Nifedipine Alone or Nifedipine Plus Indomethacin for Treatment of Acute Preterm Labor: An Open Label, Randomized Comparative Effectiveness Controlled Trial

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Nifedipine Alone or Nifedipine Plus Indomethacin for Treatment of Acute Preterm Labor: An Open Label, Randomized Comparative Effectiveness Controlled Trial

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BACKGROUND

Preterm birth

Prematurity is a leading cause of perinatal morbidity and mortality affecting 12% of approximately 4 million deliveries in the United States.[1] Though the etiology of preterm labor is largely unknown, it is defined as regular contractions of the uterus resulting in changes in the cervix that starts before 37 weeks of pregnancy. Preterm birth that occurs between 20 to 37 weeks of pregnancy, due to preterm labor and premature rupture of membranes, continues to be a challenging concern for the practicing obstetrician.[2] Since the fetus is not yet fully developed before 37 weeks of pregnancy, health problems associated with preterm birth include a variety of acute and chronic health/developmental deficiencies. Among these are acute respiratory, gastrointestinal, immunologic, CNS, hearing and vision issues, as well as cerebral palsy, cognitive, visual, hearing, behavioral, social-emotional, learning, health, and growth problems.

To mitigate the complications of preterm birth, tocolytics, a group of medications that are purported to prevent preterm delivery, are utilized. Tocolytics belong to a number of pharmacological families, including non-steroidal anti-inflammatory drugs (NSAID), β2-agonists and calcium channel blockers. Though this group of medications may postpone delivery by 48 hrs., none of the tocolytics have been proven to be effective in the treatment of preterm labor and improve neonatal outcomes.[3-5]

Current Tocolytic Therapy

Nifedipine is a commonly used calcium channel blocker used for tocolysis in the treatment of preterm labor.[1,6] Its mechanism of action is prevention of the influx of extracellular calcium ions into the myometrial cells of smooth muscles within the pregnant uterus. It has been studied in 38 randomized controlled trials involving 2511 women. Compared to placebo, nifedipine significantly decreases delivery within 48 hours, RR 0.3 (0.21-0.43).[7] When compared to other tocolytic agents including betamimetics, nitroglycerin, non-steroidal anti-inflammatory agents, magnesium sulfate and oxytocin receptor antagonist, nifedipine had similar results in prolongation of pregnancy and neonatal outcomes.

Indomethacin is another commonly used tocolytic agent from the NSAID family. The role of prostaglandins in the initiation of labor is well documented.[8] Indomethacin is a non-selective prostaglandin synthetase inhibitor that suppresses the production of prostaglandins, thus, ultimately, inhibiting preterm labor.[9,10] Prostaglandins are produced in multiple compartments of pregnancy including the cervix, amion, chorion and uterine tissue. Numerous randomized controlled trials comparing indomethacin to either placebo or other tocolytic agents have shown a significant delay in delivery of up to 48 hours in 90-94% and up to 7 days in 75% of cases when indomethacin was used.[11-21]

Nifedipine has been compared to indomethacin in 2 trials. In 2011, Kashanian randomized 40 women to nifedipine and 39 to indomethacin for the treatment of preterm labor.[22] Only 33

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women were included in the analysis, (23 nifedipine and 10 indomethacin). There were similar rates of delay in delivery and gestational age at delivery in both groups. In 2014, Klauser conducted a randomized controlled trial (RCT) of 301 women for the treatment of preterm labor; 114 received nifedipine, 90 magnesium, and 90 indomethacin.[23] The gestational age at delivery, prolongation of pregnancy >7 days were similar between groups. Maternal adverse effects were reported in all groups.

Combination Therapy in Medicine

Since the etiology of preterm labor is multifactorial, single-targeted agents for complex medical conditions may be suboptimal treatment and may explain why RCTs involving single tocolytics have not improved the neonatal outcome. Other disciplines of medicine routinely use combination medication therapy that include varying mechanisms of action to treat many medical conditions. For instance, initial treatment of myocardial infarction includes aspirin, a beta blocker and nitrate.[24] In addition, chemotherapy often involves multiple agents. [25] Moreover, hybrid medications are often prescribed for hypertension (i,e volsartan-hydrochlorothiazide), pain (i.e. acetaminophen-oxycodone) and diabetes (i.e glipizide-metformin). In certain conditions in pregnancy, this same concept occurs including broad spectrum antibiotics for postpartum endometritis (gentamicin and clindamycin)[26], preterm premature rupture of membranes (ampicillin and erythromycin)[2], and postoperative pain (i.e. opioid with non-steroidal anti-inflammatory)[27]. But for preterm labor, current practice includes single agent tocolytic therapy.

Combination Tocolytic Therapy in Pregnancy

Eleven RCTs have compared combinations of tocolytic agents.[28] The majority of studies included IV ritodrine and terbutaline. In 1978, indomethacin in combination with IV ritodrine was compared to IV ritodrine alone involving 208 women.[29] There was a statistically significant decrease in the number if women delivering before 37 weeks in the combination group, RR 0.7 (CI 0.5-0.97). A recent study of combination salbutamol (n=38) and nifedipine was compared to nifedipine alone (n=38).[30] There was no difference in the mean gestational age at or interval to delivery. Those with combination had more adverse effects. There are no studies that compare nifedipine combined with indomethacin to nifedipine alone.

Pharmacogenomics and Personalized Medicine

Pharmacogenomics is the application of genomic and molecular data in order to better target the delivery of health care, facilitate the discovery and clinical testing of new products, and/or help determine an individual's predisposition to a particular disease or condition. [Shastry] Personalized medicine is multifaceted, and includes genetic factors, historical factors (e.g. family history, and personal medical and obstetric history), and environmental factors (e.g. concurrent medication use or exposure to tobacco smoke), and synthesizes this information with the overall goal of individualizing medical care and treatment to optimize outcomes. The goal of personalized medicine is to provide the 'right dose of the right drug for the right patient at the right time'.[Shastry] The application of pharmacogenomics knowledge has the potential to individualize medications and doses, and may minimize adverse effects.

Pharmacogenomic information is currently provided by the US Food and Drug Administration (FDA) for over 130 drugs.[FDA] Many of the medications with pharmacogenomic labeling are

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commonly prescribed; it has been estimated that 25% of all outpatient prescriptions includes one of these medications.[Freuh] Currently, more than 50 genes have been implicated as genomic biomarkers; most commonly, these biomarkers pertain to polymorphisms in cytochrome P450 (CYP) enzyme metabolism. Polymorphisms in CYP enzymes are relatively common. For example, CYP2D6 is estimated to metabolize approximately 25% of drugs, and is known to be very polymorphic. Over 70 alleles have been identified in this gene, and as a result, CYP2D6 activity ranges widely, even within populations.[Zhou, Zhou] Up to 8% of European Americans will be identified as 'poor metabolizers'. Given that this is only one hepatic enzyme involved with metabolism, this attests the importance of developing companion diagnostic tests to accompany pharmaceuticals. Pharmacogenetic evidence exists in asthma, anticoagulation, antibiotics, and oncology.

Pharmacogenomics of indomethacin and nifedipine

Pharmacogenetic studies in pregnancy and pregnant women are limited. However, there are pharmacogenetic information for both indomethacin and nifedipine, which may impact the treatment for preterm labor. The polymorphic CYP3A family of metabolic enzymes in the liver metabolizes nifedipine. One small study of 14 women receiving nifedipine tocolysis genotyped participants for CYP3A5, and analyzed nifedipine concentration over time. These investigators found that the CYP3A5 genotype correlated with clearance of nifedipine: individuals with high CYP3A5 expression metabolized nifedipine more quickly and had lower serum levels of nifedipine across the study period.[Haas] In another pilot study of 20 pregnant women, Haas et al. examined 20 pregnant women receiving nifedipine for preterm labor. The CYP3A5 genotype was found to correlate with nifedipine levels. Women with the high-expression genotype were again found to have lower nifedipine levels. Importantly, this study also showed that individuals with high expression of CYP3A5 had less improvement in contraction frequency at several time points: after the loading dose, at the steady state, and in the first hour after the study dose.[Haas J Mat] Indomethacin is another commonly used tocolytic. It is metabolized by the polymorphic CYP2C9 and CYP2C19 enzymes in the liver.[Nakajima]

Rationale for this clinical trial

Our current treatment for preterm labor has not been shown to be effective in prolonging pregnancy sufficiently to improve neonatal outcomes and other treatment strategies are needed.[1] Multiple examples demonstrate that multi-agent treatments are routine clinical practice in other fields of medicine including chemotherapeutics for cancer, multi-therapeutics for myocardial infarction and broad spectrum antibiotics for pneumonia. At this time, it is unclear if a combination of tocolytic medications for preterm labor is more advantageous for women. If pregnancy is prolonged with combined tocolytic therapy, this could directly influence the treatment of preterm labor and potentially improve neonatal outcomes. There currently are no trials of combination regimens using widely used tocolytic agents, such as nifedipine and indomethacin. In addition, the pharmacogenomic profile of indomethacin and nifedipine in a clinical trial has not been investigated in a randomized controlled trial. Thus, we propose a comparative effective trial of nifedipine plus indomethacin vs. nifedipine alone for the treatment of preterm labor.

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Hypothesis
We hypothesize that preterm labor is a multifactorial condition that may respond to a combination of tocolytic agents that function by different mechanisms. Therefore, combination tocolytics may prolong pregnancy compared to single agent therapy.

Objective
Our objective is to determine whether pregnancy prolongation, defined as the time interval from the initiation of tocolytics to delivery, is significantly longer for women who receive nifedipine plus indomethacin versus those that receive nifedipine alone.

RESEARCH DESIGN & METHODS

Study Design

We propose an open label, randomized, comparative effectiveness controlled trial of nifedipine alone vs. nifedipine-indomethacin in pregnant women for the treatment of acute preterm labor. Prior studies have confirmed that tocolytic therapy can prolong pregnancy for 48 hours to achieve corticosteroid therapy in women with preterm labor.[1] Thus, administering just a placebo for tocolysis is not justified at this time.

Consenting subjects will be randomly allocated to receive one of two clinically accepted clinical regimens, either nifedipine alone or nifedipine plus indomethacin.

Inclusion Criteria
We will include pregnant women with singleton or multiple gestation between $22^{0/7}$ to $31^{6/7}$ weeks gestation who present with regular uterine contractions defined as at least one contraction every 10 minutes for 30 minutes with at least one of the following:

- cervical change of at least 1 cm or
- cervical dilation of 2 cm at the time of initial exam or
- positive fetal fibronectin and transvaginal cervical length <2.5 cm

These criteria were chosen since these findings are associated with acute preterm labor.[1]

Exclusion Criteria
We will exclude pregnant women with:

- clinical chorioamnionitis (defined as a temperature of >100.4 F and any of the following: fundal tenderness, maternal tachycardia, fetal tachycardia or purulent vaginal discharge)
- non reassuring fetal heart tones
- suspected placental abruption
- preterm premature rupture of membranes
- prior tocolytic treatment during the past 48 hours
- known adverse effect to indomethacin or nifedipine
- already receiving nifedipine for chronic hypertension

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Recruitment
Recruitment will occur on the Labor and Delivery ward at Memorial Hermann Hospital-Texas Medical Center, Lyndon B Johnson Hospital, Memorial Hermann Memorial City Hospital, Memorial Hermann Pearland Hospital and Memorial Hermann Greater Heights Hospital. All participants will be admitted for hospital care to the antepartum unit or to labor and delivery. Eligible women will be identified after it is deemed necessary by the caring physician to provide medical treatment for preterm labor. Those who meet study criteria will be approached about the study in the admission area/OB triage, antepartum unit or labor and delivery at the time of admission. A member of our research team, either a physician or research assistant/nurse, will obtain written informed consent.

Randomization
Randomization will be performed based on a computer-generated list that will be created by a non-clinical member of the research team. Randomization will be stratified by site. A permuted block randomization with a random fashion will be used to prevent imbalances between groups. The medication based on the computer generated list will be typed out on a piece of paper with the medication regimen written according to the below regimens. This piece of paper will be placed in an opaque envelope and numbered according to the computer generated list. The opaque envelopes will be kept on the obstetrical unit and be managed by the research team.

Methods
Women will receive the first dose of either nifedipine-indomethacin or nifedipine after it is determined necessary that the patient will require medications for preterm labor by the caring physician according to clinical management for a 48 hour time period. 48 hours is chosen based on current American College of Obstetrics and Gynecology recommendations, which state that tocolytic therapy should be administered to allow for administration of antenatal corticosteroids and transfer to a tertiary facility.[1] Antenatal steroids have been shown to accelerate fetal lung maturity as well as decrease the incidence of intraventricular hemorrhage and necrotizing enterocolitis.[51,52] The optimal timing of delivery after administration of corticosteroids is at least 48 hours but less than 7 days after steroid administration.[51] Data will also be collected regarding maternal demographics, pregnancy characteristics, medications administered, length of pregnancy, and maternal/ outcomes and neonatal fetal outcomes.

Medication regimen
Women randomized to nifedipine-indomethacin will receive nifedipine 10 mg orally every 20 minutes for 3 doses, then 10 mg every 6 hours for a total of 48 hours and indomethacin 100 mg orally, then 50 mg orally every 6 hours for a total of 48 hours.[5]

Women randomized to nifedipine will receive nifedipine 10 mg orally every 20 minutes for 3 doses, then 10 mg every 6 hours for a total of 48 hours.[7]

Table 1: Scheme for medication regimen

Time	Nifedipine + Indomethacin	Nifedipine
0 minutes	Nifedipine 10 mg + Indomethacin 100 mg	Nifedipine 10 mg
20 minutes	Nifedipine 10 mg	Nifedipine 10 mg

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40 minutes	Nifedipine 10 mg	Nifedipine 10 mg
6 hours.	Nifedipine 10 mg + Indomethacin 50 mg	Nifedipine 10 mg
12 hours.	Nifedipine 10 mg + Indomethacin 50 mg	Nifedipine 10 mg
18 hours.	Nifedipine 10 mg + Indomethacin 50 mg	Nifedipine 10 mg
24 hours.	Nifedipine 10 mg + Indomethacin 50 mg	Nifedipine 10 mg
30 hours.	Nifedipine 10 mg + Indomethacin 50 mg	Nifedipine 10 mg
36 hours.	Nifedipine 10 mg + Indomethacin 50 mg	Nifedipine 10 mg
42 hours.	Nifedipine 10 mg + Indomethacin 50 mg	Nifedipine 10 mg
48 hours.	Nifedipine 10 mg + Indomethacin 50 mg	Nifedipine 10 mg

The regimens above were chosen based on the regimens used in prior studies and are standard therapy for tocolysis. Nifedipine was chosen as the comparative tocolytic agent since prior studies have demonstrated that nifedipine provides reduction of uterine contractions associated with preterm labor and less adverse effects.[31] Higher dose nifedipine has not been shown to be more efficacious than lower dose nifedipine.[32] Moreover, multiple studies comparing single agent tocolysis indicate similar prolongation of pregnancy. Compared to nicardipine, nifedipine has been associated with less adverse effects, particularly when used in combination therapy.[31]

No other interventions will be performed. The clinical management regarding corticosteroids, magnesium sulfate for neuroprotection and outpatient expectant management after 48 hours hospitalization will be determined by the managing clinical provider.

Rescue regimen
If it is determined that either the nifedipine-indomethacin or nifedipine alone is not adequate or adverse effects occur during the treatment of preterm labor, then other tocolytic regimens may be administered according to the managing physicians.

Pharmacogenomics
To assess the pharmacogenomic profile, maternal blood (20 ml) will be obtained via venous puncture prior to tocolytic administration, 48 hours after tocolytic administration and at delivery. Cord blood (20 ml) will also be obtained at the time of delivery after delivery of the newborn and prior to delivery of the placenta.

Finally, a myometrial biopsy will be obtained at the time of cesarean section for those women who undergo this mode of delivery. Myometrial biopsies will be obtained from the middle of the upper margin of the lower segment of the uterine incision measuring 4cm x 2cm x 2 cm. The biopsies will be performed by the managing obstetrical physician at the time of cesarean section once the neonate and placenta are delivered. The biopsy specimen will then be placed immediately in cold physiologic saline solutions and brought to the lab. This biopsy will be taken to the lab to test whether different medicine will increase or decrease contractions in the tissue. Once this test is finished, the tissue will be frozen. Thinly cut portions of the frozen tissue will be tested for whether or not the medicines are present or absent within the tissue.

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Moreover, a portion of the tissue will be used to create cell cultures to test tocolytic drugs in vitro and determine cellular responses to these drugs.

A placental biopsy will also be obtained after it is delivered measuring 1 cm x 1 cm x 1 cm.

All biopsies and blood collected will be tested for other factors including genes, DNA material or other diagnostic tests. Any samples not used for this study will be stored and used for future research.

All information will be confidential and de-identified. The subject will be coded as a number and a linking log will be used to separate patient information from research data.

Primary Outcomes
The primary outcomes will include the following

- Percentage of women achieving 48 hours pregnancy prolongation

Secondary Outcomes
Secondary outcomes will include the following:

- Percentage of women achieving 7 days pregnancy prolongation
- Number of hours from first dose of tocolytic agent to delivery. The hours will be rounded to the closest day.
- Neonatal outcomes including birthweight, gender, need for NICU admission, length of NICU admission, length of hospital stay, rate of death, intraventricular hemorrhage, necrotizing enterocolitis, culture positive sepsis, neonatal seizures, need for mechanical ventilation, ECMO, CPAP or blow by oxygen and length of mechanical ventilation, CPAP, ECMO or blow by oxygen.
- Pregnancy outcomes including rate of cesarean delivery, clinical chorioamnionitis, preterm premature rupture of membranes, preeclampsia, need for blood transfusion
- Reported adverse effects including headache, nausea, vomiting, acid reflux, maternal hypotension including BP<90/60, maternal tachycardia with heart rate >120, syncope.

Neonatal outcomes will be followed until hospital discharge or day 120 of life.

Data analysis and sample size calculations
We have conducted a Bayesian power analysis for this study comparing combination therapy with nifedipine and indomethacin versus single agent therapy with nifedipine alone for treatment of acute preterm labor. Our primary outcome is the percentage of women that remain pregnant at >48 hours.

Design prior distributions:

Single agent outcome rate: 77% (95% CI: 68%-85%)

Combination outcome rate: 95% (95% CI: 74%-99.9%)

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Our clinically important absolute difference (combination % - single agent %) of > 10%. Pr(difference >10%)> level of certainty. Our enthusiastic prior is the same as the design prior above. Neutral prior assumes no difference between two groups, RR=1.0 with 95% CI of 0.5-1.33.

		Bayesian Power	
Level of Certainty	N per group	Enthusiastic prior	Neutral prior
67%	30	0.83	0.77
	40	0.85	0.80
	50	0.85	0.83
	100	0.86	0.83
75%	30	0.80	0.72
	50	0.83	0.79
	100	0.84	0.81
80%	30	0.77	0.68
	50	0.79	0.77
	100	0.84	0.80

For the primary outcome of % of women that remained pregnant > 48 hours, we assumed rates of 77% (95% Credible Interval: 68%-85%) in the nifedipine alone group and 95% (95% Credible Interval: 74%-99.9%) in the nifedipine-indomethacin group. We assumed an absolute risk difference (RD) of 10% between the two groups as the minimum clinically important treatment effect. We are interested in a posterior probability of 67% or more for this effect, i.e. Pr(RD>0.10) > 0.67. We conducted a simulation study to calculate the needed sample size to have 80% power to detect an RD >10% with 67% probability. We used the expected outcome rates to simulate data, and we assumed a neutral prior (meaning no a priori difference between the two groups with a RD centered at 0) for the final analysis. A sample size of n=40 per group will have 80% power of detecting a RD of 10% or more with 67% certainty.

Potential Benefits

In general, women will be a part of a study that may potentially affect how we manage preterm labor in pregnancy. As a specific benefit, half of the patients will be given nifedipine-indomethacin, a combination tocolytic regimen that could reduce preterm birth more than single agent therapy. We expect the combination tocolytic regimen to be at least as effective as each single tocolytic, with an average delay in delivery of at least 22 days as reported in

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previous studies.[23] Of course, patients may deliver prior to this time, or may have a longer pregnancy duration.

Known Potential Risks

For this comparative effectiveness trial, there is little incremental risk as patients are receiving clinically accepted treatment regimens and the research aspect of this study is being randomized to one of these two treatment regimens vs. being assigned by treating physician. It is the practice of some physicians to use multiple tocolytic agents in tandem. There is a chance of additive maternal and fetal side effects with the two drugs being used together; however, many of the side effects are mild and reversible with the cessation of the medications. In previous trials using combination tocolytics, the risk of adverse side effects was not significantly amplified in the combination therapy group. [28] It is unlikely that the side effects will be synergistic as the mechanisms of action of indomethacin and nifedipine differ significantly. Preliminary assessments of interaction on pharmacological engines such as the FDA and Epocrates show no known interactions between indomethacin and nifedipine.

Nifedipine

Nifedipine is a category C medication and is generally well tolerated. There are no associated teratogenic abnormalities in human pregnancies.[33] The most frequently reported side effect in pregnancy is headache (7.5-31.2%) which is associated with transient hypotension caused by loading doses.[22,31,34] Other reported adverse effects include flushing/heat sensation (5-43.7%), dizziness/lightheadedness/giddiness (6-27%), weakness (12%), nausea (11%), muscle cramps (8%), peripheral edema (7%), nervousness/mood changes (7%), palpitations/tachycardia (7%), dyspnea (6%) and nasal congestion/sore throat (6%).[22,33,34] Nifedipine does not appear to influence fetal movement, heart or blood flow and thus does not have any direct fetal effects.[31] In pregnancies with multiple gestations, nifedipine has been associated with increased dyspnea.

Indomethacin

Indomethacin is a category C medication.[35] Fetal exposure to indomethacin is associated with the development of antenatal closure of the ductus arteriosus, [37-39] antenatal oligohydramnios,[40,41] postnatal persistent patent ductus arteriosus,[42] necrotizing enterocolitis,[43] intraventricular hemorrhage[44] and postnatal renal failure[45] as reported clinically and in studies in rodents[46-48]. Many of these adverse events occur with prolonged courses of indomethacin administered for both greater than 48 hours and over 32 weeks gestation.[10,13,49] Other potential risks include nausea/vomiting/heartburn/abdominal discomfort (3-9%) and headache (11.7%).[35]

Although employed in clinical practice at times, combination nifedipine-indomethacin has not been studied within a randomized controlled trial. Prior studies have raised concern for combination tocolytic agents due to reported cases of pulmonary edema, myocardial infarction and cardiovascular collapse.[28,50] However, these included ritodrine, magnesium and terbutaline, which as individual agents increase this pulmonary risk. Thus, these risks are minimal with our study combination regimen.

Breastfeeding

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Tocolysis is generally administered during the antepartum period and will not be used postpartum. Therefore, nifedipine and indomethacin should not affect breastfeeding.

Blood Draw with Venipuncture Blood
Blood draws will be obtained from the maternal veins up to three times during the study. Possible side effects of obtaining blood samples are pain, bruising, bleeding or infection at the blood drawing site and rarely, nausea or a lightheaded feeling. A skilled nurse will be retrieving the maternal blood. A maximum of 2 tablespoons (20 ml) of blood will be obtained at each sampling for total maximum of 6 tablespoons (60 ml) for the duration of the study

Cord blood collection
Cord blood collection after delivery of the newborn and prior to delivery of the placenta is a routine procedure at the time of delivery. Therefore, the risk to the subject is minimal. Approximately 2 tablespoons (20 ml) of cord blood will be obtained.

Myometrial biopsy
The biopsy is performed at the site of the uterine incision as described in the methods. This uterine incision is closed using a continuous, locking suture to control bleeding. Therefore, the risk of bleeding in minimal since this site is sutured independent of this study.

Placental biopsy
The risk of a placental biopsy is minimal since this is performed after delivery of the placenta.

Neonatal follow up
Neonatal follow up in the study will not differ from the follow up of neonates whose mothers received single tocolytics. Neonates receive routine follow up by the pediatrician or neonatologist if the admitted to the neonatal intensive care unit (NICU). If neonatal exam is concerning in an infant antenatally exposed to indomethacin (hypotension, cardiac murmur that does not resolve), an echocardiogram will be obtained at that point to assess the ductus arteriosus. There are no known fetal effects of nifedipine. Enrollment in the study will not incur extra time commitment for mother or baby aside from the initial 48 hours to administer tocolytic therapy.

Cost and funding
There is no additional cost for participation in the study, and thus, no funding is necessary for the study.

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