX C: Sample Consent Forms

Oregon Health & Science University
Consent Form

IRB#: 6091

Protocol Approval Date: 4/10/2012

OREGON HEALTH & SCIENCE UNIVERSITY

Consent Form

Title: Vitamin C to Decrease the Effects of Smoking in Pregnancy on Infant Lung Function: A Randomized Trial

Principal Investigator: Cynthia McEvoy, M.D. (503) 494-8122

Sponsor: National Institutes of Health

PURPOSE:

You have been invited to be in this research study because you are pregnant and are a smoker. The purpose of this study is to see how extra vitamin C affects a newborn infant's lungs in moms who smoked during pregnancy. We know that smoking during pregnancy can affect how a baby's lungs are formed. We have early data that extra vitamin C may improve the lung function tests in these babies, but we need to test this further. If you agree to join and do not withdraw later, you will be in this study for about 2 years. Your baby will be in the study from birth to one year old.

This study includes a sub-study that collects blood for genetics research and future research. You will be asked to sign a separate form for the sub-study.

This study group will include 330 pregnant women and we will continue to follow their babies through 1 year of age. Women from the Portland, Oregon area and from Indianapolis, Indiana can join the study. We expect about half of the subjects to be recruited in the Pacific Northwest.

PROCEDURES:

Before Delivery (Prenatal)

Your participation in this study will be in addition to your routine prenatal clinic visits. As part of the study you will be asked to do the following:

Enrollment

During the first visit, we will ask you questions about how much you smoke, smoking history, your lifestyle, and your medical history. This will take about 20 minutes. If you have not yet had an ultrasound, an ultrasound to tell your baby's due date will be ordered. For the first part of the study (1 to 3 weeks), you will be given capsules to take that are placebos. A placebo is a capsule that tastes, looks, and smells like the study capsule but has no real medicine in it. You will be asked to take one capsule per day and then return to clinic in 1-3 weeks. The purpose of this period is to determine if you can take this capsule regularly.

We will give you prenatal vitamins throughout your pregnancy. We will ask that you take no other vitamin or other vitamin supplements, including extra vitamin C, other than those we give you or until you have talked about it with us. No changes in what you eat are necessary.

Assignment to Study Group

If you have been able to take your placebo capsules when you return at your second study visit, you will be assigned by chance to get a vitamin C supplement or placebo for the rest of your pregnancy. You will be asked to take one capsule each day with the prenatal vitamin. One capsule of vitamin C contains 500 milligrams of vitamin C.

Assignment to the study groups is done by chance (like the flip of a coin). You have an equal chance of getting capsules that contain vitamin C or capsules that do not. Neither you nor the clinic staff will know which capsules you are taking. The study is done this way because sometimes knowing that you are getting the capsule with vitamin C can change the results of the study. Also, sometimes people get side effects from placebos. Please ask the investigator if you have any questions at all about this kind of study.

Further Clinic Visits During Pregnancy

You will meet with the study staff at each visit. You may also get reminder calls, texts, or emails prior to the visits. We will do your study visit with your prenatal clinic visit whenever possible. The following will occur at study visits:

- We will ask that you bring left over study capsules in the original bottle. As needed, you will get extra study capsules to take.

- The study staff will review your smoking history at each visit and answer any questions or concerns you may have about the study. This will take about 10 minutes.
- We will get samples of your urine for this study. This will be tested for a substance that may be related to smoking. We will also have you do an exhaled carbon monoxide test which is a simple breathing test where you blow into a tube and it measures the level of carbon monoxide in your lungs. Carbon monoxide is a gas that can build up in the body of smokers.
- Near the start of the study, a blood sample (1-2 teaspoons) will be taken. This is taken at the same time you have blood work done as part of your routine care. One to two other blood samples (1-2 teaspoons each) will be done for the study during your pregnancy. Again, if at all possible, this will be done at the same time as other blood work that is part of your prenatal care. These blood samples will be tested for vitamin C levels so we will ask you not to have anything to eat for 6 hours before the blood is taken since this can affect the vitamin C level. A small sample of hair will be collected from you at the beginning of the study and near delivery., We will also collect these samples from you when your baby is 3 and 12 months of age and we will also have you do an exhaled carbon monoxide test at these times. The strands of hair will be cut, not pulled, from as close to the skin on the back of your head as possible. They will be used to test for the presence of nicotine use.

As often as possible during your regular ultrasound, several extra pictures will be done to do sensitive measures of blood flow in your umbilical artery and placenta. This will be done at OHSU and PHSW and will be done near the start of the study (it may be done twice if you join the study early in your pregnancy) and done again when you are closer to delivery. During the last ultrasound you will be asked to breathe 60% oxygen through a face mask for 5-10 minutes to see the effect of extra oxygen on the blood flow to your baby's lungs. This has not caused problems when done in other studies. You will not be charged for any extra ultrasound pictures done for this study.

Delivery

When you are in the hospital to deliver your baby it is usual care that you will not be allowed to eat food but may be allowed to drink fluids. If you would like to drink juices, we will offer an organic brand that does not have a lot of vitamin C in it (either naturally or added) since this may affect the levels of vitamin C that will be measured in the umbilical cord blood when your baby is born. The following things will be done for the study at delivery:

- Umbilical cord blood is usually collected for clinical care. When possible, about 1 teaspoon of this blood will be collected to measure levels of nicotine, cotinine (a substance that nicotine is changed into by the body), vitamin C and other substances such as proteins or other vitamins that may be affected by smoking and extra vitamin C.
- Several small pieces of your placenta will be tested for substances showing vitamin C use/breakdown and your placenta will be weighed, measured, and looked at for signs of infection or decreased blood flow.

After Delivery Until Baby is One Year Old

- At 3 and 12 months of age, a test of how your baby's lungs work will be done. At OHSU and PHSW, a test of how your baby's lungs work will also be done at about 48 hours of age. If this first test cannot be done before you and your baby go home, we will do it within the first month of age. The breathing tests will measure your baby's breathing and how his/her lungs work by using a mask that your baby will breathe through.
- During the breathing tests brief closures of the airway (lasting a fraction of a second) will be done. A vest will also be wrapped around your baby's chest for part of the test done at 3 and 12 months. During this part of the test, the front of the vest will be inflated and help your baby blow air out quickly. We measure the flow of air that your baby breathes out. For part of the test we will also give your baby several breaths before the vest inflation. Each lung function test takes about 15-20 minutes. We will tell you whether the test was within normal limits and send these results to your baby's doctor.
- We will not use any medication to make your baby sleepy for the lung function tests that are done while your baby is in the hospital after delivery. For the 3 and 12 month test, your baby will not be able to eat or drink for four hours before the test because we will need to give a syrup to make him/her sleepy. You will be asked to sign an extra form allowing us to give the syrup that will make your infant sleepy for about one hour. At the 3 and 12 month test we will also ask whether your baby has had any breathing problems since the last visit, whether they are on any medications, and if anyone living in the home smokes. These questions will take about 10 minutes.
- When possible (near delivery and at 3 and 12 months of age), we will get a small sample of hair from the back of your baby's head for testing for the study.
- We will contact you monthly by phone, email, text or in person until your baby is 12 months of age and ask how your baby's breathing has been. This will take about 10 minutes.

Your agreement to be in the study gives your permission for the research staff to look at your medical records for any health care you get during your pregnancy and delivery, and the medical records of your baby at delivery and through 12 months of age.

Please see the tables below, which go over your participation in the study.

Maternal Data Collection Schedule

	Screening	Consent, trial of placebo	Randomization	Monthly Visits thru Delivery	Delivery
Estimated pregnancy week ranges	**11 – 20 wks**	**11 – 20 wks**	**13 – 22 wks**	**17 – 40 wks**	**40 wks**
Medical history	**X**				
Monthly pill count and refill		**X**	**X**	**X**	

Watch for negative effects			**X**	**X**	**X**
Changes in health	**X**	**X**	**X**	**X**	**X**
Smoking Questionnaire	**X**	**X**	**X**	**X**	**X**
Urine sample *			**X**	**X**	**X**
Hair sample			**X**		**X**
Ultrasound for blood flow**				**X**	
Blood samples *			**X**	**X**	**X**
Amniotic fluid, Cord blood, placenta collection					**X**

*Two levels obtained after randomization, evenly spaced as possible; ** may be done twice during pregnancy at OHSU and PHS

Infant Data Collection Schedule

	Birth	**Month 1and 2**	**Month 3**	**Month 4-11**	**Month 12**
Weight and length	**X**		**X**		**x**
Breathing Test *	**X**		**X**		**x**
Monthly breathing questions		**X**	**X**	**X**	**x**
Hair sample	**X**		**X**		**x**

*** A syrup to make your baby sleepy will be given at the 3 and 12 month breathing test**

If you agree to be in this study, to this study, there will be about 11 visits over about 18 months. Most of these visits will be done with your routine prenatal care. These visits also include the breathing test your baby will have at 3 and 12 months of age.

We may contact you after your child is one year of age to continue to ask questions about their breathing.

If you have any questions about this study now or in the future, contact Cynthia McEvoy, M.D. (503) 494-8122.

RISKS AND DISCOMFORTS:

Vitamin C Dose: There are no documented risks of vitamin C supplementation in pregnant women with doses of up to 1000 mg per day given during pregnancy. The vitamin C dose in this study is 500 mg/day which is about half of the dose that has been used in other studies in pregnancy. However, you may experience the discomfort of an upset stomach.

Lung Function Tests: There are no known specific risks of the lung function testing. Your baby may become restless during the lung function testing. If this happens your baby will be checked by Dr. McEvoy or your baby's doctor and the test will be stopped if needed.

Blood Draws: You may feel some pain when your blood is drawn. However when possible, all blood draws for the study will be done at the same time that routine blood is drawn for your normal prenatal care. There is a small chance the needle will cause bleeding, a bruise, or an infection.

Hair Samples Collection: There is no risk to you or your baby during the collection of hair strands or nail clippings. The hairs will be cut, not pulled, and should cause no discomfort.

Questionnaires: Some of the questions asked in the study may seem very personal or embarrassing. You may refuse to answer any of the questions that you do not wish to answer.

Sedation: *For the pulmonary function tests done at about 3 and 12 months of age, OHSU's rules for helping an infant go to sleep will be followed. Chloral hydrate will be given by mouth. A nurse or physician will give the medication and be in the room watching your child closely while they are sleeping and until they are awake. We will watch how much oxygen is in his/her blood while he/she is sleeping and when he/she wakes up. Your infant may become fussy before going to sleep or after waking up. He / She may having some coughing with the medication, feel sick to their stomach, have small decreases in the oxygen in their blood, have difficulty breathing and feel light headed. You will be given a sheet that lists the things to watch out for after the test and when you take him/her home.*

There may be other unexpected risks related to this study.

BENEFITS:

You and your baby may or may not personally benefit from being in this study. However, by serving as a subject, you may help us learn how to benefit patients in the future.

ALTERNATIVES:

You may choose not to be in this study. You may also talk to your doctor about vitamin C and you may also stop smoking.

CONFIDENTIALITY:

We will not use your name or your identity for publication or publicity purposes.

Blood specimens or hair samples sent to laboratories outside of OHSU will be coded with a unique identifier. The investigators at OHSU will be the only people with access to the code. No other identifying information will be provided to those laboratories.

Your research record may be reviewed and copied by all investigators listed on page one of this consent form, others at OHSU who are participating in the conduct of this research protocol, the sponsor (NIH), Oregon Clinical and Translational Research Institute (OCTRI), the National Center for Research Resources, the OHSU Institutional Review Board, and the Office for Human Research Protections.

Under Oregon Law, suspected child or elder abuse must be reported to appropriate authorities.

COSTS:

The costs for newborn care that you would have even if you were not in this study will be billed to you or your insurance company. You will be billed for any of these costs that your insurance does not cover.

The costs of the study will be covered by the research team or a sponsor of the study. This includes, for example, the study capsule, extra blood draws, and extra pictures taking during ultrasounds specifically for the study and any other tests or procedures that are done only for the study. You will be reimbursed up to $25 for each study visit that involves blood draws or extra time for the study and up to $100 for travel mileage, childcare, and inconvenience for pulmonary function tests that require travel to the hospital for the study. You will be reimbursed up to $120 for completion of all 12 monthly breathing questions on your infants. You may receive up to $450 for the study as a whole for travel/time expenses depending on distance traveled. The study coordinator will answer any questions you may have about these reimbursements.

LIABILITY:

If you believe you have been injured or harmed while participating in this research and require immediate treatment, contact Cynthia McEvoy, M.D. (503) 494-8122.

You have not waived your legal rights by signing this form. If you are harmed by the study procedures, you will be treated. Oregon Health & Science University does not offer to pay for the cost of the treatment. Any claim you make against Oregon Health & Science University may be limited by the Oregon Tort Claims Act (ORS 30.260 through 30.300). If you have questions on this subject, please call the OHSU Research Integrity Office at (503) 494-7887.

It is not the policy of the federal funding agencies to compensate or provide medical treatment for human subjects in federally funded studies.

PARTICIPATION:

Dr. McEvoy at 503- 494-8122 has offered to answer any other questions you may have about this study. If you have any questions regarding your rights as a research subject, you may contact the OHSU Research Integrity Office at (503) 494-7887.

You do not have to join this or any research study. If you do join, and later you change your mind, you may quit at any time. If you join and later quit smoking, you still may participate in the study. If you refuse to join or withdraw early from the study, there will be no penalty or loss of any benefits to which you are otherwise entitled. Your baby's doctors may withdraw your baby from the study at any time if they believe it is in your baby's best interest.

If in the future you decide you no longer want to participate in this research, we will either destroy your samples or we will remove your name and any other identifiers from your samples (making them anonymous), and we continue to use if for research.

Your consent to participate in this study is voluntary. You may refuse to sign this consent form. If you refuse to sign this consent form, your baby's health care and relationship with OHSU will not be affected.

Your health care provider may be one of the investigators of this research study, and as an investigator is interested in both your baby's clinical welfare and in the conduct of this study. Before entering this study or at any time during the research, you may ask for a second opinion about your care from another doctor who is in no way involved with this project. This may involve extra cost that you will have to pay. You do not have to be in any research study offered by your physician.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. law. This web site will not include information that can identify you. At most, the web site will include a summary of the results. You can search this web site at any time.

SIGNATURES:

Your signature below indicates that you have read the above and agree to participate in this study. We will give you a copy of this consent form.

OREGON HEALTH & SCIENCE UNIVERSITY

INSTITUTIONAL REVIEW BOARD

PHONE NUMBER (503) 494-7887

CONSENT/AUTHORIZATION FORM APPROVAL DATE

July 11, 2012

Do not sign this form after the

Expiration date of: 04-09-2013

_____ _____

Signature of Mother Date

_____ _____

Signature and Printed Name Date

Of Person Obtaining Consent

COPIES FOR: SUBJECT CHART

INVESTIGATOR

INFORMED CONSENT – MEDICAL STUDIES

Do not write below this line – for office use only

Stamper Plate:

IRB#: 6091

Protocol Approval Date:
04/10/2012

OREGON HEALTH & SCIENCE UNIVERSITY

Consent Form – Genetic Sub-study

Title: Vitamin C to Decrease the Effects of Smoking in Pregnancy on Infant Lung Function: A Randomized Trial

Principal Investigator: Cynthia McEvoy, M.D. (503) 494-8122

Sponsor: National Institutes of Health

PURPOSE:

You and your baby are participating in the above study and are invited to participate in this sub-study that will look at how genes are involved in the different ways nicotine affects different people. Genes are units of DNA – the chemical structure carrying your genetic information—that determine many human characteristics such as the color of your eyes, your height, and whether you are male or female.

This study may help explain why some people smoke, why some people never smoke, and why some people who start smoking have great difficulty stopping. Looking at these genes may also help us tell which children born to smokers are more likely to get asthma. The blood samples provided will be analyzed to see if there are differences in the genes of people who smoke versus those who don't.

If you agree, your blood, hair, urine, and placenta samples and your baby's blood, hair, and urine will also be stored indefinitely to be used for future research that might also include other genetics research.

PROCEDURES:

About 1 teaspoon of your blood will be collected, as often as possible, at the same time as a routine blood draw at one of your routine prenatal visits. About 1 teaspoon of blood will be taken at the time of delivery from the umbilical cord blood sample which is already being collected as part of the main study and small pieces of the placenta will be taken which is already collected as part of the main study. If we are unable to collect umbilical cord blood, we may ask to collect up to 1 teaspoon of blood from your infant when possible with another blood draw.

SUBJECT ACCESS TO GENETIC INFORMATION:

The results of these studies will not be made available to you because the research is still in an early phase and the meaning of the results is unknown.

RISKS AND DISCOMFORTS:

This research involves genetic research. A Federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information. Be aware that this new Federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

Although we have made every effort to protect your identity, there is a small risk of loss of confidentiality. If the results of these studies of your genetic makeup were to be accidentally released, it might be possible that the information we will gather about you as part of this study could become available to an insurer or an employer, or a relative, or someone else outside the study. Even though there are discrimination protections in both Oregon law and Federal law, there is still a small chance that you could be harmed if a release occurred.

Whenever possible, blood will be collected at the time of other blood draws so there will be no additional pain.

BENEFITS:

You and your baby will not personally benefit from being in this study. However, by serving as a subject, you may help us learn which children are most likely to smoke or get breathing problems so that programs can be developed to help these children from getting these problems.

ALTERNATIVES:

You may choose not to be in this study.

CONFIDENTIALITY AND PRIVACY OF YOUR PROTECTED HEALTH INFORMATION:

A code number will be assigned to you and your genetic information, as well as to information about you and all personal identifiers will be removed from blood samples. Only the investigators named on this consent form will be authorized to link the code number to you. Other investigators who may receive samples of your blood for research will be given only the code number which will not identify you.

All other parties including employers, insurance companies, personal physicians, and relative will be refused access to the information or to the samples, unless you provide written permission, or unless we are required by law to do so.

We will not use your name or your identity for publication or publicity purposes.

Your research record may be reviewed and copied by all investigators listed on page one of this form, others at OHSU who are participating in the conduct of this research protocol, the OHSU

Institutional Review Board, the Oregon Clinical and Translational Research Institute (OCTRI), the National Center for Research Resources (NCRR), and the Office for Human Research Protections (OHRP) which is a part of the US Department of Health and Human Services.

Under Oregon Law, suspected child or elder abuse must be reported to appropriate authorities.

COSTS:

There is no cost to you or your baby for participating in this research.

COMMERCIAL DEVELOPMENT:

By consenting to participate, you authorize the use of your samples for the research described in the PURPOSE and PROCEDURES sections of this document. In addition, you acknowledge that OHSU may make any lawful use of your samples, including, but not limited to, future research studies, destroying them, or transferring them to a public or private entity.

Samples obtained from you in this research may be used to make a discovery that could be patented of licensed to a company. There are no plans to provide financial compensation to you should this occur. However, should OHSU ever provide your samples to anyone else for research or commercial use, it will do so in such a way to protect your privacy and confidentiality as stated in the CONFIDENTIALITY section of this document. Further, you will have no responsibility or liability for any use that may be made of your samples.

LIABILITY:

If you believe you have been injured or harmed while participating in this research and require immediate treatment, contact Cynthia McEvoy, M.D. (503) 494-8122.

You have not waived your legal rights by signing this form. If you are harmed by the study procedures, you will be treated. Oregon Health & Science University does not offer to pay for the cost of the treatment. Any claim you make against Oregon Health & Science University may be limited by the Oregon Tort Claims Act (ORS 30.260 through 30.300). If you have questions on this subject, please call the OHSU Research Integrity Office at (503) 494-7887.

It is not the policy of the federal funding agencies to compensate or provide medical treatment for human subjects in federally funded studies.

Oregon Health & Science University is subject to the Oregon Genetic Privacy law (ORS 192.531 through ORS 192.549) and its requirements concerning confidentiality and the legal remedies provided by that law for breach of its requirements. You have not waived your legal rights by signing this form. For clarification on this subject, or if you have further questions, please call the OHSU Research Integrity Office at (503) 494-7887.

PARTICIPATION:

If in the future you decide you no longer want to participate in this research, we will remove your name and any other identifiers from your blood, but the material will not be destroyed and we will continue to use it for research.

Dr. McEvoy at 503- 494-8122 has offered to answer any other questions you may have about this study. If you have any questions regarding your rights as a research subject, you may contact the OHSU Research Integrity Office at (503) 494-7887.

You do not have to join this or any research study. If you do join, and later change your mind, you may quit at any time. If you decline to join or withdraw early from the study, there will be no penalty or loss of any benefits to which you are otherwise entitled. If you withdraw early, neither you nor your baby will not be expected to complete any further testing. You will be informed of any new findings that may affect your decision to continue in the study.

Your health care provider may be one of the investigators of this research study, and as an investigator is interested in both your baby's clinical welfare and in the conduct of this study. Before entering this study or at any time during the research, you may ask for a second opinion about your care from another doctor who is in no way involved with this project. You do not have to be in any research study offered by your physician.

We will give you a copy of this form.

SIGNATURES:

Your signature below indicates that you have read the foregoing and agree to participate in this study.

OREGON HEALTH & SCIENCE UNIVERSITY

INSTITUTIONAL REVIEW BOARD

PHONE NUMBER (503) 494-7887

CONSENT/AUTHORIZATION FORM APPROVAL DATE

July 11, 2012

Do not sign this form after the

Expiration date of: 04-09-2013

_____ _____

Signature of Mother Date

_____ _____

Person Obtaining Consent Date

COPIES FOR: SUBJECT CHART
INVESTIGATOR

INFORMED CONSENT – MEDICAL STUDIES

Do not write below this line – for office use only

Stamper Plate: