Intrapartum maternal oxygen supplementation:

Effects on the mother and neonate

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Specific Aims

Intrapartum oxygen (O_2) supplementation is nearly ubiquitous on Labor and Delivery units, with over 60% of the 2 million laboring patients per year in the U.S. receiving supplemental O_2 at some point during labor(1). O_2 is most commonly administered to laboring patients with Category II electronic fetal monitoring (EFM) – a broad class of EFM used to identify patients at risk for fetal acidemia – with the theoretic rationale that maternal O_2 supplementation increases O_2 transfer to the fetus, thereby reversing hypoxia and preventing acidemia (2). This practice has become standard of care, yet there is no evidence of benefit and even concern for possible harm (1, 3-5). Therefore, there is an urgent need to fill this important knowledge gap.

Our central hypothesis is that prolonged intrauterine exposure to O_2 during labor, compared with room air (RA), generates free radicals that have downstream biochemical and clinical consequences to the neonate. This hypothesis is founded on several lines of evidence. *First*, neonatal resuscitation *post-delivery* with 100% O_2 is associated with an increased risk of free radical disease including retinopathy, hypoxic ischemic encephalopathy, and cerebral palsy (6). *Second*, preliminary studies on *intrapartum* O_2 administration suggest possible harm to the fetus. In patients undergoing scheduled cesarean deliveries, administration of just 60% O_2 was associated with increased free radical activity in both the mother and fetus (8). In other small clinical trials comparing O_2 to RA in laboring women, those receiving O_2 were up to four times more likely to have neonates with decreased umbilical artery pH and an increased need for delivery room resuscitation. (4,5) However, none of these studies investigated mechanisms for harm with excess O_2 *during labor* or neonatal consequences, such as neurodevelopmental outcomes, past the delivery room. (7) *Third*, our preliminary data from a large observational cohort showed that intrauterine hyperoxemia, compared to normoxemia, was associated with an increased risk of neonatal morbidity in acidemic neonates. *Finally,* prior animal and human magnetic resonance imaging (MRI) studies have demonstrated distinct changes in the hypothalamus, hippocampus, and white matter in neonates exposed to O_2 *after birth,* revealing potential regions of the brain susceptible to hyperoxia-associated injury mediated by free radicals (7-9). Taken together, these data suggest that the use of RA for intrauterine resuscitation may be safer than O_2. Analysis of a recently completed pilot RCT at our institution comparing O_2 to RA in laboring women with Category II EFM demonstrated feasibility of a randomized trial in this setting. Therefore, we will test our hypothesis by pursuing the following specific aims:

Primary Aim: **Conduct a randomized controlled trial of maternal O_2 supplementation versus RA in patients with Category II EFM in labor to:**

a) **Determine the effect of RA, compared with O_2, on maternal and fetal free radical activity.** We will test the *hypothesis* that RA, compared with O_2, for Category II EFM in labor will result in lower maternal and umbilical cord levels of malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), byproducts of free radical mediated lipid peroxidation and markers of oxidative stress that is associated with neonatal morbidity.
b) **Investigate the effect of *duration* of RA or O_2 exposure on maternal free radical accumulation.** We will test the *hypothesis* that the magnitude of maternal free radical activity is directly related to duration of O_2 exposure by serially measuring maternal MDA and 4-HNE levels during labor.
c) **Investigate the effect of O_2 exposure on placental oxidative stress.** We will test the hypothesis that intrapartum O2 exposure is associated with increased local oxidative stress in the placenta, as represented by 4-HNE.

Secondary Aim 1 (Exploratory): Determine the impact of RA, compared with maternal O_2 supplementation, on neonatal outcomes in women with Category II EFM in labor. We will evaluate a composite outcome of neonatal morbidity including neonatal death, hypoxic-ischemic encephalopathy, seizures, hypothermia treatment, intubation, mechanical ventilation, and meconium aspiration syndrome in patients randomized to O_2 or RA in labor.

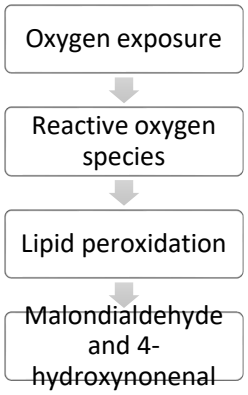
Secondary Aim 2: **Determine the relationship between maternal serum and urine markers of oxidative stress during labor**

The expected outcome of this proposal is high-quality mechanistic evidence for the pathophysiology of in-utero oxygen exposure. If RA is proven to be a more effective and safer option than O_2, it will change the clinical approach to intrauterine resuscitation of millions of women who labor and deliver each year in the U.S.

Background

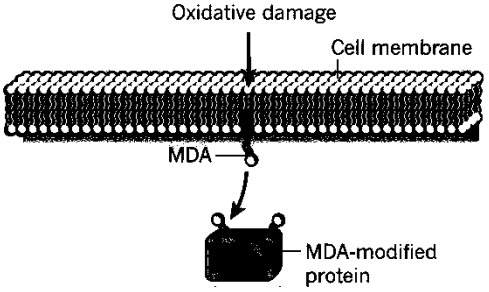
Oxygen use on Labor and Delivery: Maternal oxygen (O_2) administration for electronic fetal monitoring (EFM) changes is common practice on Labor and Delivery units in the Unites States. In fact, two-thirds of laboring patients receive supplemental O_2 at some point during their labor [1]. Category II EFM, as defined by the National Institute of Child Health and Human Development (NICHD), is a broad class of EFM designed to identify those at risk for neonatal acidemia from prolonged hypoxia [17]. O_2 is administered to the mother for the theoretical benefit of increasing O_2 transfer to the fetus, thereby preventing or reversing fetal acidemia. Maternal O_2 supplementation is recommended as an intrauterine resuscitative measure for Category II EFM by the American Congress of Obstetricians and Gynecologists with little to no evidence on the safety, duration, or dose of O_2 that is appropriate for use. In a recent review, Hamel et al concluded that there is insufficient evidence for routine O_2 administration for intrauterine resuscitation and that such administration may even be *harmful* to the mother and fetus via production of free radicals [1].

Figure 1: Oxidative stress and production of malondialdehyde



Oxidative stress from hyperoxygenation: Excess O_2 exposure leads to the production of free radicals, an unstable and reactive chemical species [18]. Cells produce free radicals, or reactive oxygen species (ROS), as byproducts of O_2 metabolism. Hyperoxia and reoxygenation, particularly after a period of *hypoxia*, may induce free radical generation [18, 19]. The production of free radicals causes a domino effect in which additional free radicals subsequently form and accumulate, leading to oxidative stress [20]. The downstream effects of oxidative stress include cell membrane and DNA damage with potential for carcinogenesis[21]. Malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), well-studied markers of oxidative stress [22-24], are surrogates for ROS induced lipid peroxidation in the cell membrane (Figures 1 and 2). Markers of oxidative stress such as MDA and 4-HNE are standardly used to quantify free radical activity because direct measurement of free radicals is limited by their short half-lives.

Figure 2: Lipid peroxidation in the cell membrane. Adapted from Cruz-Guilloty et al[2]



Although several markers of oxidative stress exist, MDA and 4-HNE are particularly relevant as it has been linked to both adult and neonatal morbidity. In adults, increased MDA levels are associated with "disruption of surface receptor pathways and clinical diagnoses such as atherosclerosis and cardiovascular disease [25, 26]. Elevated MDA levels in the neonate are associated with hyperbilirubinemia and hypoxic-ischemic encephalopathy [27-29]. There is also evidence that MDA can be transplacentally transported and thus, the mother and fetus are at risk for downstream effects [30]. Elevated 4-HNE is associated with injury to developing neurons[34,35] and has been implicated in the pathogenesis of morbid diseases such as Alzheimer's disease, atherosclerosis, and malignancy.

Excess intrauterine oxygen exposure may be harmful to the neonate: Several studies evaluating hyperoxygenation in adults and animals have demonstrated detrimental cardiac, pulmonary, and cerebral effects [16, 31-34]. Over-oxygenation in neonates is associated with free radical diseases including retinopathy, bronchopulmonary dysplasia, hypoxic ischemic encephalopathy, and cerebral palsy [1, 5-9]. As a result, the American Academy of Pediatrics now recommends initial neonatal resuscitation with room air (RA) instead of O_2 [35]. The detrimental postnatal effects of hyperoxia call for a closer look at intrauterine O_2 exposure. The two small published clinical trials comparing O_2 to RA in laboring patients with reassuring EFM found that more neonates in the O_2 group required delivery room resuscitation and that O_2 exposed neonates were more likely to have umbilical artery (UA) pH values <7.20 [11, 12]. Our preliminary data also suggest an increased risk of neonatal morbidity in acidemic neonates with hyperoxia (*see C1-1*)[36]. *The results of these*

clinical studies raise important safety questions about risks to the neonate with liberal use of intrapartum O_2 but do not address the primary indication for O_2 use: Category II EFM.

Research Design and Methods

Overview

We propose a randomized controlled trial to investigate maternal and fetal free radical activity in laboring patients with Category II EFM exposed to O_2 or RA. A randomized controlled trial, the 'gold-standard" of study design, was chosen to produce high quality evidence on the safety of O_2 administration for Category II EFM. *Our central hypothesis* is that prolonged intrauterine exposure to O_2 during labor, compared with RA, generates free radicals that have downstream biochemical and clinical consequences to the neonate. This trial will be conducted on Labor and Delivery (L&D) at Washington University Medical Center (WUMC). Patients ≥37 weeks gestational age in active labor (≥6 centimeters cervical dilation) with Category II EFM necessitating intrauterine resuscitation (as determined by the care provider team) will be randomized to RA or maternal O_2 administration of 10L/min via nonrebreather face mask until delivery. All other resuscitation techniques (maternal repositioning, intravenous fluid bolus, etc) will be left to the discretion of the care provider team. EFM strips will be read and interpreted by the study nurse coordinator. Maternal blood, collected at randomization and within 1 hour post-delivery, placenta and umbilical artery blood collected at the time of delivery will be used to run MDA analyses. Maternal urine will be collection on enrollment (prior to rupture of membranes or cervical dilation <6cm), at time of randomization, and within 1 hour of delivery. At any of these time points, if patient has a foley catheter, the urine may be collected from the catheter. If patient does not have a catheter, either a straight catheterization can be performed or a clean catch sample can be obtained. The same commercially available assays used to quantify MDA and 4-HNE in our blood samples will be used for the urine samples. Study staff will extract data from medical records including maternal demographics, antepartum history, intrapartum management, delivery outcomes, and neonatal outcomes. The patient's primary nurse will perform real-time documentation of O_2 or RA initiation, any disruption in intervention, administration of amnioinfusion and additional resuscitation techniques performed (number of times patient is repositioned, total intravenous fluids, discontinuation of oxytocin).

Conduct of Trial

Study site and population: There are approximately 4000 deliveries per year at Barnes Jewish Hospital (BJH). BJH is an academic, tertiary care center. As a large urban referral hospital, there is a diverse patient population composed of 55% Caucasian, 30% African American, and 15% other racial groups. Patients are largely managed by resident physicians under the supervision of faculty of the Washington School of Medicine (WUSM) and non-academic community OB/GYN physicians.

Inclusion and Exclusion criteria: Patient meeting the eligibility criteria in Table 1 will be approached for enrollment in the trial:

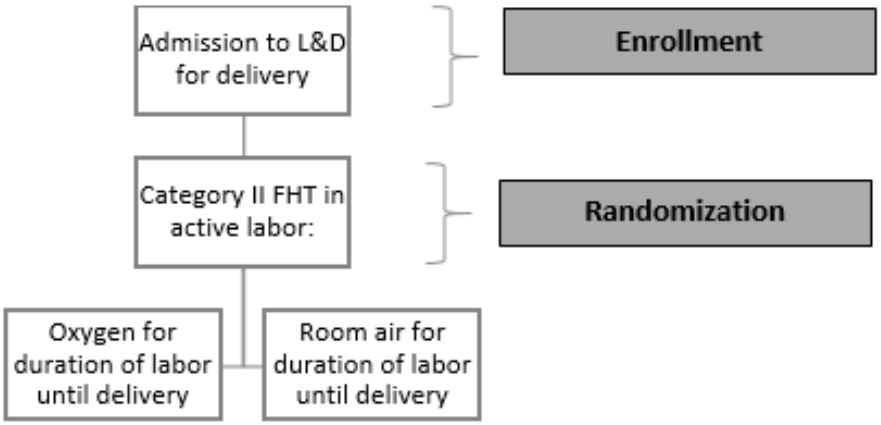
Table 1: Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria (Rationale)

• Singleton term pregnancy: gestational age ≥37 weeks • Admitted for spontaneous labor or induction of labor • English speaking • Ability to give informed consent • COVID-19 Negative Result within the last 5 days, previouslyCOVID-19 positive, but meet CDC criteria considered to be “non-infectious”, have a pending COVID-19 test with no symptoms of COVID-19, and/or has been vaccinated for COVID-19 and no recent COVID-19 exposures.	• Major fetal anomaly (increased a priori risk for neonatal morbidity) • Multiple gestation (unique considerations for labor management) • Category III EFM (need for expedited delivery) • Maternal hypoxia (Absolute indication for O2 administration) • Umbilical artery Doppler abnormalities (placental insufficiency that impacts O2 transfer to fetus) • Preeclampsia (increased a priori risk for oxidative stress) • Intrauterine growth restriction (increased a priori risk for oxidative stress) • Pregestational diabetes (increased a priori risk for oxidative stress) • Current tobacco smoker • COVID-19 Positive and do not meet CDC criteria for “non-infectious" • Declined COVID-19 Testing

Recruitment of Subjects and Randomization: We will use established recruitment techniques employed in recent RCTs performed on L&D at BJH. All women admitted to L&D in spontaneous labor or induction of labor will be screened with the above inclusion and exclusion criteria. Eligible subjects will be approached for written consent to participate on admission to L&D, irrespective of starting cervical dilation. Randomization will be performed only when the patient is in active labor (defined as cervical dilation ≥6cm) and develops Category II EFM necessitating intrauterine resuscitation as determined by the care provider team **(Figure 3).** Using a computer-generated sequence in block sizes of 4, patients will be randomized in a 1:1 ratio to either O_2 or RA.

Figure 3: Enrollment and Randomization of Study Participants



Intervention

1. **O_2 group:** Patients in this group will receive O_2 via nonrebreather face mask at 10L/min (FiO_2 approximately 0.80) [55]

2. **RA group**: Patients in this group will be exposed to RA only and not wear an O_2 mask unless required for maternal hypoxia (FiO_2 approximately 0.21).

Primary Aim 1

Determine the effect of RA, compared with O_2, on maternal and fetal free radical activity.

1. Hypothesis: RA, compared with O_2, for Category II EFM in labor will result in lower mean maternal and umbilical cord levels of malondialdehyde, a byproduct of free-radical-mediated lipid peroxidation and a marker of oxidative stress.
2. Rationale: MDA and 4-HNE are downstream products of free radical buildup from excess O_2 and are known markers of oxidative stress.. The current use of O_2 for Category II EFM may result in prolonged exposure that generates free radicals and resultant oxidative stress.
   To explore the feasibility of MDA analysis in laboring patients and to provide preliminary data for sample size calculation, we performed the Thiobarbituric Acid Reactive Substances (TBARS) assay for MDA quantification in a subset of maternal (n=27) and UA (n=33) samples exposed to O_2 in the above mentioned pilot trial. The mean maternal MDA was 6.5 μM ± 1.0 and the mean UA MDA was 8.3μM ± 2.0. Although no conclusions regarding oxidative stress can be made from this data alone, these results demonstrate feasibility of MDA analysis in this setting.
3. Data collection**:** We will compare maternal and fetal free radical mediated oxidative stress between O_2 and RA groups by measuring MDA and 4-HNE in maternal and umbilical cord blood and placenta samples. Maternal blood will be drawn at time of randomization and within 1 hour of delivery. Per the BJH universal cord gas policy, a segment of the umbilical cord and placenta will be removed after delivery of the infant and trained providers will immediately collect UA and umbilical vein samples for routine cord gas analysis. An additional UA sample will be collected for the purpose of this study. We will employ the services provided by the WUMC Women and Infants Health Specimen Consortium to collect samples in EDTA tubes, centrifuge them, and store the plasma in-80°C freezers.
4. Outcome: The *primary outcome* is mean maternal and UA MDA and 4-HNE at time of delivery. We will collaborate with the Washington University at St Louis (WUSTL) Core Laboratory for Clinical Studies (CLCS) to perform the Thiobarbituric Acid Reactive Substances (TBARS) assay for MDA quantification[24, 56] This assay is performed by adding TBA to plasma and performing an ELISA to fluorometrically measuring the resultant MDA-TBA complexes. The CLCS will use the commercially available OxiSelect™ TBARS Assay Kit (Cell Biolabs, Inc) to perform these studies. We will use the Oxiselect™ HNE ELISA kit (Cell Biolabs, Inc) to quantify 4-HNE. 4-HNE quantification in placental villi will be determined using immunohistochemistry (IHC). Secondary outcomes include UA cord gases and clinical measures of neonatal morbidity including NICU

admission, neonatal death, intubation, mechanical ventilation, hypoxic ischemic encephalopathy, meconium aspiration syndrome and need for hypothermia therapy.

5. Data analysis: The primary analysis will be by intention-to-treat. Baseline demographics and secondary outcomes will be compared between RA and O_2 groups using χ^2, Fisher exact test, Mann-Whitney *U* test, or Student's *t* test as appropriate. The primary outcome, mean maternal and UA MDA, will be compared between groups using the Student's t test if normally distributed. If non-normally distributed, the median maternal and UA MDA levels will be compared between groups using the Mann-Whitney U test. Linear regression will be used to adjust for relevant covariates. We will perform supplementary analyses including 1) stratified analysis by presence of maternal comorbidities or fetal growth restriction to assess if the effect of O_2 on oxidative stress varies in patients with fetal growth restriction or comorbidities. (2) as-treated analysis to determine whether compliance with the assigned group affects outcomes.
6. Sample size calculation: Based on pilot trial data, the mean UA MDA in 33 patients receiving O_2 for Category II EFM in active labor is 8.3 ± 2.0 μM. Assuming a two-sided alpha of 0.05, a total **212 patients (106 O_2 and 106 RA)** will afford 80% power to detect a 10% difference mean UA MDA between O_2 and RA groups, while accommodating for 15% loss due to UA samples. A 10% difference in UA MDA levels may be meaningful as neonatal MDA differences as small as 3-5% are associated with free-radical mediated injuries such as hypoxic ischemic encephalopathy [28, 57] There is limited published data on maternal MDA levels and associated adverse outcomes. However, this sample size will afford >90% power to also detect a 10% difference in maternal MDA between groups. To accommodate a compliance run-in of 6 patients, a total of **218 patients** will be recruited.

   Feasibility of the sample size: BJH delivers approximately 4,000 patients per year. Based on results of our pilot trial, 32% of the approached patients meeting eligibility criteria consented to participation in the study. Of the patients enrolled, 40% met criteria for randomization (Category II EFM necessitating intrauterine resuscitation after 6cm dilation). In total, 114 patients were randomized over the study period of 12 months. Therefore, we anticipate successful enrollment of 212 patients within 2.5 years (70-72 patients/year).

Secondary Aim 1:

Investigate the relationship between duration of RA or O_2 exposure and magnitude of oxidative stress

1. Background, rationale, and hypothesis: Khaw et al demonstrated a dose response relationship between maternal MDA concentration and duration of O_2 exposure in a RCT of patients randomized to 60% O_2 by facemask or RA during scheduled cesareans [10]. In an analysis of 27 maternal MDA samples among patients randomized to O_2 in our pilot trial, we found higher median MDA levels in patients who received O2 for 30-60 mins compared to those who received O_2 for <30 mins. This trend in maternal MDA, particularly in the first 60 minutes of O_2 exposure, suggests a dose-dependent relationship between

duration of O_2 exposure and maternal oxidative stress (unpublished, Raghuraman et al). Here, we will test the hypothesis that duration of O_2 exposure correlates with levels of maternal MDA and 4-HNE.

2. Data collection*:* Study staff will draw maternal blood at time of randomization and within 1 hour of delivery. Because patients will be randomized to O_2 or RA when they have Category II EFM at any point after 6cm cervical dilation, durations of exposure will vary within groups. Since it is not feasible to sample the umbilical cord at multiple time points, this analysis will be restricted to maternal MDA and 4-HNE.
3. Outcome and data analysis: The primary outcome is mean maternal MDA and 4-HNE in O_2 exposed patients. We will compare mean MDA and 4-HNEacross 5 pre-specified durations of O_2 exposure (<30 mins, 30-45 mins, 45-60 mins, 60-90 mins, or >90 minutes) using the one-way ANOVA test. If results of the ANOVA test suggests unequal means, the Tukey post hoc test will be used to identify the groups that differ. Linear regression will also be used to assess the effect of duration of exposure on MDA and 4-HNE concentrations.
4. Estimated statistical power: Maternal MDA differed significantly between 22 patients exposed to O_2 for <30 mins and 20 patients exposed to O_2 for 30-60 mins (unpublished, Raghuraman et al). Based on these data we estimate that the sample size of 212 (106 in the O_2 group) will provide adequate power to detect differences between at least 2 of the 5 groups.

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Secondary Aim 2: **Determine the relationship between maternal serum and urine markers of oxidative stress during labor**

1. *Background, rationale, and hypothesis*: The majority of studies investigating MDA concentrations in the pregnant population have utilized serum or plasma samples. [48] In the non-pregnant population, urinary markers of oxidative stress have been proposed as an alternative to blood because they provide a less invasive way to assess oxidative status, can be used in large-scale studies, and may provide more accurate measurements of oxidative stress than blood because it is less liable to artificial increase of oxidative markers during sample collection and storage.[49] However, the effect of pregnancy physiology or labor on urine assessment of oxidative stress remains unknown. For example, glomerular filtration rate, renal plasma flow, proteinuria, and glucosuria increase in pregnancy[50]. The effect of these changes in renal physiology on oxidative stress marker analysis is unknown. Furthermore, urine may reflect changes in oxidative stress that are less acute than changes detected in blood (i.e longer time to detect a difference). Given limited data on urine analysis of MDA and 4-HNE in pregnancy and especially during labor, I will study the relationship between MDA and 4-HNE concentrations in paired maternal serum and urine samples to test the hypothesis that urine can serve as a less invasive and readily available alternative to serum to quantify and trend oxidative stress in labor.

2. *Data collection*: Among patients enrolled in the RCT that are undergoing an induction of labor, urine samples will be collected by study staff at three time points. First, at the time of admission prior to the onset of labor in

order to obtain a baseline assessment of oxidative stress. Second, at the time of randomization in active labor which will be a paired specimen with maternal blood collected at the same time. Third, within 1 hour of delivery which will also be a paired specimen with maternal blood collected at the same time. Urine samples will be collected either as-mid void clean catch specimens or directly obtained from foley catheters in patients with epidurals. Table 2 outlines all planned sample collections in this trial. Urine samples will be centrifuged and stored at -80°C. The same commercially available assays used in Aims 1 and 2 will be used for MDA and 4-HNE quantification. Urine specific gravity will be measured in all samples to account for degree of urine dilution.

Table 2: Timing of all sample collection

Sample	**Time of collection**
Maternal blood (serum)	-At randomization (active labor with Category II EFM) -Within 1 hour of delivery
Umbilical artery blood (serum)	-At delivery
Maternal urine	-On admission or at enrollment (prior to labor onset) -At randomization -Within 1 hour of delivery

3. *Outcome and data analysis*: The primary outcome is MDA and 4-HNE urine concentrations. The primary outcome will be compared between room air and O_2 groups using Student's t test or Mann-Whitney *U* test, as appropriate. Linear regression will be performed to test the correlation between serum and urine concentrations. The mean MDA and 4-HNE urine concentrations throughout the three time points in labor (Table 3) will be graphed to assess baseline trends in oxidative stress over time within the room air group.
4. *Estimated statistical power*: Due to the exploratory nature of this aim, we will use a sample size of convenience and perform urine assessments in 100 patients (50 room air and 50 O_2). This aim will only include patients undergoing induction of labor, which comprises 70% of our cohort. These results will provide preliminary data regarding the feasibility of urinary oxidative stress analysis in labor and may allow for future oxidative stress studies to maximize patient participation by limiting invasive blood draws.

Safety Monitoring: The study team will review all neonatal adverse events and establish a data and safety monitoring board (DSMB), which will meet after 50% of participants have been recruited to review any adverse neonatal or maternal events among the study cohort and determine safety of continuing the study to completion. Reportable severe adverse neonatal outcomes include intrapartum stillbirth and neonatal death. Reportable non-severe adverse neonatal outcomes include need for hypothermia protocol, seizures, and prolonged NICU stay >72 hours. The study statistician will perform an interim analysis after 50% recruitment. The Haybittle-Peto stopping rule will be used in which a $p < 0.001$ for the difference in the primary outcome between groups will guide further conduct of the trial.

Anticipated challenges and safeguards: A potential limitation of the study is the inability to blind patients and healthcare providers to the allocation, however, the assessors will be blinded to allocation to limit bias in data analysis. Allocation concealment via use of opaque sealed envelopes will also limit selection bias. Bias could result from nurses and physicians managing subjects differently based on knowledge of their group assignment. To minimize this, providers will be specifically instructed to apply all other intrauterine resuscitation techniques as needed, irrespective of their group assignment. Any additional intrauterine resuscitation that is performed will be documented. To maximize adherence to protocol, we will conduct nursing and physician education sessions.

Protection of Human Subjects/Approvals

1. Risks to human subjects-
1A. Human subjects' involvement, characteristics, and design- The study will enroll 212 patients to investigate whether intrapartum O_2 administration in patients with Category II EFM is associated with oxidative stress. The study population will be English-speaking. Amongst the patients enrolled in the pilot trial, 77% were African American, 14% were Caucasian, 4% were Hispanic, and 5% were of other ethnicities. Dr. Raghuraman and the other study members have completed the training module required by the WUSM Human Research Program Office and received education on the responsible conduct of research. The preceding pilot trial was approved by the IRB at WUSTL (IRB ID 201602164) and registered at ClinicalTrials.gov (ID NCT02741284).

1B. Sources of materials- The study will involve collection of data from twokey components:
1) *Clinical Data Collection* from the medical records of mother and child. For all subjects, these data will be stored in a secure, password-protected REDCap database anonymized with study identifier only.
2) *Maternal and cord blood samples:* All blood samples will be stored in a locked freezer that only the research team has access to. The maternal/ cord blood and placenta outcomes assessed in this study are additional materials collected for the purpose of the study and are not a part of routine prenatal care. Maternal and cord blood/placenta malondialdehyde levels are not used clinically and at present, cannot be directly linked to clinical outcomes. This will be disclosed to all study participants.

1C. Potential Risks: The proposed study involves potentially rare risks to the subjects, including loss of confidential health information, provision of poor feedback to families regarding outcome data, physical risks of serial blood draws, and excessive demands on patients. We anticipate minimal risk to the neonate by "withholding" oxygen treatment for Category II EFM because (1) the clinical evidence thus far suggests that oxygen provides no benefit, and (2) there were no adverse outcomes amongst neonates in the room air group of my pilot trial. Protections against anticipated risks are outlined below under "Adequacy of Protection Against Risk."

2. Adequacy of protection against risks-
2A. Recruitment and Informed Consent: Pre-screening for eligibility criteria via medical record review will be conducted, and potential participants will be approached on Labor and Delivery. None of the data obtained in pre-screening will be saved. The study design, expectations, and risks/benefits will be explained, and potential subjects will be given a written informed consent form by a study team member. Consent will be documented by using a signed consent form. Trained study team members will have sufficient time to explain the study and the informed consent carefully in a private room, and eligible patients may decide to enroll at any time as long as they still meet eligibility criteria. Of note, our team has significant experience recruiting patients and neonates as part of prior investigations in this population.

2B. Protections Against Risk- Oxygen administration for Category II EFM is the current standard of practice. The safety of randomization to room air for Category II EFM has been demonstrated by the preceding pilot trial. All neonatal adverse events will be reviewed by the data and safety monitoring board (DSMB), which will meet after 50% of participants have been recruited to review any adverse neonatal or maternal events among the study cohort and determine safety of continuing the study to completion. The PI will be responsible for reporting all adverse events to the IRB.

Informed Consent: Informed consent will always be obtained before a subject participates in any component of the protocol. For all participants, the consent form will be explained to participants as well as having them read the consent themselves. This consent will contain a detailed description of all study procedures, as well as any possible risks and/or benefits. Patients will be asked to sign the consent form. It will be clearly communicated to the patients during the consent process that their decision to participate will not affect their obstetric care. A copy of the consent forms will be kept in a locked cabinet in the research office and participants will be given a copy of the consent forms for their own records.

Blood draws: The malondialdehyde (MDA) test results are for research purposes only. MDA levels are not used for clinical purposes. They will not be available to the care provider team and will only be accessible to the research team. Samples will be stored in a locked freezer. Any future laboratory studies performed on

these samples will be for research purposes only. To minimize the potential physical risks of serial blood draws including bruising, bleeding, and discomfort, trained and experienced research nurses will perform the blood draws. Umbilical cord blood and placenta sampling has minimal risks given the universal cord gas policy at our institution.

Burden to the Patient: The patients will not be charged for any evaluations, nor will they be obligated to continue with the study if they wish to withdraw. Such withdrawal will not affect any programs or services for which the child and/or mother would be otherwise eligible.

Additional Protections for Children: Per Missouri state law, any suspected case of child abuse or neglect must be reported to the Missouri Department of Social Services Children's Division. All participants will be informed of this risk during the informed consent procedures.

2C. Protection of Confidentiality*:* As with any research, there is a risk of accidental loss of privacy and inadvertent release of protected health information, and this can lead to psychological, social, or legal distress. Although these are serious risks for study subjects and their families, the likelihood of adverse effects from these potential risks within this protocol are extremely low when using the system of patient identifiers and unique research code numbers located in different locations and password protected datasets with limited accessibility. Patients will be consented in private rooms and will be notified that their decision to participate will not affect their medical care. Clinical data will be entered into Research Electronic Data Capture (REDCap), a secure, web-based application available through the Clinical and Translational Science Award (CTSA) at WUSM. Access to our REDCap system is protected by multiple layers of security, running on Unix platforms configured for strict user permission policies. Our REDCap database is hosted on Unix servers on a secure computer network. User access is provided via an external secure web server. All web communication between the user web browser and the web server is SSL encrypted. Further protections are provided via strict Unix permission configurations on the servers themselves. Access to the data username/password keys is restricted in accordance with our University's HIPAA policy.

3. Potential Benefits of the Proposed Research to Human Subjects and Others- As above, in the event that clinically significant findings and/or unanticipated abnormalities are found on the infant's brain scans, the family will be notified and appropriate referrals will be made in coordination with the primary clinical team. Beyond this, there will be no direct benefits to individuals participating in the proposed study other than the knowledge that they are contributing to an improved understanding of the safety of intrapartum oxygen use that can affect millions of laboring patients in the future.

4. Importance of the Knowledge to be Gained- If our hypothesis is confirmed that O_2, compared to RA, increases oxidative stress in laboring patients with Category II EFM, the results will revolutionize labor management for millions of patients annually. Room air would be a safer, perhaps more cost-effective, intervention for management of Category II EFM. Furthermore, knowledge about the relationship between duration of O_2 exposure and oxidative stress will guide future use of O_2 on Labor and Delivery. The data collected on neonatal morbidity will provide more information on the risks of O_2 exposure and guide future larger studies. Findings from this research will be published in peer-reviewed medical journals and presented at local, national, and international conferences.

5. Data and Safety Monitoring Plan- Data and research monitoring will be undertaken annually by the study's PIs and the WUSM IRB. Additionally, a Data and Safety Monitoring board (DSMB) will be established. The DSMB plan includes, but is not limited to, the following features:

Review of adverse events: All personnel in contact with participants and/or data are prepared to identify adverse events and have been instructed to report their occurrence immediately to the study PIs.

Reporting of serious adverse events: In the unlikely event of a serious adverse event, the PIs, in collaboration with any relevant personnel, will take the appropriate actions to report the event to the HRPO/IRB.

Criteria for stopping the study: An interim analysis will be performed after 50% recruitment. Using the Haybittle-Peto stopping rule, a P <0.001 for the difference in the primary outcome between groups will guide further conduct of the trial.

Inclusion of Women and Minorities: We anticipate that the majority of the subjects in this study will be African American. In the recently completed pilot trial, 77% of the patients were African American, 14% were Caucasian, 4% were Hispanic, and 5% were of other ethnicities.

Inclusion of Children: Pregnant minors are considered emancipated under Missouri state law and will be eligible to enroll in the study.

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