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Maternal Buprenorphine-naloxone Treatment and the Infant

JHM IRB - eForm A – Protocol

1. Abstract

Medical, psychosocial and financial problems associated with prenatal opioid dependency and Neonatal Abstinence Syndrome (NAS) have reached epidemic proportions in the US. Solutions include optimizing the treatment for opioid dependent pregnant women while mitigating the severity of NAS and other neurobehavioral consequences of prenatal opioid exposure. Currently, only methadone maintenance is offered standardly, with more providers prescribing buprenorphine-only in the US due to milder NAS. Methadone treatment, although advantageous for many women, is a difficult choice due to the frequency and severity of the NAS. Buprenorphine-only has a high diversion/abuse potential, is not always readily availble, and can have unpleasant side effects in some women. Buprenorphine-naloxone (B+N) treatment of pregnant women may be an attractive and effective strategy due to the antagonist component, which can result in reduced abuse liability, reduced risk of diversion and increased drug effectiveness by increasing medicaiton adherence, all of which can serve to decrease NAS severity. However, there are currently no published reports that provide a prospective assessment of maternal, fetal and infant functioning with maternal B+N maintenance.

The purpose of this study is to evaluate the effects that maternal B+N maintenance have on the neurobehavioral development of the fetus and infant. To accomplish this, we will study a sample of *n*=80 (considering a recidivism rate of 50% for *n*=40 protocol completers) opioid dependent pregnant women that will receive B+N as part of substance abuse treatment at a comprehensive care treatment facility for pregnant and parenting women with substance use disorders, or stable patients receiving B+N from other treatment providers who are willing to a) complete the Addiction Severity Index (ASI) around the time of consent, b) consent to a review of medical records, c) leave weekly research urines for the study team and d) plan for delivery at the JHBMC hospital. A matched sample of *n*=80 (considering a recidivism rate of 50% for *n*=40 protocol completers) methadone maintained women will be recruited as a comparison sample. Fetal neurobehavior and maternal physiology will be assessed, via an established maternal-fetal data acquisition system, at 4 points during gestation: 24, 28, 32 and 36 weeks. Infant birth parameters and NAS spectrum display will be evaluated at birth, and infant neurodevelopment will be assessed during the first month of life. We will compare the neurodevelopment of the B+N-exposed fetuses and infants to that of the comparison group of methadone and a historical (2012-2016) group of buprenorphine-only exposed fetuses and infants. Additionally, among a subsample of participants who wish to and are eligible to breastfeed their infants, we will evaluate maternal plasma, breast milk and infant plasma concentrations of buprenorphine, naloxone and their major metabolites.

2. Objectives (include all primary and secondary objectives)

Aim 1: Determine the acute effects of B+N and methadone exposure on the fetus by comparing fetal neurobehavior at times of peak and trough maternal B+N levels during the second half of gestation (24,28,32 and 36 weeks).

Hypothesis 1a: Fetal heart rate and motor activity patterns indicative of fetal well-being will be attenuated when maternal circulating B+N and methadone is at its peak as compared to trough concentration.

Hypothesis 1b: Differentials in fetal heart rate and motor activity patterns between peak and trough will increase over the course of gestation as neurobehavioral development becomes more organized.

Hypothesis 1c: The differential in fetal heart rate and motor activity patterns at peak and trough will be reduced in fetuses of B+N maintained mothers as compared to a matched cohort of methadone exposed fetuses, and will show either no difference or greater attenuation when compared to buprenorphine-only exposed fetuses.

Aim 2: Evaluate the development of the B+N-exposed fetus, and compare this with two other treatment exposures (a matched prospective methadone maintained group and a recent historical buprenorphine-only group).

Hypothesis 2a.: Fetal heart rate and motor activity patterns indicative of greater fetal well-being, and their developmental trajectory, will either not differ between B+N and buprenorphine-only groups at 24, 28, 32 and 36 weeks gestation, or they will be indicative of more optimal neurodevelopment. Separate comparisons will be made at both trough and peak levels to allow examination of both acute and chronic effects.

Hypothesis 2b: Fetuses of B+N maintained mothers, as compared to a matched cohort of methadone exposed fetuses, will exhibit fetal heart rate and motor activity patterns indicative of greater fetal well-being.

Aim 3: Determine the differential effects on the infant's NAS expression in B+N as compared to the other two treatment exposures (matched methadone exposed and historical buprenorphine exposed infants).

Hypothesis 3a: B+N-exposed infants will have mild NAS

Hypothesis #3b: B+N exposed infants will have less severe NAS symptomatology than methadone exposed infants, consist with current literature.

Hypothesis #3c: B+N exposed infants will have similar NAS severity relative to buprenorphine-only exposed infants.

Hypothesis #3d: An additional exploratory subaim will seek to identify other maternal physiologic and/or fetal neurobehavioral markers that may predict NAS severity.

Aim #4: Will evaluate the longitudinal neurobehavioral developmental profile of B+N exposed infants up to 1 month of age. Infant neurobehavioral development will be compared to a matched cohort of methadone exposed and a historical cohort of buprenorphine only exposed infants.

Hypothesis#4a: B+N-exposed infants will evidence mild neurobehavioral alterations in the first month.

Hypothesis #4b: B+N-exposed, as compared to methadone-exposed infants will exhibit less severe neurobehavioral alterations.

Hypothesis #4c: The neurobehavioral functioning of the B+N and buprenorphine-only exposed infants will be similar.

Aim #5: Will evaluate concentrations of B+N in breast milk, maternal and infant plasma among breastfeeding women maintained on B+N.

Hypothesis #5a-c: B+N concentrations in a) maternal plasma, b) breast milk c) infant plasma will be low

Hypothesis #5d: B+N concentrations in breast milk, maternal plasma and infant plasma will be related to maternal dose of B+N.

3. Background

The PI's main clinical focus for the past 26 years has been the development of a highly successful model of care for the population of infants and children of women with substance use disorders. The PI has directed

the pediatric component of the Center for Addiction and Pregnancy since the program's inception and continues to provide pediatric care to the children of women with substance use disorders who attend comprehensive drug treatment. Research based on this population has been afforded by an initial K-award and two subsequent investigator-initiated awards. All aforementioned awards evaluated maternal maintenance medication effects on fetal and infant development. This research has followed the clinical uses of various medications in pregnancy for the treatment of opioid dependency. The initial K award (2000-2005) and first RO1 (2005-2010) evaluated methadone (i.e. full agonist) effects, the second RO1 (2011-2016) evaluated buprenorphine (i.e. mixed agonist-antagonist) effects. The next logical step in the progression of this research is the evaluation of B+N (i.e. buprenorphine (mixed agonist-antagonist) + full antagonist) use in pregnant opioid dependent women and fetal and infant effects. All studies to date have recruited subjects form the CAP program, and all have used the same computerized maternal physiology and fetal neurobehavioral assessment paradigm. Inclusion and exclusion criteria are essentially the same for all projects.

4. Study Procedures

a. Study design, including the sequence and timing of study procedures:

No procedures described here are part of routine patient care.

Participants will be selected from a population of women with OUDs in substance use disorder treatment at the Center for Addiction and Pregnancy (CAP), a comprehensive care treatment facility located in Baltimore and providing treatment for substance use disorders, mental health services, obstetric care and pediatrics (Jansson, 1996), which has been the site for all previously described research activities. Additional participants may include stable pregnant patients who are maintained on B+N in treatment by other providers who are willing to leave weekly research urines for toxicology testing and undergo fetal testing procedures. Total subject enrollment will be 160 (80 methadone and 80 B+N) participants; using a conservative estimate of 50% non-completion, the final sample size will be 80 (40 methadone and 40 B+N) mother/infant dyads.

Women accepting or requesting buprenorphine treatment will be offered study participation at the time of their contact with CAP intake staff after indicating a willingness to talk to study staff regarding research. The methadone comparison sample (*n*=80) will be drawn from the population of CAP methadone maintained women and matched on . severity of OUD (route of administration (IV vs other)
Eligibility for study enrollment and B+N maintenance will be determined by the project obstetrician and psychiatrist prior to study enrollment. B+N dose after induction to medication will be determined and regulated by the psychiatrist with adjustments made at the recommendation of patient counselors based on subjective and objective indicators of withdrawal or over-medication symptoms. All medication dosing will take place at an observed medication window. Take-home dosing will be afforded for holidays, inclement weather, or at the discretion of the project psychiatrist with input from the subject's counselor for other reasons on an individualized basis. Participants enrolled from other treatment programs will be informed of the study at their treatment program and asked to contact study personnel if they are interested.

Stable buprenorphine or B+N women wishing to be enrolled in the protocol will not undergo induction, but will be admitted to the study after confirmation from their current prescribed of their medication status and dose. They will receive their current dose, with adjustments to be made at the discretion of their therapist and the overseeing psychiatrist.

<u>B+N induction protocol</u>: Subjects will be asked to not use heroin or other opioids for at least 10 hours and be in mild withdrawal at the time of enrollment. B+N filmstrips will be administered sublingually at the discretion of the prescribing physician. Participants will be instructed to hold the filmstrips under their tongue until dissolved without talking. All dosing will be observed. All alterations in dose during the induction period and beyond will be at the discretion of the project psychiatrist(s) and obstetrician.

Maximum dose on day 1 and 2, except as determined by project physicians, is 10 and 16 mg, respectively. The co-investigator obstetrician will be aware of all B+N inductions and will facilitate transfer to the on campus Labor and Delivery suite for management of somatic complaints that are not manageable in the outpatient setting. All women will provide urine for toxicology testing prior to the initial dose each day to assure that no agonist opioids have been taken. Subjects that take illicit or licit opioids during the evenings of the induction period, by history or urine toxicology screening, may be removed from the protocol.

All participants receiving B+N will receive the following instructions regarding dosing: No smoking for 15 minutes prior to dosing. The participant will be asked to take a drink of water to increase stickiness of filmstrip. Hold medication under the tongue until dissolved.

Control and confounding measures: Research on pregnant women with opioid use disorder presents unique and well-known challenges. Polydrug licit and illicit substance use, other psychiatric comorbidities, and correlates of unhealthy lifestyles all may contribute independently to fetal neurobehavioral development and pregnancy outcomes. Highly restrictive criteria for enrollment to exclude factors, particularly those related to polydrug exposure so that effects of B+N can be isolated, results in small and non-representative samples. Less restrictive exclusionary criteria but subsequent controlling for confounders will result in larger samples and more generalizable data, but raises threats to interpretation of final results. The PI's 26 years of clinical experience working with this population has provided extensive expertise regarding the nature and extent of these factors and the complexities inherent in pregnant opioid dependent women's lives. The proposed project period will use a less restrictive enrollment approach, inducting women to B+N maintenance and following subsequent substance use closely; and not dis-enrolling them in the event of relapse. Fetal testing will not proceed if women have positive urine toxicology indicating illicit substance use or licit substance misuse at the time of testing to exclude the occurrence of fetal measures affected by acute effects of substances. An exception will be a positive toxicology for marijuana if the subject has denied recent (within the last 24 hours) use, as chronic marijuana users will have urine toxicology that remains positive for many weeks after cessation of use. The marijuana positive group will be analyzed as a subpopulation.

Maternal medical and obstetrical history. Maternal health records will be abstracted for past/current medical history, past/current obstetrical history and current medications.

Maternal drug abuse history, demographic information and psychosocial history. Information will be abstracted from maternal health records and from the Addiction Severity Index (ASI; McLellan, 1992) which will be administered to all subjects at the time of enrollment. This standardized clinical interview provides data regarding substance use history (lifetime; past 30 days), and functional impact and use severity in 7 areas. The domains of drug and alcohol use and psychological functioning will be reviewed and abstracted for study purposes. Information abstracted from maternal health records will include current/past obstetrical and medical histories, drug abuse treatment history and current B+N dose. Program intake assessment and medication records for each subject are reviewed at the time of consent as well as at the time of testing.

Withdrawal symptomatology. To verify appropriate dosing, women will be asked for a subjective report using two questionnaires at the time of fetal monitoring. On the Adjective Questionnaire (Eissenberg,1996; Preston,1988) respondents use a 5-point scale to rate 21 withdrawal symptoms (i.e. watery eyes, muscle cramps) intermixed with 16 agonist adjectives (i.e. nodding, itchy skin). The Clinical Opiate Withdrawal Scale, (COWS; Wesson, 2003) assesses 11 common signs and symptoms of opiate withdrawal, with each item rated on a scale; scores below 12 reflect minimal or mild withdrawal. While we regard this as a necessary control measure, no subject in the previous project period had been determined to be in significant opioid withdrawal at the time of fetal monitoring.

Ascertainment of illicit substance use/licit substance misuse: Quicktest urine screening, which will include testing for buprenorphine (10 ng/mL), amphetamines (1000 ng/mL), barbiturates (300 ng/mL), benzodiazepines (300 ng/mL), cocaine (300 ng/mL), methadone (300 ng/mL), methamphetamines (1000 ng/mL), opiates (300 ng/mL), oxycodone (100 ng/mL), and THC (50 ng/mL), for a total of once weekly screening, will occur for CAP participants and those from other treatment programs. These urine toxicology screens will be performed by research staff. Participants will undergo urine toxicology screening at the time of each fetal monitoring session. Urine toxicology screening that is positive, i.e. representative of illicit substance use or licit substance misuse, at the time of fetal testing will result in cancellation of the fetal monitoring procedures for that day, with the exception of marijuana (see above).

Ascertainment of other substances. Alcohol is a well-known fetal teratogen, and chronic fetal exposure to alcohol will be excluded in the study populations. Any woman meeting DSM V or ASI criteria for alcohol use disorder will not be enrolled. We will also attempt to identify women using alcohol acutely; recent alcohol use will be verified with participant interview. Positive clinical screens for alcohol use will also be tracked via chart review. Women having evidence of or reporting recent alcohol use more than three times during the period of study enrollment will be removed from the protocol; however alcohol use emerged as a disenrollment criteria in only one participant in the prior study, likely due to our exclusion of alcohol use disordered participants based on ASI screening. In contrast to the low levels of alcohol use in our participants, cigarette smoking is routine, reflecting the larger CAP clientele: 88.5% use nicotine. Self-report data will be collected from each participant regarding the average number of cigarettes smoked per day upon enrollment and at each testing point. Women will be asked to refrain from smoking for 2 hours prior to each maternal-fetal recording. Nicotine withdrawal is unlikely in this time frame, and pregnant women are less likely to report any nicotine withdrawal in the first 24 hours of abstinence than non-pregnant women (Ussher, 2012).

Maternal psychiatric characteristics. Approximately 75% of CAP clients report a current history of psychological problems at program intake, and more are diagnosed during treatment. All CAP clientele are referred for psychiatric evaluation after admission and near 40% receive medication, primarily selective serotonin reuptake inhibitors (SSRIs) prescribed for depression, the major Axis I disorder among CAP clients. However, many choose not to take these medications. Psychiatric diagnoses, medications prescribed and medications taking will be recorded for each subject.

Maternal-fetal monitoring protocol

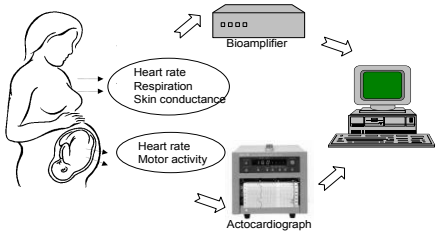
Maternal-fetal monitoring will occur at the 24th, 28th, 32nd and 36th weeks of gestation. These periods reflect the most intensive developmental trajectories in fetal neurobehaviors, which become more complex as term advances, with a shift in the steepness of the developmental trajectory in most parameters between 28 and 32 weeks (DiPietro,2004). Quality continuous fetal heart rate monitoring is difficult prior to 24 weeks due to movement artifact. Monitoring sessions will last for 60 minutes and be conducted at 9 AM (the time of trough maternal B+N level) and again at 2 ½ hours after a single sublingual dose of B+N which corresponds to the time of peak maternal medication level. Women who are recruited for study participation will have their dose administered around 10 AM for 1 week prior to the time of fetal testing, and be on a stable dose of B+N during that time period. On the day of fetal testing, women will be instructed to eat breakfast 1½ hours prior to testing to control variation in blood glucose. Concurrent maternal-fetal monitoring will be digitized at 1000 Hz using an external analog to digital board using streaming software (Snapstream, HEM, Inc).The methodology and data processing techniques described have been used in our prior studies and are comprehensively described elsewhere (DiPietro, 2015).

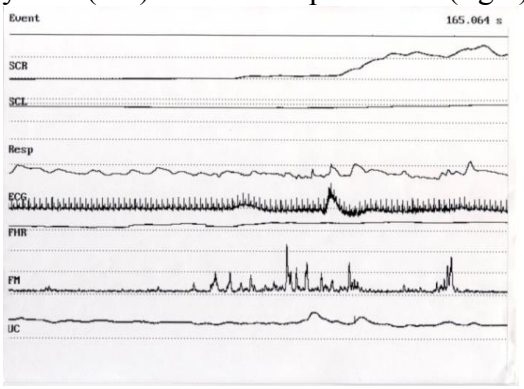
Fetal data. Fetal data will be generated from a Toitu (MT516) fetal actocardiograph, which detects fetal movement (FM) and fetal heart rate (FHR) through a single wide array transabdominal Doppler transducer and processes this signal through a series of filtering techniques. The actograph detects FM by preserving the remaining signal after band-passing frequency components of the Doppler signal that are associated

with FHR and maternal somatic activity. Reliability studies comparing actograph-based to ultrasound-visualized FMs have found this monitor to be accurate (Besinger & Johnson, 1989; DiPietro, 1999; Maeda, 1999). Data will be analyzed off-line using software developed by DiPietro (GESTATE; James Long Company, Caroga Lake, NY). A series of algorithms will be applied to detect physiologically improbable changes in FHR while maintaining the temporal nature of the data, using serial expansion and contraction of acceptable limits in progressive intervals, based on moving averages (medians) of preceding data points. FM data represent raw voltage values generated from the actograph, calibrated by multiplying by a conversion factor with adjustment for offset, and scaled from 0 to 100 in arbitrary units. Derived fetal variables include those centered on heart rate alone, movement, and their interrelation. Cardiac variables will include FHR, FHR variability (root mean square) and FHR accelerations (i.e., episodic excursions in FHR amplitude above baseline). Movement is characterized as number of bouts, duration, and total motor activity. Linkage between the two domains of function will be quantified through ascertaining the degree to which there is somatic-cardiac (FM-FHR) coupling based on a priori criteria (i.e., the proportion of observed FMs associated with excursions in FHR of a predetermined criteria for a predetermined length of time; (DiPietro, 1996) and the temporal linkage between the two, computed in seconds (DiPietro, 2001). FM-FHR coupling reflects coactivation of the sympathetic and parasympathetic components of the autonomic nervous system since changes in heart rate and movement are temporally, but not necessarily causally related, leading to the conclusion that the two activities are centrally coordinated (Timor-Tritsch,1978) with a predictable developmental trajectory during gestation.

Maternal data. Maternal physiologic signals will be amplified using a multi-channel, electrically isolated, bioamplifier. Electrocardiogram will be recorded from 3 carbon fiber disposable electrodes in triangulated placement; data will undergo R-wave detection, manual editing for artifact, and quantified as heart period (interval between R-waves in msec), heart period variability (standard deviation of successive heart periods), and an indicator of respiratory sinus arrhythmia which reflects vagal, predominantly parasympathetic influences on heart rate variation (Grossman & Kollai, 1993). Vagal tone will be indexed using the analytic technique developed by Porges (1986), which applies a 21-point polynomial to detrend sequential heart periods and a band-pass filter to extract the variance within the frequency band consistent with respiration within this age group (i.e., 24 to 1.04 Hz). Electrodermal activity (skin conductance) will be monitored from two electrodes with gelled skin contact area placed on the distal phalanxes of the index and middle fingers of the non-dominant hand affixed with adhesive And skin conductance will be measured by administering a constant 0.5 volt root-mean-square 30 Hz AC excitation signal and detecting the current flow; scaling will be from 0 to 25 microsiemens. Respiratory data will be collected via a bellows gauge and quantified in terms of period (sec between breaths). Maternal data will be time synchronized and analyzed in conjunction with fetal data using the GESTATE program. Blood pressure will be measured at the start of each session using a sphygmomanometer. All maternal and fetal measures, with the exception of blood pressure, will be computed in 1-minute intervals and averaged over the 60 min recording.

Below are a schematic of the maternal-fetal monitoring system (left) and raw output values (right).





Postnatal protocol

Neonates: All neonates born to participants will be evaluated for signs and symptoms of neonatal abstinence syndrome (NAS) using a modified Finnegan Scale scoring system, previously described (Jansson, 2009c**)** to determine the severity of the infant's NAS display and the need for pharmacologic treatment. Scored items will be coded by symptom and severity of each symptom during the infant's hospitalization by nurses trained and experienced in administration. NAS parameters will be evaluated every 3-4 hours for a minimum of 4 days post-delivery and throughout the infant's hospitalization. Since NAS measurements are subjective, additional measures of NAS severity will include amount of medication (morphine sulfate, mg) used to treat NAS, the need or not for second medication to treat NAS, days of pharmacotherapy required, and length of hospital stay.

Control and confounding measures: All neonates at the hospital of record have a urine toxicology screen done at birth for benzodiazepines, opiates, THC, cocaine, methadone, barbiturates, phencyclidine, amphetamines and buprenorphine. All urine screens will be monitored and tracked for evidence of recent substance exposure Infants will be excluded from further evaluation in the event of a significant medical complication which would include (but is not limited to): sepsis requiring antibiotic therapy, significant congenital malformation, birth at or transfer to another hospital, or preterm (<37 weeks) delivery.

Neonatal health status. Chart review will be used to extract salient data regarding labor, delivery, and neonatal parameters including infant sex, size, maturation, Apgar scores and any medical compromise and resuscitation methods employed. Maternal and infant toxicology results will be used to ascertain fetal exposures late in pregnancy. Additional infant medical history will be abstracted from infant medical records at the time of hospital discharge and will include all medications received and amounts, and any medical complications.

Infants: Infants will be evaluated through one month of age using a standardized neurobehavioral evaluation of high risk, especially substance exposed infants, the NICU Network Neurobehavioral Scale (NNNS; Lester, 2001). Evaluation of infant neurobehaviors can inform care providers (pediatricians, neonatologists, nurses, occupational / physical therapists, etc.) and parents regarding optimal care practices for this group. The NNNS is a comprehensive assessment of neurologic integrity and behavioral functioning, including withdrawal and signs of stress. Descriptive statistics for this scale have been provided from a prospective, large longitudinal study (Lester, 2002). The 13 NNNS summary scores (habituation, attention, handling, quality of movement, regulation, non-optimal reflexes, asymmetric reflexes, stress/abstinence, arousal, hypertonia, hypotonia, excitability and lethargy) have been normatively described for both substance exposed and non-exposed infants (Tronick, 2004). We have previously described the NNNS profile for methadone-exposed infants (Velez, 2009). Assessments will be performed by trained, certified investigators on days 3, 14 and 30 of life in the hospital or in a quiet lab adjacent to the infant's hospital room.

Breastmilk: *Maternal samples*: Subjects will provide plasma and breast milk at times of peak maternal medication levels (i.e. 2 – 2 ½ hours after maternal dose of B+N) on days 2, 3, 4, 14 and 30 post-partum.
Infant samples: Infant plasma will be obtained on day 14 coincident with a heel stick for routine pediatric care (i.e. PKU test) when feasible. NAS scores will be abstracted per the parent protocol.
Analysis of breast milk and plasma samples: Plasma and breast milk samples will be analyzed by Sam Houston State University in Huntsville, Texas. These investigators have extensive analytical experience with gas chromatography-mass spectrometry (GCMS) and liquid chromatography tandem mass spectrometry (LCMSMS) for many drugs of abuse, their metabolites and licit pharmacotherapies. This plasma method investigates buprenorphine and norbuprenorphine concentrations as well as the glucuronide metabolites. New analytical methods will be developed for B+N and metabolites in breast milk based on the analysis of these analytes in multiple matrices.

b. Study duration and number of study visits required of research participants.

Study duration and the number of study visits depends on when during gestation (up to 34 weeks of gestational age) the participant enters the protocol. One hour long maternal-fetal monitoring sessions will occur up to 8 times, twice on 4 separate days at monthly intervals (i.e., 24, 28, 32 and 36 weeks). The number of monitoring visits depends on the gestational age at enrollment; participants enrolling after 24 weeks will have fewer visits. There are no study visits for neonatal testing, as all data are abstracted from neonatal charts as standard of care. Infants will be enrolled for 1 month after birth, and make 3 study visits (days 3, 14 and 30). For those participants enrolled in the lactation arm, study visits will occur on days 2,3,4,14 and 30 postpartum for sampling.

c. Blinding, including justification for blinding or not blinding the trial, if applicable.

This trial is not an open label trial; therefore, there is no blinding of study participants.

d. Justification of why participants will not receive routine care or will have current therapy stopped.

All CAP participants will receive standard substance use disorder, prenatal and pediatric care through the CAP program and in the newborn nursery or NICU. All participants from other treatment programs will receive care through their providers in those programs.

e. Justification for inclusion of a placebo or non-treatment group. NA

f. Definition of treatment failure or participant removal criteria.

There are several scenarios under which participants may terminate the study voluntarily or as a result of safety or compliance concerns. These include: patient choice (e.g., does not like buprenorphine-naloxone); voluntary or enforced discharge from the CAP or other treatment program (e.g., from incarceration, relocation, violation of CAP rules); physician choice (e.g., toxicity or side effects related to buprenorphine-naloxone); and ineligibility (e.g., violation of the study protocol, missing 5 consecutive days of buprenorphine-naloxone dosing, loss of pregnancy, development of fetal or maternal health condition).

Participants will be notified in the event that the research staff has determined that termination is necessary. Upon voluntary discontinuation or termination, participants will have the option of either being switched to methadone maintenance or continuing on B+N or buprenorphine provided there is a provider outside of CAP who will accept a B+N or buprenorphine transfer patient. It is expected that most participants who leave the study prematurely will request transfer to standard CAP methadone maintenance, as in previous protocols.

g. Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.

Women who are either voluntarily disenrolled from the protocol or whose study participation is terminated or completed will be transferred from buprenorphine-naloxone to buprenorphine or methadone maintenance. Transfer from buprenorphine to methadone is less complicated than transfer from methadone to buprenorphine. There is no risk of withdrawal precipitated by the first dose of methadone after buprenorphine. However, it may take several days for all buprenorphine to be eliminated from a participant's system. Clinical titration of methadone doses is essential to stabilize patients as quickly as possible. Commencement of methadone will begin not less than 24 hours after the last dose of buprenorphine

5. Inclusion/Exclusion Criteria

Fetal, Neonatal and Infant protocol**:** *Inclusion criteria*: 1. Current opioid use disorder (OUD) as defined by DSM 5 criteria 2.18-44 years of age with singleton pregnancies, generally uncomplicated by conditions that jeopardize pregnancy outcome 3. Gestation less than 34 weeks

Exclusion criteria: 1. Complications of pregnancy, including gestational diabetes, polyhydramnios, hypertension, placenta previa or significant risk of preterm delivery; 2. Evidence of fetal malformation detected by prenatal ultrasound; 3. Significant general maternal health problems that can affect fetal functioning, including Type I or gestational diabetes, alterations in thyroid functioning, HIV infection or hypertension; 4 Significant maternal psychopathology that would preclude informed consent, such as unstable schizophrenia; 5. Alcohol use disorder per DSM 5 criteria; 6. Significant benzodiazepine use which would portend seizure activity during the induction period. This will be determined for all women reporting benzodiazepine use at the time of program intake by the project psychiatrist(s). 6. For the B+N group: Women stable on methadone maintenance (defined as more than 3 consecutive days of dosing); 7. For the B+N group: Women coming to treatment reporting "street" methadone use (for more than 3 consecutive days); 8. Women planning for adoption of their infant.

Breastmilk protocol: 10 B+N exposed infants and their mothers from the fetal study who meet criteria for breastfeeding (i.e. illicit drug use/licit drug misuse abstinence in the last 90 days prior to delivery or counselor endorsement of breastfeeding in the case of abstinence 30-90 days prior to delivery, HIV negative status, no concurrent prescription medication use that is contraindicated in lactation, and legal custody of their infant at delivery) will be enrolled.

Inclusion criteria: 1. B+N maintained mother, enrolled in parent study. 2. Free of illicit drug use and licit drug misuse after 36 weeks gestation or 30-90 days prior to delivery by standardized urine toxicology drug screening and drug treatment compliant.3. Delivery at 37 weeks or later determined by second trimester ultrasound which is standard of care at the facility of recruitment.

*Exclusion criteria: 1.*Concurrent Axis I diagnosis, or the presence of a serious psychiatric illness that would preclude informed consent. 2. Presence of maternal medical illness or condition that would preclude or impair the infant's ability to breast feeding. (i.e. respiratory concerns such as meconium aspiration, perinatal asphyxia, etc. These will be individually determined on a case-by-case basis).3. Major congenital malformation or minor congenital malformation that would impair the infant's ability to breastfeed (i.e. Pierre-Robin syndrome, cleft palate). Determined on a case by case basis. 4. Mother or infant positive for illicit substances at birth, indicating recent substance abuse and non-suitability for breastfeeding in the perinatal period (per ABM (Jansson, 2009a) guidelines). 5. Poor compliance with drug treatment and/or high risk of relapse to illicit substances as determined by mental health counselor making breastfeeding unadvisable, 6. Delivery at another institution. 7. Failure to leave specimens as per protocol.

6. Drugs/ Substances/ Devices

a. The rationale for choosing the drug and dose or for choosing the device to be used.
b. Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.

There is a limited body of literature that supports the safe and efficacious use of B+N during pregnancy. Small US studies evaluating maternal and infant outcomes in B+N exposed pregnancies have found no obvious significant adverse effects related to medication use in mothers or infants (Debelak, 2013; Lund, 2013), however, both studies suffer from small *n*s (10 each). One recent study found favorable results in B+N, as compared to methadone-exposed infants in terms of reduced NAS severity in a larger cohort (*n*=62), but did not compare results to buprenorphine-only exposed infants (Weigand, 2015).

c. Justification and safety information if non-FDA approved drugs without an IND will be administered.

NA, an amended IND was applied for (with no response from the FDA).

7. Study Statistics

a. Primary outcome variable.

Fetal variables. Derived fetal variables include those centered on heart rate alone, movement, and their interrelation. Cardiac variables will include FHR, FHR variability (root mean square) and FHR accelerations (i.e., episodic excursions in FHR amplitude above baseline). Movement is characterized as number of bouts, duration, and total motor activity. Linkage between the two domains of function will be quantified in two ways: somatic-cardiac (FM-FHR) coupling based on a priori criteria (i.e., the proportion of observed FMs associated with excursions in FHR of a predetermined criteria for a predetermined length of time; (DiPietro,1996) and through time series techniques to quantify linkage without a priori criteria (DiPietro, 2001). With the exception of accelerations, motor bouts, and somatic-cardiac coupling which are either counts or proportions. Variables are quantified in 1-minute epochs and mean values for each 60 minute recording are used for analysis. Fetal measures will be derived at trough (just prior to dosing) and peak (2 hours after dosing) maternal buprenorphine levels.

Maternal autonomic variables. Maternal ECG data will undergo R-wave detection, manual editing for artifact, and quantified as heart period (interval between R-waves in msec), heart period variability (standard deviation of successive heart periods), and an indicator of respiratory sinus arrhythmia which reflects vagal, predominantly parasympathetic influences on heart rate variation (Grossman & Kollai, 1993). Maternal variables also include respiratory period (s) and skin conductance. As with the fetal data, variables are quantified in 1-minute epochs and mean values for each 60 minute recording are used for analysis.

Neonatal variables. The primary newborn study measures are Neonatal Abstinence Syndrome (NAS) scores as assessed every four hours for the duration of the infant's hospital stay after delivery. Variables will include mean NAS scores on days1 through 4 minimally (all opioid exposed infants are hospitalized for a minimum of four days after delivery for observation) or for the infant's hospitalization, and highest score. NAS severity will also be quantified as the total amount of medication (oral morphine sulfate, mg) used to treat NAS, days of pharmacologic treatment required, and total days of hospital stay.

Infant variables. Infant testing using the NNNS generates 13 summary scores (habituation, attention, handling, quality of movement, regulation, non-optimal reflexes, asymmetric reflexes, stress/abstinence, arousal, hypertonia, hypotonia, excitability and lethargy) that have been normatively described for both substance exposed and non-exposed infants (Tronick, 2004). Exploratory data analysis techniques will be used to determine distributions for each measure to determine how many of these will be used in the final analysis.

Breastmilk variables: Concentrations of B+N and major metabolites in breast milk, maternal and infant plasma

b. Statistical plan including sample size justification and interim data analysis.

Fetal studies: Individual analyses will be performed for three cardiac measures (FHR, FHR variability and accelerations) and two motor variables (overall activity, bout duration), and two synthetic measures (event and time series-based FM-FHR coupling, as described in the Procedures section. We will also evaluate effects of BN on maternal physiological function (heart period, respirations, cardiac vagal tone, skin conductance, and blood pressure).

Models for aims 1 and 2 will be longitudinal mixed effects linear regression models with random intercepts (Fitzmaurice, 2011). Additional models with fetal sex interactions will be fit to evaluate whether boys are differentially affected by either exposure, as we have observed in previous evaluating neurobehavior in methadone exposed infants (Jansson, 2010). Additionally, all analyses will be adjusted for maternal age, race, and non-opioid substance use.

For Hypothesis 1A, we will fit a model where the outcome for the ith individual at the jth timepoint (y_{ij}) = $\beta_0 + \beta_1 peak + \beta_{2\text{-}p} x_{2\text{-}p} + \varepsilon_{ij}$, and β_1 represents the difference, across timepoints, in the outcome between peak and trough measurements. For Hypothesis 1B, we will fit a similar model, $y_{ij} = \beta_0 + \beta_1 peak + \beta_2 time +$

$\beta_3$peak*time+$\beta_{4\text{-}p}x_{4\text{-}p}+\varepsilon_{ij}$, where β_3 represents the rate of change in the difference in outcome between peak and trough over time. For Hypothesis 1C, we will fit a model where $y_{ij} = \beta_0+ \beta_1$peak + β_2BN + $\beta_3$peak*BN+$\beta_{4\text{-}p}x_{4\text{-}p}+\varepsilon_{ij}$, where β_3 represents the between-treatment (BN-Methadone) or (BN-Buprenorphine) difference in peak-trough differences. Additionally, we will fit a model with a 3-way interaction between peak, time, and BN to determine if changes over time in peak-trough differences vary between BN and Buprenorphine.
For Hypothesis 2A, we will fit models separately for peak and trough measures where $y_{ij} = \beta_0+ \beta_1$BN + $\beta_{2\text{-}p}x_{2\text{-}p}+\varepsilon_{ij}$ where β_1 represents the difference in outcome between BN and Bup. We will also fit models where $(y_{ij}) = \beta_0+ \beta_1$BN + $\beta_2$time + β_3BN*time+$\beta_{4\text{-}p}x_{4\text{-}p}+\varepsilon_{ij}$, and β_3 represents the difference in outcome trajectory over time between BN and Buprenorphine. For Hypothesis 2B, we will fit a model similar to 2A but only using data from 36 weeks to compare methadone and BN.

Neonatal study: For hypothesis 3A, we will use descriptive statistics to characterize NAS in BN-exposed infants, as measured by mean daily NAS scores on days 1-4 of life, highest NAS score, amount of medication (morphine sulfate, mg) required to treat NAS, number of days treated for NAS, and total days hospitalization. For hypothesis 3B, we will fit models of NAS severity (measured by Day 3 Finnegan scores, total medication used to treat NAS, total days of NAS treatment and total days of hospitalization), as a function of BN exposure (compared to methadone exposure), adjusting for maternal age, race, and non-opioid substance use. The models for hypothesis 3C will be analogous, but with buprenorphine exposure as the comparator. For hypothesis 3D, we will examine additional predictors of NAS severity including infant sex (Warren, 2015), daily and cumulative dose of BN, other medications including SSRIs (dosage and categorical), illicit drug use based on number of "dirty" urines and specific substance exposures, cigarette smoking (cigarettes per day and CO levels).

Infant study: For hypothesis 4A, we will use descriptive statistics to characterize BN-exposed infants' NNNS scores on each of 13 summary scales; based on previous work we will focus on habituation, arousal, excitability, and hypertonicity. We will fit a model to determine if NNNS scores change over time during the first month of life, and we will fit an additional model with an interaction between infant sex and time to determine if sex affects NNNS trajectories. For hypothesis 4B, we will fit models where $NNNS_{ij} = \beta_0+ \beta_1$BN + $\beta_{2\text{-}p}x_{2\text{-}p}+\varepsilon_{ij}$, and β_1 represents the difference, across timepoints in the NNNS between BN and methadone exposed infants. We will then fit a model where $NNNS_{ij} = \beta_0+ \beta_1$BN + $\beta_2$time + β_3BN*time+$\beta_{4\text{-}p}x_{4\text{-}p}+\varepsilon_{ij}$, where β_3 represents the difference in NNNS trajectories over time between BN and methadone-exposed infants. For hypothesis 4C, we will fit analogous models to 4B, but with buprenorphine-exposed infants as the comparator. These models will be adjusted for maternal age, race, dosing, and other substance use.

Breastmilk study: Basic descriptive statistics (mean, standard deviation, range) will be used to report concentrations of B+N in breast milk of lactating women on days 2-4, 14 and 30. Additional considerations for associations between maternal dose, maternal plasma and breast milk concentrations will be examined using linear regression. Descriptive statistics will be used to report plasma concentrations of B+N in breastfeeding infants and linear regression to evaluate potential associations between infant B+N plasma concentrations and maternal B+N dose. Although potentially underpowered, we will also fit linear regression models for associations between maternal dose, maternal plasma concentrations and breast milk concentrations with maternal dose as the main predictor. Although limited power exists for longitudinal models in this study due to small subject numbers, we will fit mixed effects models to examine changes over time in breast milk B+N concentrations for each sampling time. The analyses proposed here are not meant to be exhaustive and cannot be due to space limitations. However, they illustrate our approach for examining the relations among the complex set of independent, dependent, and confounding variables collected in this proposal.

Computation of Power: Sample size estimates for each study were premised with a power of .8, alpha =.05, and large effect sizes as observed in our prior studies (Jansson, 2005, 2007a), which were conducted with a similar experimental design. Based on a conservative estimate of 50% non-completion, the total number of participants to be enrolled will be 160. In comparison samples, we will have 40 for buprenorphine, and 40 for methadone. Using available pilot data, for hypothesis 1, we would have 80% power to detect a mean difference of 4.23 (SD 6.68) in peak/trough fetal heart rate. For the mixed effects models, via simulation using pilot data, we would be powered to detect a trajectory difference of .35 per week between groups.
For hypothesis 2, we would have 80% power to detect a mean difference of 4.12 (SD 6.49) in peak fetal heart rate. For the mixed effects models, via simulation, we would be powered to detect a trajectory difference of .32 per week between BN and buprenorphine.
For hypothesis 3, we would have 80% power to detect a mean effect size of ..63 for difference in NAS severity between groups
For hypothesis 4, we would have 80% power to detect a group difference of .44 (SD 0.70) in NNNS subscales scores. For the mixed effects models, via simulation using pilot data on the arousal subscale, we would be powered to detect a trajectory difference of .04 per day between groups.

No interim data analysis is planned for this study.

c. Early stopping rules.

The study may be modified, suspended, or terminated at the recommendation of the Johns Hopkins IRB or at the direction of the DSMB in the interests of protecting study participants. Reasons the JHU IRB or DSMB may recommend modification, suspension, or discontinuation of the trial could be based on the AE or SAE reports or the failure to maintain any participants in the study due to premature attrition during induction with buprenorphine.

8. Risks

a. Medical risks, listing all procedures, their major and minor risks and expected frequency.

There is a growing body of literature to support the relative safety and efficacy of buprenorphine use during pregnancy (Jones, 2010; Fischer, 2005; Jansson, 2010; Kakko, 2008, Lejeune, 2006), and the use of naltrexone, a compound similar to naloxone, produces no untoward effects when used in pregnancy in general to the mother or infant. . One study evaluating fetal effects of maternal naloxone treatment at term found mild increases in fetal heart rate accelerations, fetal breathing and fetal movements (Arduini, 1986) all signs of fetal well-being. More recently, several observational studies have reported the relatively good outcomes in buprenorphine-naloxone exposed infants (Debelek, 2013, Dooley, 2016, Weigand, 2015, Gawronski, 2014) However, any medication used during pregnancy can present some risk to the mother and/or fetus. Risk to the neonate includes Neonatal Abstinence Syndrome (NAS), which occurs with less severity in buprenorphine, as compared to methadone exposed infants (Jones, 2010) however, there are no additional risks to the acquisition of NAS data. There are no known risks to fetal monitoring procedures for mothers or fetuses. There is no risk to the acquisition of breathalyzer testing, CO testing, or urine toxicology testing to subjects. Subjects will have additional urine toxicology screenings that CAP patients. There are no known risks of the NNNS exam that will be administered to infants.
For Buprenorphine-naloxone in particular, side effects are:
<u>More Common reactions</u>: Constipation (14%), Nausea (23%), Vomiting (11%), Xerostomia (5% or greater), Dizziness (16%), Headache (36%), Somnolence (14%) Withdrawal syndrome (25%), pain (22%), Diaphoresis (14%)
<u>Less Common reactions</u>: Vasodilatation (9%) Vomiting (8%)
<u>Serious Reactions, but rare</u> (<1%): Syncope, Application site reaction, Anaphylaxis, Hypersensitivity reaction, Loss of consciousness, Respiratory depression, Respiratory distress, Respiratory failure, Drug withdrawal.

Further, plasma and breast milk samples might induce minor bleeding, discomfort and bruising.

b. Steps taken to minimize the risks.

There could be a potential risk of buprenorphine-naloxone induction for pregnant opioid dependent patients, which would involve some degree of opioid withdrawal and/or stress; effects of maternal opioid withdrawal on the fetus are not known. There might be some risk to the sobriety of a methadone maintained patient who would undergo withdrawal from methadone to accept B+N. Therefore, this protocol will enroll only methadone naïve subjects (3 or less days of "street" methadone intake or methadone dosing) to minimize this risk for the B+N cohort. Subjects will have additional urine specimens collected for urine toxicology screening during the period of study enrollment, however, these results will not be shared with CAP or other treatment program staff and therefore present no risk to the subject's status in substance use disorder treatment.

c. Plan for reporting unanticipated problems or study deviations.

Any adverse events or serious adverse events will be reported to the IRB, the NIDA project officer, the FDA (via the holder of the IND for this study TBD) and to the study Data Safety Monitoring board.

d. Legal risks such as the risks that would be associated with breach of confidentiality.

There is some risk to subjects from breach of confidentiality of their status as drug dependent people. The study will take place within the Center for Addiction and Pregnancy, a drug treatment facility, where patient status is kept confidential in compliance with federal regulations. Study records will be stored by study staff in a de-identified database, and records will be kept in a locked filing cabinet within a locked room.

e. Financial risks to the participants.

None anticipated.

9. Benefits

a. Description of the probable benefits for the participant and for society.

Subjects and their infants may benefit from participation by receiving B+N as opposed to methadone. Methadone treatment is the standard of care for opioid dependent pregnant women in the US and at CAP. There is a growing interest in the use of B+N during pregnancy due to the reduced incidence of severe NAS in infants. Infants may benefit from the NNNS examination, which may provide information regarding the infant's neurobehavioral strengths and difficulties that may be beneficial to the caretaker.

Benefits to society are larger. It has been established that buprenorphine is an acceptable alternative to methadone for use during pregnancy, and its use may confer an advantage to both fetal and infant functioning. But there is no information on the combination drug B+N, and many women maintained on this medication with success are currently required to switch to an alternative preparation when they become pregnant due solely to a lack of evidence regarding effects on the fetus and infant. Specific hypotheses for this study are based on the premise that B+N maintenance during pregnancy will generate fewer adverse effects on offspring than methadone, and either the same degree or lesser deleterious effects as conferred by buprenorphine. Confirmation of hypotheses will directly benefit the care given to women with opioid use disorders who seek medication assisted treatment during pregnancy and to society, as adding naloxone to buprenorphine-only will reduce abuse liability and drug diversion, and likely increase drug effectiveness by allowing medication adherence throughout pregnancy to women vulnerable to relapse.

10. Payment and Remuneration

a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol.

Pregnant women will be compensated $25 per fetal testing day, for a maximum of $100, which will be delivered at the completion of the final testing session in the form of a gift card for baby supplies. Mothers will receive a $20 gift certificate for each study visit involving an infant assessment (days 3, 14 and 30) for a total of up to $60. The total possible compensation for participation is $160.

11. Costs

a. Detail costs of study procedure(s) or drug (s) or substance(s) to participants and identify who will pay for them.

Study medication and procedures will be provided at no cost to subjects. Should the study be funded by NIDA, study medications for CAP participants provided by the manufacturer (Indivior) per written agreement with the study PI. Medications received in other treatment programs will be provided by those treatment programs.