Fetal Metabolic Consequences of Late Preterm Steroid Exposure

NCT number NCT03076775
Document Date 08/09/2018

Fetal metabolic consequences of late preterm steroid exposure

PROTOCOL

Prepared by:
Ashley Battarbee, MD

The University of North Carolina at Chapel Hill
Department of Obstetrics and Gynecology
Division of Maternal Fetal Medicine
3010 Old Clinic Building Campus Box 7516
Chapel Hill, NC 27599
(919) 966-1601

August 9, 2018

1 Table of Contents

1. Introduction

1.1. Study Abstract

In the United States, preterm birth (PTB) at < 37 weeks is the greatest contributor to neonatal death. Late PTBs between 34 and 36 weeks comprise over 70% of all PTB.[1] Compared to term neonates, those born late preterm are more likely to have respiratory distress, hypothermia, and poor feeding. This increases hospital stay, NICU admissions, and hospital readmissions.[2] In a randomized clinical trial, maternal betamethasone (BMZ) given to women at risk of delivery in the late preterm period lowered neonatal respiratory complications, but increased neonatal hypoglycemia, compared to placebo.[3] Previous studies have demonstrated an association between antenatal BMZ and maternal hyperglycemia[4] as well as antenatal BMZ and fetal hyperinsulinemia.[5] Thus, neonatal hypoglycemia observed after antenatal BMZ for threatened late PTB is likely due to transient steroid-induced maternal hyperglycemia.

This protocol describes a multisite randomized controlled trial to define the fetal metabolic and hormonal response to late preterm BMZ exposure, and the effect of maternal glucose control on fetal metabolic and hormone levels.

1.2. Primary Hypothesis

BMZ exposure in the late preterm period is associated with fetal hyperinsulinemia as measured by umbilical cord blood C-peptide level. Compared to usual care (no glucose screening), regular screening for and treatment of maternal hyperglycemia following antenatal BMZ administration in pregnancies at risk for late preterm birth will result in lower umbilical cord blood C-peptide level.

1.3. Purpose of the Study Protocol

This protocol describes the background, design and organization of the randomized clinical trial and may be viewed as a written agreement among the study investigators. The Data and Safety Monitoring Board (DSMB) will review the protocol. Before recruitment begins, the protocol will be approved by the Institutional Review Board (IRB) of each clinical center. Any changes to the protocol during the study period require the approval of the IRBs; major changes also require the approval of the DSMB.

2. Background

2.1. Incidence and Complications of Late Preterm Birth

Preterm birth is the greatest contributor to neonatal death in the United States. In 2013, preterm births made up 11.4% of all live births and over 70% of all preterm births were between 34 weeks 0 days and 36 weeks 6 days gestation.[6] Based on a consensus statement of the NICHD workshop entitled "Optimizing Care and Outcome of the Near-Term Pregnancy and the Near-Term Newborn Infant," neonates born during this time period are considered to be "late preterm" neonates.[7]

While the etiology for late preterm birth can vary from spontaneous labor or premature rupture of membranes to iatrogenic delivery for maternal or fetal indications, studies have shown that regardless of indication, neonates born during the late preterm period are at increased risk for complications compared to those born at term. In a study of 21,771 late preterm births, specific neonatal morbidities such as ventilator-treated respiratory distress, transient tachypnea of the newborn, grade 1 or 2 intraventricular hemorrhage, sepsis, and

hyperbilirubinemia were all more common in late preterm compared to term neonates.[2] Other studies have also shown an increased risk for problems with temperature regulation and difficulty feeding with resulting hypoglycemia.[8,9] While mortality remains rare in the late preterm period, neonatal death after birth at 34, 35, and 36 weeks is still higher compared to death after delivery at term.

2.2. Effect of Antenatal Corticosteroids on Neonatal Morbidity

In 2016 an important step toward reducing respiratory morbidity in neonates born in the late preterm period occurred. A study of 2827 late preterm pregnancies showed a significant reduction in respiratory complications among neonates exposed to BMZ in utero compared to placebo (11.6 v 14.4%, p=0.02), however, neonatal hypoglycemia was more common in the BMZ compared to placebo group (24% vs 15%, p<0.001). There was no significant reduction in NICU admission (41.8 v 44.9%, p=0.09), suggesting that there is potential for additional improvement in late preterm neonatal outcomes.[3] Other studies have also demonstrated an increase in neonatal hypoglycemia after exposure to BMZ,[10,11] although the mechanism has not yet been studied.

2.3. Rationale for Neonatal Hypoglycemia after Antenatal Corticosteroids

Previous studies have shown that antenatal corticosteroids are associated with transient but significant maternal hyperglycemia. Almost all non-diabetic pregnant women who receive antenatal BMZ experience at least 1 capillary blood glucose (CBG) level above 140mg/dL, which persists for 3-4 days for most women.[4,12,13] Current clinical practice for non-diabetic women who receive BMZ does not include any CBG monitoring, and thus the opportunity to correct any maternal hyperglycemia is missed. While the neonatal effects of maternal hyperglycemia have not been studied in this context, umbilical cord blood studies have demonstrated increased fetal C-peptide levels after antenatal BMZ exposure.[5] This BMZ-induced fetal hyperinsulinemia suggests that the neonatal effects after BMZ may be similar to those seen in neonates of diabetic mothers with hyperglycemia.

Maternal hyperglycemia, even if transient, can lead to fetal hyperglycemia with subsequent fetal insulin production, placing the neonate at risk for hypoglycemia at birth. BMZ acts on multiple maternal tissues to increase maternal glucose: 1) BMZ decreases pancreatic insulin release, 2) BMZ increases insulin resistance in the liver, increasing glucose production, and 3) BMZ increases insulin resistance in the muscle and fat cells, decreasing glucose uptake. Elevated circulating maternal glucose crosses the placenta and subsequently stimulates the fetal pancreas to produce insulin. Neonates delivered during this transient period of hyperinsulinemia are then at risk for hypoglycemia at birth. Fundamental gaps in understanding the adverse maternal and fetal metabolic effects of antenatal late preterm steroids have limited our ability to achieve better neonatal outcomes.

2.4. Rationale for a Randomized Clinical Trial

Late preterm births at 34 weeks 0 days – 36 weeks 6 days gestation comprise over 70% of all US preterm births.[1] In the US annually over 300,000 neonates are born in the late preterm period. While a recent study of 2827 late preterm pregnancies showed a significant reduction in respiratory complications of neonates born to mothers treated with BMZ compared to placebo, this exposure was also associated with an increase in neonatal hypoglycemia.[3] The Society of Maternal-Fetal Medicine (SMFM) Statement in August 2016 acknowledged that "concern has been raised about the potential increased risks associated with neonatal hypoglycemia" following antenatal BMZ. SMFM reassured both obstetricians and neonatologists that "these [risks] should be minimized by the utilization of routine [postnatal] testing for all late preterm newborns, as advocated by the Committee on Fetus and Newborn of the American Academy of Pediatrics.[14]

Our proposal is innovative because we are challenging the current dogma regarding the management of non-diabetic women receiving antenatal BMZ in the late preterm period. The current approach of disregarding maternal hyperglycemia potentially diminishes neonatal benefits of antenatal BMZ. Despite the impact of late preterm BMZ on neonatal respiratory morbidity, the best strategy to optimize overall neonatal outcome, including preventing hypoglycemia, is unknown. We contend that neonatal hypoglycemia associated with antenatal BMZ in the late preterm period can be prevented, just like that seen in diabetic mothers.[15] Our proposal to test maternal glucose in non-diabetic women who receive BMZ in the late preterm period addresses the challenge of understanding the metabolic effects of antenatal BMZ. We expect to find that treatment of BMZ-induced maternal hyperglycemia decreases the frequency of neonatal hypoglycemia after birth. ***Completion of our study lays the foundation for a future multicenter trial with potential to change clinical practice for treatment of women with threatened late PTB.***

3. Study Design

3.1. Primary Research Question

This study will address the following primary research questions:
Aim 1: What is the association between late preterm steroid exposure and fetal C-peptide levels, compared to no steroid exposure?
Aim 2: What is the effect of screening for and treatment of BMZ-induced maternal hyperglycemia on fetal C-peptide levels.

3.2. Secondary Research Questions

Secondary research questions this study will address are:

- What are other fetal metabolic and hormonal effects of late preterm steroid exposure, compared to no steroid exposure?
- What are other fetal metabolic and hormonal effects of screening for and treatment of BMZ-induced maternal hyperglycemia, compared to usual care (no glycemic control)?
- What is the prevalence of neonatal hypoglycemia in the steroid exposed vs unexposed groups? Does screening for and treatment of steroid-induced maternal hyperglycemia decrease the prevalence of neonatal hypoglycemia?
- What is the degree and duration of neonatal hypoglycemia?
- Does screening for and treatment of steroid-induced maternal hyperglycemia decrease the prevalence of NICU admission?
- What is the effect of timing of delivery on fetal metabolic and hormonal levels?

3.3. Design Summary

This proposal includes use a retrospective cohort of 75 mother-neonate dyads and umbilical cord samples collected during the Maternal Oral Therapy to Reduce Obstetric Risk (MOTOR) trial, as well as a prospective cohort of 144 mother-neonate dyads prospectively enrolled in our randomized clinical trial.

Women who had enrolled in the MOTOR trial, now completed, will serve as unexposed controls for this new proposal, as antenatal BMZ was reserved for threatened PTB less than 34 weeks' gestation at the time the trial was conducted. The MOTOR trial was a randomized, blinded trial of 1,760 pregnant women with periodontal disease to measure the impact of periodontal treatment during pregnancy on maternal and neonatal outcomes.20 Of these, there are approximately 1500 stored umbilical cord blood specimens from otherwise healthy, non-diabetic women, 5% of whom delivered between 34 weeks 0 days and 36 weeks 6 days. We have

been granted access to use 75 umbilical cord blood samples which meet inclusion criteria as unexposed late PTB controls to compare to the samples we collect in our randomized clinical trial.

For this randomized clinical trial, we will enroll 144 non-diabetic women with singleton pregnancies who are receiving betamethasone between 34 weeks 0 days and 36 weeks 5 days in anticipation of late preterm delivery. Eligible, consenting women will be randomized to one of two management schemes:

1. Intervention group: maternal capillary blood glucose (CBG) screening with treatment of hyperglycemia with insulin per existing institution protocol for diabetic women. Guidelines based on the existing UNC-CH algorithm for treatment of diabetic women are demonstrated Tables 1 and 2 below.

Table 5 Maternal insulin algorithm if NPO

CBG q 1-2hr	IV Regular Insulin
<110mg/dL	Stop insulin infusion
111-140mg/dL	**1** U/hr
141-180mg/dL	**1.5** U/hr
181-220mg/dL	**2** U/hr
>220mg/dL	**2.5** U/hr & titrate

Table 6 Maternal insulin sliding scale if eating general diet

CBG fasting and 1hr post-prandial	SQ Insulin
<110 mg/dL	0 units
111-140 mg/dL	**1** unit if fasting BG*
141-180 mg/dL	**1** unit
181-220 mg/dL	**2** units
>220 mg/dL	**4** units & consider infusion

2. Control group: usual care with no CBG screening or treatment as is current protocol at UNC-CH as well as other participating institutions

3.4. Eligibility Criteria

3.4.1. Inclusion Criteria

1. Singleton Pregnancy. A multiple gestation reduced to singleton (either spontaneously or therapeutically) before 14 weeks 0 days by project gestational age (see Section 3.4.2 below) is acceptable.
2. Gestational age at time of 1st dose of betamethasone administration between 34 weeks 0 days and 36 weeks 5 days confirmed by study criteria (see Section 3.4.2 below)
3. Receiving or already received betamethasone for clinical indications within 6 hours of randomization.
4. Likelihood of delivery in the late preterm period (any one of the following):
    a. Membrane rupture as defined by visible leakage of amniotic fluid from the cervix, Positive AmniSure test, or the presence of any two of the following:
        i. Pooling of fluid in the vaginal vault
        ii. Positive nitrazine test
        iii. Ferning of vaginal fluid
    b. Preterm labor with intact membranes. Preterm labor is defined as at least 6 regular uterine contractions in 60 minutes and at least one of the following:
        i. Cervix greater than or equal to 3cm dilated
        ii. At least 75% effaced
    c. Maternal or fetal indication for delivery (either by induction of labor or cesarean section) at less than 37 weeks' gestation (e.g. placenta accreta).
    d. Delivery expected in no less than 12 hours and no more than 7 days, as deemed necessary by the provider. An induction must be scheduled to start by 36 weeks 5 days at the latest, whereas a cesarean delivery must be scheduled by 36 weeks 6 days at the latest.

5. Planned inpatient management until delivery.

3.4.2. Gestational Age Determination

Gestational age is determined in the following manner, and is denoted "project gestational age". The "project EDD", which is based on the project gestational age, cannot be revised once a determination has been made.

3.4.2.1. Spontaneous Pregnancies

This algorithm is also applicable to pregnancies where ovulation induction or artificial insemination was performed and is based upon a comparison of LMP and gestational age as assessed by the earliest dating ultrasound. If a patient has never received an ultrasound at the time of screening, she is automatically ineligible.

To qualify as the earliest dating ultrasound:

- If a very early ultrasound, it must be based on a fetal pole with a crown-rump length
- Gestational age cannot be determined by gestational sac measurement
- There must be a report or an image showing the ultrasound measurements or a note in the patient's chart documenting the date of the ultrasound and the ultrasound measurements or exact EDC or GA.

The first day of the last menstrual period (LMP) is determined, and a judgment made as to whether the patient has a "sure" LMP date.

1. If the LMP date is unsure, the ultrasound measurements obtained at the patient's first dating ultrasound examination are used to determine the project gestational age (Table 1). This ultrasound must have been performed before the time of screening.
2. If the LMP date is sure, project gestational age is determined by a comparison between the gestational age by LMP and by the earliest dating ultrasound. This ultrasound must have been performed before the time of screening.
    a. If the earliest dating ultrasound confirms the gestational age by LMP within the number of days specified in Table 1 below, the LMP-derived gestational age is used to determine the project gestational age.
    b. If the ultrasound determined gestational age does not confirm the LMP generated gestational age within the number of days specified in Table 1, the ultrasound is used to determine the project gestational age.

Table 1. Cutoffs for Using LMP to Determine Gestational Age for Sure LMP

Gestational age at first ultrasound by LMP	Method of measurement	Ultrasound agreement with LMP
≤ 8 6/7 wk	CRL	More than 5 days
9 0/7 wk to 13 6/7 wk		More than 7 days
14 0/7 wk to 15 6/7 wk	BPD, HC, AC, FL	More than 7 days
16 0/7 wk to 21 6/7 wk		More than 10 days
22 0/7 wk to 27 6/7 wk		More than 14 days
≥ 28 0/7 wk		More than 21 days

3.4.2.2. In-Vitro Fertilization

One exception to the algorithm above is the case where the patient has undergone in-vitro fertilization (IVF) to achieve pregnancy. If in-vitro fertilization is used (standard IVF, IVF with donor egg, or IVF with ICSI) and

- the embryo is transferred at 3 days of age, the project EDD is 263 days after the date of transfer;
- the embryo is transferred at 5 days of age, the project EDD is 261 days after the date of transfer.

3.4.3. Exclusion Criteria

1. Diabetes, pregestational or gestational
2. Any prior antenatal corticosteroid course during the current pregnancy
3. Systemic corticosteroid administration during pregnancy (at least 5mg oral prednisone or its equivalent for at least 3 weeks)
4. Planned outpatient treatment with antenatal corticosteroids
5. Current multiple gestation or multiple gestation reduced to a singleton gestation at or after 14 weeks 0 days by project gestational age either spontaneously or therapeutically
6. Fetal demise, or known major fetal anomaly, including cardiac anomaly and hydrops
7. Maternal contraindication to insulin: history of hypersensitivity reaction to any components of the medication
8. Delivery planned within 12 hours of randomization
9. Delivery planned at or after 37 weeks 0 days gestation
10. Delivery planned outside the E-ALPS Consortium
11. Participation in another interventional study
12. Participation in this trial in a previous pregnancy

3.5. Informed Consent Criteria

Written informed consent must be obtained before entry into the study. Full disclosure of the nature and potential risks of participating in the trial is to be made.

Each center will develop its own consent forms according to the requirements of its own institutional review board using the model consent forms in the Appendix. Each center will also develop its own patient research authorization documents, as required by the HIPAA Privacy Rule, following the guidelines of its own institution. A copy of the signed consent form for the study will be provided to the patient.

Women who are not fluent in English will be enrolled by a person fluent in their language. Both verbal and written informed consent and authorization will be obtained in that language; if this is not possible the patient will be excluded.

3.6. Randomization Method

The randomization sequence will be prepared in a permuted block design, stratified by study site and gestational age at trial entry (34 weeks 0 days – 34 weeks 6 days and 35 weeks 0 days – 36 weeks 5 days) to assure balance between the two groups with respect to anticipated differences within the patient populations and possible differences in other aspects of patient management. The study statistician will create the randomization scheme, and assignments will be centrally assigned using the Research Electronic Data Capture (REDCap) application. Enrolled subjects will be randomized in a 1:1 ratio to usual care (no maternal glucose screening or treatment) or to study intervention (regular maternal glucose screening and treatment).

The randomization sequence will be generated by a statistician and uploaded into REDCap. This sequence will be unknown by any other study personnel and will be unable to be accessed in REDCap by anyone other than the statistician. As such there is full concealment of randomization group until a patient is screened, meets all eligibility criteria, signs the consent form, and the randomization function in REDCap is utilized to place the subject in the control or intervention group. Once the subject is assigned to a group, this randomization cannot be undone.

4. Study Procedures

4.1. Screening for Eligibility and Consent

All pregnant women with a singleton pregnancy who are receiving betamethasone between 34 weeks 0 days and 36 weeks 5 days because of anticipated delivery before 37 weeks are potentially eligible for screening. The inclusion/exclusion criteria will be reviewed with each patient's chart. If a patient is eligible, the study will be explained to her and she will be asked to sign the informed consent form.

4.2. Randomization

Eligibility will be confirmed just prior to randomization. Subjects will be randomized by central assignments using the Research Electronic Data Capture (REDCap) application prior to or less than 6 hours after betamethasone administration. Once a patient's study identifier has been entered on the randomization log, that patient remains randomized regardless of whether the management protocol in each group (screening and treatment vs usual care) is adhered to.

4.3. Baseline Procedures

In addition to information collected for eligibility, project gestational age, and project EDD determination, the following information will be obtained at randomization from a patient interview followed by a review of her medical record:

- Demographic and anthropometric information: age, race, pre-pregnancy weight, current weight, and height
- Social history: marital status, years of education, alcohol use, tobacco use, and other maternal drug use
- Medical/surgical history: pre-existing maternal medical problems such as cardiac disease, asthma, infections, thyroid disorder
- Obstetrical history including value of diabetes testing in this pregnancy (1hr 50g glucose screen and 3hr 100g oral glucose tolerance test) and outcome of all prior pregnancies
- Current pregnancy complications including membrane rupture, preterm labor, bleeding, hypertensive disorders of pregnancy, oligohydramnios, non-reassuring fetal status, etc.

4.4. Patient Management and Follow-up

Other than the timing and frequency of maternal glucose screening and recommended thresholds for treatment of hyperglycemia detailed in the study protocol, women will receive standard care as defined by their clinical provider. Women randomized to the intervention arm will undergo screening for and treatment of maternal hyperglycemia until delivery or hospital discharge for a maximum of 5 days following BMZ.

All study neonates will have CBG testing within 2 hours of life. This is standard of care for neonates born at less than 37 weeks. As part of the study protocol, CBG will be measured within 2 hours of life on those neonates who are delivered at or after 37 weeks and 0 days. The remainder of neonatal care will be according to the clinical provider. Umbilical cord blood and placental samples will be collected after delivery to evaluate fetal metabolic status in utero as well as other genetic changes. All study specimens will be labeled with a unique study ID code. Personnel responsible for lab assays will receive the specimens in a coded fashion without any documentation of PHI or study arm.

- Placental biopsies:
    - 15 punch biopsies will be obtained from each placenta using a standard 3mm punch biopsy tool. Biopsies should be obtained 2 cm from the cord insertion site.
    - Three punch biopsy samples will be placed into 5 separate aliquot tubes.
    - 0.5mL AllProtect solution will be placed in 3 of the 5 aliquot tubes
    - The other 2 aliquot tubes will be empty
- Placental portions:
    - 2 placental portions should be removed using sharp dissection (scalpel or scissors). Each portion should measure approximately 0.5 by 0.5 inches and should traverse the complete depth of the placenta.
    - Each placental portion should be placed into a 15mL falcon tube
    - The distance from the cord insertion should be recorded. One portion should be removed 2 cm from the cord insertion. The other should be removed 2 cm from the periphery.

While maternal CBG will only be performed for maximum of 5 days following BMZ, maternal postpartum data will be collected through hospital discharge. Neonatal data will be collected through hospital discharge or until 28 days of life.

4.5. Adverse Event Reporting

Detailed information concerning adverse events will be collected and evaluated throughout the conduct of the protocol. Patients will be questioned in a non-directed manner regarding side effects or symptoms associated with the management plan daily after receipt of betamethasone for maximum of 5 days.

In addition, an Adverse Event Form will be completed for any event that is serious, deemed potentially related to the study, and/or unexpected in nature, severity or frequency. The Adverse Event form will be sent by fax within forty-eight hours to the Primary Investigator. The Primary Investigator will be notified immediately (within 24 hours) of any maternal, fetal, or neonatal deaths. If a death is reported, a copy of the patient's medical record will be made. Adverse events will be reported to the Data and Safety Monitoring Board (DSMB), and will be considered along with other interim safety data in their deliberations.

The Common Terminology Criteria for Adverse Events (CTCAE) scale will be used to evaluate adverse events in a consistent manner across all sites. Maternal hypoglycemia is a known adverse event which can result from overtreatment with insulin. The following grading scale will be used to define the severity of the adverse event.

Adverse Event	Grade 1 (Mild)	Grade 2 (Moderate)	Grade 3 (Severe, medically significant)	Grade 4 (Life-threatening consequences)	Grade 5 (Death)
Hypoglycemia (mg/dL)	< LLN – 55	< 55 – 40	< 40 – 30	< 30 or seizures	Death

Subject stopping rules will include:

- CTCAE grade 4 or 5 hypoglycemia
- Patient requests to withdraw from study

Study stopping rules will include:

- CTCAE grade 4 or 5 hypoglycemia in more than 33% of patients receiving intervention
- CTCAE grade 3 hypoglycemia in more than 50% of patients receiving intervention

Other potential reasons for stopping the entire study include: occurrence of any grade 3 (severe) or grade 4 (life-threatening) SAE in 50% percent of subjects that an independent medical monitor and DSMB believe is likely related to active treatment.

4.6. Study Outcome Measures and Ascertainment

4.6.1. Primary Outcome

Fetal C-peptide levels (in mg/dL), measured in umbilical venous blood

4.6.2. Neonatal Secondary Outcomes

1. Fetal cortisol, insulin-like growth factor, leptin, and other hormonal and metabolic markers
2. NICU admission and length of stay
3. Neonatal hypoglycemia, defined as CBG < 40mg/dL within the first 48 hours of life
4. Neonatal blood glucose nadir (in mg/dL)
5. Timing of neonatal blood glucose nadir (in minutes of life)
6. Severe neonatal hypoglycemia, defined as composite of:
    a. CBG < 25mg/dL at 0-4 hours of life
    b. CBG < 35mg/dL at 4-24 hours of life
7. Symptomatic hypoglycemia, defined as CBG < 40 and one of the following: poor feeding, irritability, tremors, jitteriness, exaggerated Moro reflex, lethargy, seizure, poor tone, persistent hypothermia
8. Neonatal hypoglycemia treatment (i.e. measurable feeding, glucose gel, glucose-containing IVF)
9. First repeat neonatal blood glucose after treatment
10. Seizures or encephalopathy due to hypoglycemia
11. Neonatal mortality

4.6.3. Maternal Secondary Outcomes

1. Maternal hyperglycemia, defined as intrapartum CBG >100 mg/dL, fasting CBG >95mg/dL or 1-hour postprandial CBG >140mg/dL
2. Maternal treatment with insulin (total units required)
3. Maternal hypoglycemia, defined as CBG< 60 mg/dL or < 80 mg/dL with symptoms including hunger or nausea, lightheadedness or dizziness, sweating or chills, irritability, tingling or numbness in lips or tongue, weakness or fatigue, seizure, loss of consciousness

5. Statistical Considerations

5.1. Sample Size and Power

For the ALPS trial at UNC-CH we annually enrolled 40-50% of all late PTBs. Thus, during the 18 month recruitment for this proposed study, we expect ~300 women to present with threatened late PTB and 144 who will enroll and be randomized (72/group). We expect no more than 10% loss to follow-up with a total of 70% delivering within 3 days of BMZ (50/group) during the period of maternal hyperglycemia when our intervention has the greatest benefit.

Table 4. Power for Sample Size = 144 (α=0.05)

Power (%) if 10% loss to follow-up (n=130)	Power (%) for subgroup if 70% deliver in 3 days (n=100)	Mean difference in C-peptide level (mcg/L)	Standard deviation	Sample size needed
95	85	0.5	0.75	84 (42/group)
95	**90**	**1**	**1.5**	**98 (49/group)**
90	80	1.5	2.5	90 (45/group)
98	90	1.5	2	78 (39/group)

As shown in Table 4, this sample size provides reasonable power to detect a clinically meaningful difference in C-peptide levels between the intervention and usual care groups after BMZ exposure. This calculation was performed using a range of expected primary outcomes based on previously reported values (normal C-peptide 0.6-1.0mcg/L, 95th percentile C-peptide 1.77mcg/L, C-peptide after antenatal BMZ exposure 2.85mcg/L) under conservative scenarios. Further, this sample size will be sufficient to detect differences in cortisol and insulin-like growth factor between these groups with more than 80-90% power based on previously reported values and the same assumptions above.

In addition to these 144 women enrolled prospectively, we will have access to 75 umbilical cord blood samples from the MOTOR trial. With this available sample size based on the same conservative scenarios in Table 4, we will have ≥80% power to compare C-peptide levels between unexposed controls and women exposed to BMZ who are randomized to usual care.

5.2. Interim Analysis

The Data and Safety Monitoring Board meets monthly; however, a formal interim analyses for overwhelming efficacy is planned after 50% of recruitment is completed (n=75). For the planned interim analysis, a formal detailed statistical report will be written by Jeffrey Laux, PhD, Biostatistician including protocol adherence, the primary outcome, and adverse events reported as well as center performance in terms of recruitment, data quality, loss to follow-up and protocol violations.

The main statistical issue relevant to interim analysis is the problem of performing multiple tests of significance on accumulating data. A number of procedures have been developed to handle this situation. For this trial, the group sequential method will be used to control the type I error with the Lan-DeMets spending function and the O'Brien-Fleming type boundary. Assuming that the analysis is conducted at precisely 50%, this implies that the alphas to be used will be 0.003 and 0.049 at the interim and final analyses, respectively. However, this should not preclude the Data and Safety Monitoring Board recommending termination earlier if there is evidence of harm. Pre-specified stopping rules for safety will be developed.

It is recognized that any decision to terminate the study would not be reached solely on statistical grounds but on a number of complex clinical and statistical considerations.

1.1. Analysis Plan

A detailed Statistical Analysis Plan will be written before the first subject is enrolled into this study. The following is a summary of the proposed plan. Jeffrey Laux will be responsible for statistical computations.

All statistical analyses will be based upon the total cohort of patients randomized into the trial. Although data on some patients may be missing, all relevant data available from each patient will be employed in the analyses. Patients will be included in the treatment group to which they were randomly assigned regardless of treatment actually received (intention to treat).

Analysis Plan for Aim 1 (MOTOR subjects not exposed to BMZ vs BMZ-exposed subjects in usual care group):
Umbilical cord blood C-peptide levels will be compared between controls not exposed to BMZ from the MOTOR study and BMZ-exposed subjects prospectively randomized to usual care, using a linear model. We will estimate

the mean difference between groups along with a 95% confidence interval. For this comparison between controls not exposed to BMZ from the MOTOR study and BMZ-exposed subjects prospectively randomized to usual care, we will adjust for the following potential confounding factors: periodontal treatment in the MOTOR trial, gestational age at delivery, maternal BMI on admission, and fetal gender. Unfortunately we will not be able to accurately ascertain who among the women prospectively enrolled in the randomized trial has periodontal disease, and thus we will not be able to adjust for periodontitis in our analysis. Periodontitis as an unmeasured confounder is unlikely to bias the analysis of our primary outcome, umbilical cord blood C-peptide, but could bias the analysis of other secondary outcomes which are related to inflammation, such as cortisol. We will plan to state this as a limitation of the study in the final manuscript and interpret the results accordingly. A planned subgroup analysis will be performed comparing the BMZ-unexposed controls to women who were randomized to usual care and delivered within 3 days of BMZ exposure. Other 2° outcomes including umbilical cord blood cortisol, insulin-like growth factor, and leptin levels will be tested in similarly. We will not adjust for multiple comparisons, but will present results for all comparisons regardless of "significance."

Analysis Plan for Aim 2 (BMZ-exposed subjects in usual care group vs intervention group):
Cord blood C-peptide levels will be compared between randomized arms (usual care vs. screening/treatment after BMZ) using a linear model, controlling for gestational age at randomization. We will estimate the mean difference between groups along with a 95% confidence interval. Similar models will be used to compare 2° metabolic measurements such as cord blood cortisol, insulin-like growth factor, and leptin levels between BMZ-exposed usual care vs. BMZ-exposed intervention group. Metabolic and hormonal analytes will be correlated with our exploratory outcomes: neonatal hypoglycemia (defined as neonatal glucose < 40mg/dL within 48 hours of birth), NICU admission, and NICU stay $\geq$ 72 hours.

If the 2 groups show a difference in the 1° outcome, interactions will be evaluated using linear regression models and subgroup analyses conducted to determine whether the effect differs across particular subgroups of patients. In particular we will assess subgroups defined by gestational age (dichotomized at 36 wks), interval from BMZ exposure to delivery (dichotomized at 3 days), maternal obesity (defined as BMI $\geq$ 30 kg/m^2 at time of delivery), race/ethnicity, and expected indication for late preterm delivery at trial entry (preterm premature rupture of membranes vs. preterm labor vs. obstetrical/medical indication). We will conduct 2° analyses to examine neonatal sex (male vs. female) on the 1° outcome.

We will use linear regression models to assess the association between randomization arm, time since first dose of BMZ until delivery, doses of BMZ received, and the interactions of the latter two with each of the study arm approaches (usual care vs. screening/treatment). We will estimate the association between time interval to delivery and number of BMZ doses separately in each study arm and compare the associations between arms.

Loss to follow-up will be defined as the inability to ascertain whether the neonate was born alive and if born alive umbilical cord blood not collected. Those defined as lost to follow-up will not be included in the primary analysis. It is expected that the loss to follow-up rate will be very low, less than 10 percent. However, a sensitivity analysis will be performed including patients lost to follow-up with "worst case" assumptions regarding their outcome to determine whether the results are robust.

In general, analyses of data will be conducted to address the primary and secondary research questions of the trial. All hypothesis tests that are observed to be *not* statistically significant will be reported as being inconclusive.

2. Data Collection

2.1. Data Collection Forms

Data will be collected on standardized forms on which nearly all responses have been pre-coded. Each form is briefly described below:

- Randomization and Baseline Characteristics Log - completed for all randomized patients and records project gestational age, assigned study number, and information on the management arm.
- Maternal Demographics - includes detailed maternal demographics, social data, and history.
- OB History – includes detailed obstetric history
- Maternal Glucose Log - documents maternal blood glucose measurements and any insulin administered prior to delivery.
- Labor and Delivery Summary - documents specific pregnancy complications since randomization, labor, delivery and postpartum information.
- Neonatal Baseline Form - records date and time of birth, delivery data and status at delivery, for each neonate.
- Neonatal Glucose Log - records neonatal blood glucose measurements and any treatments required for hypoglycemia
- Patient Status Form - documents loss to follow-up/withdrawal status, last date of contact for lost to follow-up patients, and side effects since the last dose of steroids.
- Adverse Event Form - records adverse events.

2.2. Web Data Entry System

For this protocol, web data entry screens in REDCap corresponding to the study forms listed above will be developed and maintained by the Primary Investigator, Ashley Battarbee. Clinical center research staff will enter data into the REDCap database. The data are edited online for missing, out of range and inconsistent values. A Users' Manual documenting this system is provided to the centers by the Primary Investigator.

2.3. Centralized Data Management System

Monthly data conversions from the REDCap database create up-to-date STATA datasets. Data are reviewed monthly using edit routines similar to those implemented online during data entry, as well as additional checks for data consistency within or across forms. A database of resulting potential data problems is generated for initial review by the Primary Investigator, who then evaluate the comments keyed in association with edits on missing or unusual values. Valid edits will be flagged in REDCap for resolution at the clinical centers.

At regular intervals, specialized data reviews comparing data availability and consistency across forms are run by the Primary Investigator on the entire database or on a specific subset of data. These reports are also submitted to the centers for correction or clarification.

An audit trail, consisting of all prior versions of each data form as entered in the computer for each patient, is maintained so that the succession of corrections can be monitored.

2.4. Performance Monitoring

Each E-ALPS Study site-PI will be responsible for coordinating day-to-day data collection, data entry, and query resolution at their site. The Primary Investigator will present regular reports to the Scientific Review Committee, Steering Committee and the Data and Safety Monitoring Board. These will include:

- Recruitment Reports – reports of the number of women screened and enrolled by month and by clinical center are compiled monthly for the co-PIs and program manager (Dorman) and provided quarterly to the site-PIs and Scientific Advisory Board
- Semi-Annual Steering Committee Reports – reports detailing recruitment, baseline patient characteristics, data quality, incidence of missing data and adherence to study protocol by clinical center, are provided semi-annually to the Steering Committee
- Data and Safety Monitoring Board Reports – for the planned interim analysis meeting of the DSMB (at 50% enrollment), a report is prepared which includes patient recruitment with center performance information, protocol adherence, and efficacy data with respect to the primary outcome. Semi-annual reports will also be prepared which include adverse events and loss to follow-up as described previously in this protocol.

3. Study Administration

3.1. Organization and Funding

3.1.1. Participating Clinical Centers

The participating Co-Investigators of the clinical centers (see table below) have agreed to abide by the study protocol, to have comparable staff, facilities and equipment and to ensure the proper conduct of the study at each of their centers including: recruitment and treatment of patients as specified in the protocol, accurate data collection and the transmission of the information to the Principal Investigator.

Site PI	Study Site
Ashley Battarbee, MD Kim Boggess, MD	UNC-CH
Alan Peaceman, MD	Northwestern U

3.2. Committees

3.2.1. Steering Committee

This committee consists of five members – the Primary Investigator, Co-Investigators at each of the clinical centers, the Biostatistician and Study Coordinator.

3.2.2. Data Safety and Monitoring Board

The Data and Safety Monitoring Board (DSMB) is made up of a group of individuals employed by the University of North Carolina at Chapel Hill but who are not directly involved with the study (see Appendix for charter). Before the trial can begin, the protocol must be approved by the committee. During the conduct of the study, the board is charged with monitoring the emerging results for efficacy and safety, in addition to center performance and protocol.

3.2.3. Scientific Advisory Board

The PIs have convened an Advisory Committee of experts to meet with the study team quarterly to discuss progress and provide scientific oversight in the event of unexpected findings or challenges and to aid in interpretation of findings for translation into clinical practice. The Advisory Committee members and areas of expertise are listed in the table below.

Scientific Advisory Board

	Area of expertise
Neeta Vora MD, chair	Maternal Fetal Medicine, translational research
Matthew Laughon MD, MPH	Neonatal medicine, pediatric pharmacology, clinical trials
Stephen DeCherney MD	Medical endocrinology, diabetes, clinical trials

4. Study Timetable

4.1. Study Staff Training

Once the study start date is set, one month start-up time is allotted to complete study staff training. It is assumed that all other preparation for the trial will have been completed, including obtaining IRB approval and implementation of the data entry and management system.

Each participating center must complete training to start the trial before recruitment at that center can begin. Each center is required to obtain IRB approval for the study before they are certified to begin the trial.

4.2. Recruitment and Data Collection Period

In the MFMU ALPS study, there were 24,133 women who were screened for participation at 17 sites over a total of 53 months. Of these, 2,831 were eligible and underwent randomization. Specific enrollment data was reviewed for the 4 sites participating in this study. As shown below, enrollment increased over time.

ALPS Enrollment	UNC	Northwestern	**Total**
Oct 2010 – Dec 2010	3 patients (1pt/mo)	1 patient (0.3pt/mo)	**4 patients (1.3pt/mo)**
Jan 2011 – Dec 2011	46 patients (3.8pt/mo)	31 patients (2.6pt/mo)	**77 patients (6.4pt/mo)**
Jan 2012 – Oct 2012	74 patients (7.4pt/mo)	59 patients (5.9pt/mo)	**133 patients (13.3pt/mo)**

Assuming a similar consent rate since there are no major safety concerns yields approximately 150 subjects per year. Therefore it would be reasonable to assume that approximately 144 patients could be comfortably enrolled over an 18-month period. This translates to an enrollment rate of approximately 8 patients per month.

4.3. Final Analysis

After a two-month period for completion of data entry for the trial and close-out, the data set will be locked and available for analysis. Approximately 6 months will be required to complete the final report to the Primary Investigator and to submit the study's primary report for publication.

References:

1. National Center for Health Statistics, final natality data. Retrieved November 15, 2016, from www.marchofdimes.org/peristats.
2. McIntire DD, Leveno KJ. Neonatal mortality and morbidity rates in late preterm births compared with births at term. Obstet Gynecol 2008; 111:35-41.
3. Gyamfi-Bannerman C, Thom EA, Blackwell ATN, Tita UM, Reddy GR, et al. Antenatal betamethasone for women at risk for late preterm delivery. N Engl J Med 2016; 1-10.
4. Jolley JA, Rajan PV, Petersen R, Fong A, Wing DA. Effect of antenatal betamethasone on blood glucose levels in women with and without diabetes. Diabetes Research and Clinical Practice 2016;118:98-104.
5. Sifianou P, Thanou V, Karga H. Metabolic and hormonal effects of antenatal betamethasone after 35 weeks of gestation. J Pediatr Pharmacol Ther 2015; 20(2):138-43.
6. Osterman MJK, Kochanek KD, MacDorman MF, Strobino DM, Guyer B. Annual summary of vital statistics: 2012-2013. Pediatrics 2015;135(6):1115-25.
7. Raju TN, Higgins RD, Stark AR, et al. Optimizing care and outcome for late preterm (near-term) infants, a summary of the workshop sponsored by the National Institute of Child Health and Human Development. Pediatrics 2006; 118:1207-14.
8. Laptook A, Jackson DL. Cold stress and hypoglycemia in the late preterm ("near-term") infant: impact on nursery of admission. Semin Perinatol 2006;30:77-80.
9. Neu J. Gastrointestinal maturation and feeding. Semin Perinatol 2006;30:24-7.
10. Ramadan MK, Hussein G, Saheb W, Rajab M, Mirza FG. Antenatal corticosteroids in the lat preterm period: a prospective cohort study. J Neonatal Perinatal Med 2016;9:15-22.
11. Porto AM, Coutinho IC, Correia JB, Amorim MM. Effectiveness of antenatal corticosteroids in reducing respiratory disorders in late preterm infants: randomized clinical trial. BMJ 2011;342:d1696.
12. Langen ES, Kuperstock JL, Sung JF, Taslimi M, Byrne J, El-Sayed YY. Maternal glucose response to betamethasone administration. Am J Perinatol 2015; 32:143-148.
13. Shelton SD, Boggess KA, Herbert WNP. Effect of betamethasone on maternal glucose. J Matern Fetal Neonatal Med. 2002; 12:191-195.
14. Society for Maternal-Fetal Medicine (SMFM) Publications Committee. Implementation of the use of antenatal corticosteroids in the late preterm birth period in women at risk for preterm delivery. Am J Obstet Gynecol 2016;215(2):B13-5.
15. Pregestational diabetes mellitus. Practice Bulletin No. 60. American College of Obstetricians and Gynecologists. Obstet Gynecol 2005;105(3):675-85.