MULTIPLE VS. SINGLE COURSES OF ANTENATAL CORTICOSTEROIDS FOR PRETERM BIRTH

by

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A thesis submitted in conformity with the requirements for the degree of Masters of Science
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Abstract

Multiple vs. Single Courses of Antenatal Corticosteroids for Preterm Birth

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A single course of antenatal corticosteroids (ACS) decreases the risk of death, respiratory distress syndrome (RDS) and possibly intraventricular hemorrhage in babies at risk of preterm delivery. The effects on RDS are most significant if babies are born between 24 hours and 7-10 following treatment. As a result, many centers repeat courses of ACS every 7-10 days.

Systematic reviews of studies in humans and animals show some benefits and risks for multiple courses of ACS.

I designed multi-center randomized controlled trial to assess the effects of multiple vs. single courses of ACS on neonatal outcomes. A pilot study of this trial suggests a multi-center trial is feasible, but care should be given to the strategy for recruiting pregnant women.
Dedicated to the Memory of My Mother...
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# Table of Contents

Chapter 1. Background........................................................................................................1
   A. Introduction........................................................................................................... 1
   B. Physiology............................................................................................................. 2
   C. Pharmacology....................................................................................................... 4
   D. Risks and Benefits of a Single Course of Antenatal Corticosteroid Treatment......4
   E. Risks and Benefits of Multiple Courses of Antenatal Corticosteroid Treatment....6
   F. Animals Studies..................................................................................................... 7

Chapter 2. Systematic Review of Published Literature in Humans...............................10
   A. Objective.............................................................................................................. 10
   B. Sources............................................................................................................... 10
   C. Methods.............................................................................................................. 10
   D. Results............................................................................................................... 11
   E. Discussion.......................................................................................................... 15

Chapter 3. Systematic Review of Published Literature in Animals...............................25
   A. Objective.............................................................................................................. 25
   B. Sources............................................................................................................... 25
   C. Methods.............................................................................................................. 25
   D. Results............................................................................................................... 26
      D.1 Lung Function..............................................................................................26
Chapter 4. The Multiple Courses of Antenatal Corticosteroids for Preterm Birth

Study Design........................................................................................................40

A. Research Questions............................................................................................40
   A.1 Primary Research Question...........................................................................40
   A.2 Other Research Questions............................................................................41

B. Methods............................................................................................................41
   B.1 Research Design..........................................................................................41
   B.2 Selection Criteria for Participants...............................................................41
List of Figures

Figure 5.1 .................................................................................................................. 76
List of Tables

Table 2.1 Characteristics of Included Studies in Humans........................................20
Table 2.2 Handling of Potential Confounders for Size at Birth & Clinical Outcomes....21
Table 2.3 Multiple (vs. single) Courses of Antenatal Corticosteroids and Neonatal
Outcomes..............................................................................................................22
Table 2.4 Multiple (vs. single) Courses of Antenatal Corticosteroids and Maternal.
Outcomes..............................................................................................................24
Table 3.1 Characteristics of Included Studies in Animals.........................................38
Table 5.1 Baseline Information of Participants and Non-participants.......................77
Table 5.2 Compliance with The Study Treatment..................................................79
Table 5.3 Treatment..............................................................................................80
Table 5.4 Neonatal Outcomes..............................................................................81
Table 5.5 Primary Outcome of the Designed RCT................................................83
Table 5.6 Maternal Outcomes..............................................................................84
Table 5.7 Maternal Hormones..............................................................................85
Table 5.8 Cord Blood Hormones........................................................................86
Table 5.9 Questionnaire.......................................................................................87
List of Appendices

Appendix A. Patient Information Form and Consent from..................................................103
Appendix B. Patient Card..................................................................................................107
Appendix C. Patient and Physician Information Letter....................................................109
Appendix D. Working Protocol .......................................................................................113
Appendix E. Data Forms.................................................................................................128
1 Background

A. Introduction

Preterm birth is a major cause of neonatal death and infant morbidity. Preterm morbidity includes respiratory distress syndrome (RDS), intraventricular haemorrhage (IVH), periventricular leukomalacia (PVL), necrotising enterocolitis (NEC), bronchopulmonary dysplasia (BPD), sepsis, patent ductus arteriosus (PDA), and retinopathy of prematurity (ROP). RDS is often the most acute problem of the preterm infant affecting 40-50% of infants delivered before 32 weeks of gestation. RDS, along with IVH, account for a high proportion of neonatal deaths. Preterm infants often require intensive care after birth, which is associated with a substantial cost to the health care system.

Today in Canada, approximately 7% of infants are born preterm. Despite advances in medical technology, the incidence of preterm birth in Canada has increased. This appears to be secondary to an increase in the incidence of multiple gestations, obstetric interventions, and may be related to the increased use of ultrasound to estimate gestational age. The rates of neonatal morbidity and mortality associated with preterm birth have decreased secondary to advances in antenatal and neonatal care, including the use of antenatal corticosteroids (ACS) and surfactant.
In 1972, following a randomized controlled trial (RCT), maternal ACS treatment was first shown to be effective in reducing the risk of RDS.\(^4\) In 1994, a National Institutes of Health (NIH) consensus conference recommended broad use of ACS for women at risk of preterm birth to decrease the incidence of RDS and other complications of preterm birth.\(^5\) A Cochrane review (Crowley) reported ACS to be associated with a substantial decrease in RDS, IVH, and neonatal death.\(^2\) These benefits extended to a broad range of gestational ages and were not limited by sex of the infant or race.\(^2\)

The benefits of ACS have been demonstrated to be most significant 24 hours following treatment and to continue for up to 7-10 days.\(^6\) Extrapolating the benefits of a single course, some physicians have suggested that multiple courses of ACS be administered at weekly intervals.\(^7\) In some centers, this approach has become routine. In a recent survey of Australian obstetricians, 85% of obstetricians indicated they prescribe multiple courses of ACS for women who remain at increased risk of preterm birth.\(^8\) In a survey of maternal fetal medicine specialists, 96% stated that they would give more than one course of ACS and 58% would give 6 or more courses.\(^9\) In the UK, 98% of obstetricians indicated that they prescribe multiple courses of antenatal corticosteroids.\(^10\) In Canada 69% of obstetricians stated they would use more than one course of ACS and several stated that they routinely use up to 10 courses. (Liu and Hannah, Meeting of SOGC; Ottawa, 1999).

B. Physiology

Alveolar epithelial type II cells synthesize and secrete pulmonary surfactant, a complex of lipids and proteins [surfactant protein (SP)-A, SP-B, SP-C and SP-D].\(^6\) Pulmonary surfactant maintains alveolar stability and normal lung function.\(^6\) Its deficiency in the newborn often leads to RDS.\(^6\) Corticosteroids are known to stimulate cytodifferentiation
and/or cause precocious changes in developmentally regulated proteins in at least 12 tissues, accelerating the rate of tissue differentiation without altering the sequence of developmental events. By contrast, adrenalectomy or hypophysectomy delays the differentiation process. Studies in rabbits and lambs indicate that steroid administration stimulates structural development and production of surfactant. This suggests that both airway epithelial cells and mesenchymal fibroblasts, the major cell types in the fetal lung, respond to corticosteroids. Corticosteroids increase lung compliance and maximal volume independent of surface active material. Corticosteroids have the potential of directly influencing the transcription of genes related to epithelial growth and differentiation during lung development. It is established that corticosteroids induce all components of surfactant including phospholipid, enzymes and proteins (A, B, C, and D). In addition, corticosteroid treatment appears to reduce protein leak from the pulmonary vasculature into the airspace and appears to accelerate clearance of lung liquid prior to delivery. These benefits represent precocious maturation and are essential in the transition to air breathing.

The reversibility of corticosteroid effects in cultured tissue is of interest with regard to clinical management. It has been shown that cortisol increases surfactant protein (SP)-B and SP-C mRNA levels in cultured lung, but that levels decline to control after removal of cortisol. The same pattern occurs for SP-A and inducible enzymes of lipid synthesis, although the kinetics vary with relative stabilities of mRNA and proteins. These observations suggest that increases in surfactant proteins in vivo may be reversible events as hormone is cleared after corticosteroid treatment.

It is uncertain whether effects of an administered steroid on surfactant proteins/enzymes are reversible in vivo, and if so, whether levels return to the original.
pretreatment value or to a new, higher basal level that would normally occur as a result of continuing tissue differentiation. At present, however, available experimental evidence indicates that induction of surfactant in fetal lung is a reversible process.\textsuperscript{16,17}

C. **Pharmacology**

Dexamethasone and betamethasone are the preferred corticosteroids for antenatal therapy. These compounds are identical in biologic activity and readily cross the placenta in their biologically active forms.\textsuperscript{18} They are devoid of mineralocorticoid activity, relatively weak in immunosuppressive activity, and act for longer than cortisol and methylprednisolone.\textsuperscript{18} Treatment with 2 doses of 12 mg of betamethasone given intramuscularly 24 hours apart or 4 doses of 6 mg of dexamethasone given intramuscularly 12 hours apart have been shown to be effective.\textsuperscript{5} The bioavailability of corticosteroids to the fetus is reduced secondary to placental metabolism.\textsuperscript{4} The umbilical vein concentrations of betamethasone are approximately 25-30\% of maternal venous concentrations in singletons.\textsuperscript{5} However, the corticosteroids do not remain in the fetal circulation for long. In one study, when the levels of betamethasone, administered prior to birth, were assayed in cord blood, the drug was undetectable 40 hours following the injection.\textsuperscript{19}

D. **Risks and Benefits of a Single Course of Antenatal Corticosteroids Therapy**

The greatest benefit of a single course of ACS therapy is a substantial reduction in mortality [odds ratio (OR) 0.60 (95\% CI): 0.48,0.75] and RDS [OR (95\% CI): 0.53 (0.44, 0.63); risk difference (95\% CI): -8.7 (-11.9, -6.3)] in all babies.\textsuperscript{2} A single course of ACS reduces the risk of RDS from 46\% to 20\% in babies born before 32 weeks of gestation.\textsuperscript{2} In addition there may be reduction in the incidence of IVH. Other benefits of a single course of ACS therapy include less need for surfactant therapy, improved circulatory stability and
reduced requirements for oxygen and ventilatory support.\textsuperscript{5,20} Thus far, a single course of ACS appears to have few adverse side effects in humans. There is no evidence of an increase in risk of infection in treated infants, even in the presence of preterm prelabour rupture of membranes (PPROM).\textsuperscript{3,21,22} No adrenal suppression has been shown with the use of a single course of ACS.\textsuperscript{2,5} To date, follow up studies of infants enrolled in RCTs have not demonstrated any long-term adverse effects following a single course of ACS.\textsuperscript{23-25} One of these studies followed children to 12 years of age. There were no significant differences between the children that received a single course of ACS, as compared to those who did not, in terms of growth, or in terms of lung, neurologic or ophthalmologic function.\textsuperscript{24} In a 20-year follow up of young adults whose mothers had participated in a randomized trial to receive one course of ACS or placebo, no differences were found between the corticosteroid treated and placebo groups as to medical or psychological variables and systolic blood pressure was actually significantly lower in the corticosteroid group.\textsuperscript{62}

Potential adverse maternal effects include infection, hyperglycemia, and pulmonary edema. Maternal infections of concern include endometritis, intra-amniotic infection or chorioamnionitis and wound or episiotomy infection. In the Crowley overview the frequency of maternal infection was not increased in the treated group compared with controls, although in one small study of 42 women in whom the fetal membranes were ruptured for more than 24 hours before delivery, maternal infection was increased.\textsuperscript{5} In addition, a meta-analysis which incorporated data from over 1400 women with preterm prelabor rupture of membranes (PPROM) found that a single course of ACS was associated with a decrease in RDS (RR 0.56:0.46-0.70 CI), IVH (RR 0.47:0.31-0.70 CI) and NEC (RR 0.21:0.05-0.82 CI).\textsuperscript{26}
Antenatal corticosteroids can also accentuate glucose intolerance. Studies have shown that glucose control during steroid administration was difficult to achieve and required an intravenous infusion of insulin.27 Finally, maternal pulmonary edema has been reported to occur with the combined use of ACS and tocolytic agents.28,29 This complication is more commonly associated with maternal infection, fluid overload, and multiple gestation.30 Pulmonary edema has not been reported when ACS were used alone.

As ACS therapy may be less effective in babies who are born seven or more days following initial treatment, there may be benefits in repeating the courses of ACS in women who remain undelivered seven or more days following the first course and continue to be at increased risk of preterm birth.

E. Risks and Benefits of Multiple Courses of Antenatal Corticosteroid Therapy

There are insufficient data from RCTs as to the benefits and risks of multiple courses of ACS, although several RCTs are currently in progress. The data from a recently completed RCT of 502 pregnant women randomized to a single vs. multiple courses of ACS showed no difference in risk of adverse perinatal outcome (one or more of RDS, BPD, neonatal sepsis, NEC, or neonatal death).31 Another small RCT comparing multiple courses of ACS to placebo found that ACS was associated with a significant reduction in RDS and death, but over 50% of randomized fetuses were excluded from the analysis, and the effect found may have been the result of the initial versus the subsequent courses.32

Several retrospective studies suggest that multiple courses of ACS decrease the risk of RDS and pulmonary disease.33-35 One retrospective analysis reported a significant decrease in use of oxygen in infants who received multiple courses of ACS.36 One study has reported a
trend toward less risk of IVH in infants who received multiple courses of ACS as compared to those who received only one course.  

The risks associated with multiple courses of ACS may include, potentially, increased risk of infection as a result of immune suppression, Cushingoid changes, and adrenal insufficiency resulting from prolonged suppression in both mothers and infants. A few adverse effects of multiple courses of ACS have been reported. Small human studies have reported a slight trend toward an increased risk of NEC.  

Others have reported a slight trend towards an increase in neonatal mortality. Two studies reported a reduction in head circumference and birth weight. Some recent studies reported an increased risk of neonatal infection following multiple courses of ACS. However, others have not found an increase in neonatal sepsis or disturbance in growth. Other adverse effects reported include a case of neonatal Cushingoid syndrome following seven courses of ACS, and hyperactive behavior in children at 3 years of age. A three year follow-up study of children who received single vs. multiple courses of ACS showed no difference in growth or disabilities between the two groups. Another, 2 to 6 year follow up study reported no adverse effects on blood pressure or growth.  

Given the known benefits of a single course of ACS (i.e. decreased risk of RDS, neonatal mortality and possibly IVH), it follows that multiple courses could continue to benefit an infant at increased risk of preterm birth, but there may be an increased risk of some adverse outcomes.

F. Animals Studies

Studies in animals have found progressive improvement in postnatal lung function following multiple doses of ACS. However multiple doses of ACS in animals have been
associated with a decreased birth weight, growth restriction, and altered myelination and restricted growth in the central nervous system.\textsuperscript{43,48,49} In addition, prenatal glucocorticoids can cause elevated hypertension and glucose intolerance in adult offspring in the rat.\textsuperscript{50,51} Prenatal glucocorticoids may program specific effects in the brain, particularly upon the hypothalamic-pituitary-adrenal (HPA) axis. Prenatal programming of the HPA axis is mediated, at least in part, via alteration in glucocorticoid receptor gene expression in the hippocampus, which is an important locus of feedback control on the HPA axis.\textsuperscript{52}

Chapter 3 of this thesis is a systematic review of the published literature to determine the effects of multiple vs. a single dose of ACS in animals. There have been many animal studies which have looked at long term effects of prenatal glucocorticoid especially on the brain. These studies mostly looked at different doses or multiple doses of glucocorticoids without comparing this to a single dose. Many of these studies have investigated the mechanism of action of glucocorticoids, rather than looking at a clinical outcomes. These studies are not included in the systematic review as they do not meet the criteria to answer the specific question of the effects of multiple vs. single doses of ACS on lung, nervous system and growth in animals.

This thesis consists of a comprehensive review of effects of multiple courses of ACS in humans and animals; the design of a multi-center RCT to determine the effects of multiple vs. single courses of ACS on perinatal and neonatal mortality or morbidity and maternal morbidity in women at increased risk of preterm birth. 7 or more days following a single course of ACS; and a pilot study to determine the feasibility of the proposed RCT. The results of the pilot study should provide useful information for revising in the design of the
proposed RCT. The results of this thesis should also provide the background for future research and clinical practice.
A. Objective

The objective of this systematic review of published studies, and meta-analysis where possible was to determine the effects of multiple courses of ACS vs. a single course on perinatal, neonatal, infant and maternal outcomes.

B. Sources

We searched MEDLINE (1966-2000), Embase (1980-2000), and Cochrane library using the following strategy. For the MEDLINE search, we combined adrenal cortex hormones, glucocorticoids, betamethasone, and dexamethasone to identify corticosteroid therapy, with lung, fetal organ maturity and respiratory distress syndrome and then again with pregnancy and pregnancy complications in two separate sets to identify all related articles. We used a similar search strategy for Embase. We also reviewed the references from included articles and from personal files to search for additional articles.

C. Methods

We included all full articles published in English, which compared multiple courses of ACS vs. a single course in women at increased risk of preterm birth and reported on perinatal, neonatal, infant and/or maternal outcomes. Studies that did not control for
differences in gestational age at birth and those published in abstract form only were excluded. Two authors (Fariba Aghajafari, Kellie Murphy) determined eligibility and independently abstracted the data. Discrepancies were resolved by consensus.

We assessed each study for methodologic quality. Specifically, we checked as to whether the study was a randomized clinical trial or a cohort study, plus for cohort studies whether they were prospective or retrospective in terms of the association between ACS and the outcomes of interest. If the data were collected prospectively but as part of another study or as part of normal care, and not specifically to answer the research question posed, we considered this a retrospective cohort study. We also checked for differences between the multiple and single course groups in regards to the following potential confounders: gestational age at first course, time from last course to delivery, preterm prelabour rupture of membranes (PPROM), pre-eclampsia or hypertension, and multiple pregnancy. We also checked as to whether the use of multiple vs. single courses was part of a routine hospital policy or whether this varied among clinicians.

Baseline information was analyzed descriptively. If the gestational age at delivery was documented to be similar between groups or if the results were presented by gestational age subgroup, the data were included in a meta-analysis (Review Manager 4.1), using a random effects model. For each meta-analysis, we calculated an odds ratio (OR) and 95% confidence interval (CI) and checked for heterogeneity using chi-square test. A $P$ value $< 0.05$ was considered statistically significant.

D. Results

Eight studies met the inclusion criteria for this systematic review. These studies included 1682 fetuses who were exposed to a single course of ACS and 1116 fetuses
who were exposed to multiple courses of ACS. All studies were retrospective, although for one study the 3 year outcomes were assessed prospectively\textsuperscript{39} and for another study, the data were collected as part of a prospective randomized controlled trial of thyrotropin releasing hormone (TRH).\textsuperscript{38} The characteristics of the studies are reported in Table 2.1.

In five studies the gestational age at delivery was documented to be similar between groups.\textsuperscript{39,54-57} For two studies, the neonatal outcomes were presented by subgroup of gestational age at delivery.\textsuperscript{36,38} In one study, a regression analysis was used to control for differences in gestational age at delivery to determine the effects of multiple courses of ACS on RDS.\textsuperscript{35} Some studies also controlled for gestational age at first course of ACS, time from last course to delivery, PPROM, preeclampsia and multiple pregnancy (Table 2.2).

There was no significant heterogeneity in any of the meta-analyses except for the meta-analyses of the trials assessing the effects of multiple vs. single courses of ACS in terms of neonatal sepsis and chorioamnionitis. Seven studies were included in the meta-analysis to determine the effect of multiple courses of ACS on RDS.\textsuperscript{36,38,39,54-57} Multiple courses of ACS were associated with a significantly decreased risk of RDS (OR 0.79. 95% CI 0.64. 0.98), a trend toward an increased risk of bronchopulmonary dysplasia (BPD) (OR 1.30. 95% CI 0.96. 1.78) and a significantly decreased risk of patent ductus arteriosus (PDA) (OR 0.56. 95% CI 0.35. 0.90). The risk of IVH, necrotizing enterocolitis (NEC), sepsis and neonatal death were not significantly different between multiple vs. single courses of ACS (Table 2.3).

One study which did not contribute data to the meta-analysis, but controlled for differences in gestational age at delivery, birth weight, and surfactant use, found a decreased
risk of RDS (adjusted OR 0.35, 95% CI 0.18, 0.70), with multiple vs. single courses of ACS.\textsuperscript{35}

Abbasi et al\textsuperscript{4} reported on the neonatal outcomes according to time between the last dose of ACS and delivery in those infants who were exposed to multiple vs. single courses of ACS. For those babies born within one week of the last dose of ACS, there was a lower incidence of RDS in infants who received multiple courses of ACS vs. a single course (32% vs. 47%. \(P=0.029\)). However, the study found a similar risk of RDS and other clinical outcomes in infants who were born 1 to 4 weeks after the last dose of ACS in both groups. French et al\textsuperscript{9} reported on the outcomes of infants who were delivered more than 7 days after the last dose of ACS compared with those who delivered within 7 days of the last dose of ACS. They found no significant difference in risk of mortality (5.3% vs. 8.3%. \(P=0.65\)), RDS (32% vs. 28%. \(P=0.763\)) and BPD (33% vs. 25%. \(P=0.469\)) between the two groups. Banks et al\textsuperscript{38} undertook a regression analysis to determine the clinical outcomes of neonates delivered either 1-6 days or 7-13 days after the last dose of ACS. After adjusting for gestational age, multiple pregnancy, and the number of courses of ACS, there was no significant difference in mortality (adjusted OR 0.96, 95% CI 0.5, 1.9). RDS and BPD for those delivered within 1-6 days vs. 7-13 days after the last dose of ACS. Vermilion et al\textsuperscript{27} reported a shorter interval from last dose of ACS to delivery for infants who were exposed to multiple courses of ACS vs. those who were exposed to a single course (2.0 ± 1.0 vs. 3.8 ± 3.1 days, \(P<0.001\)). However, in their regression analysis, the time interval was not independently associated with their significant outcomes being early onset neonatal sepsis and death. Only French et al\textsuperscript{39} reported on gestational age at first course of ACS which was significantly lower for infants who were exposed to 3 or more courses of ACS compared to
those who were exposed to 1 or 2 courses of ACS. Other potential confounders such as multiple pregnancy, preeclampsia, and PPROM were excluded from the analysis in some studies (Table 2.2).

As the selection to receive multiple courses of ACS was not randomized, this was a source of bias in all studies. However, in four studies the potential for bias was greater as the decision to use multiple courses of ACS was based on hospital policy. That is, the only women eligible to receive repeated courses had to remain undelivered for at least 7 days following an initial course and could not have contraindications to repeated courses of ACS. Thus these women may have been less likely to have had adverse fetal outcomes (Table 2.2).

French et al. performed a regression analysis to examine the effects of multiple courses of ACS on infant size. They found that infants who were exposed to 3 or more courses of ACS had a lower birth weight (a reduction of 9%) than the sample mean of 1377 g ($P=0.014$). In neonates receiving >1 course of ACS, Banks et al. reported a 39 g decrease in birth weight compared to those receiving 1 course after adjusting for gestational age at birth and multiple gestation ($P=0.016$). However, Abbasi et al. found no difference in birth weight between the group which received multiple courses vs. a single course of ACS after correction for gestational age at birth and preeclampsia. In the meta-analysis, there was no difference in risk of small for gestational age at birth (birth weight less that 10th percentile) between the two groups (OR 1.21, 95% CI 0.58, 2.54) (Table 2.3).

Abbasi et al. reported a smaller head circumference at birth of 0.46 ± 0.19 cm ($P=0.013$) in the group which received multiple courses of ACS after correction for gestational age at birth and preeclampsia. French et al. also reported a smaller head
circumference (a reduction of 4%) in infants who received 3 or more courses of ACS compared to those who were exposed to 1 course of ACS.

In a follow-up study of children at 3 years of age, French et al.\textsuperscript{39} showed no significant difference in weight, height, and head circumference for those receiving multiple courses of ACS compared to a single course. They also showed no significant difference in the likelihood of cerebral palsy (OR 0.24, 95% CI 0.01, 4.49) or disability (OR 0.21, 95% CI 0.03, 1.70) following multiple courses of ACS compared to a single course.

Four studies reported on maternal outcomes.\textsuperscript{35,54,55,57} In the meta-analysis, although the rate of chorioamnionitis was not significantly increased (OR 1.46, 95% CI 0.47, 4.59), the risk of postpartum endometritis was increased with the use of multiple courses vs. a single course of ACS (OR 3.22, 95% CI 1.90, 5.45). Finally, Pratt et al.\textsuperscript{36} reported no significant difference in the rates of confirmed maternal infection with multiple courses of ACS (39% vs. 45%, \( P=0.23 \)) (Table 2.4).

E. Discussion

It has been shown in randomized controlled trials that a single course of ACS decreases the risk of RDS, death and possibly IVH and in preterm babies.\textsuperscript{2} Corticosteroids are known to accelerate maturation of developmentally regulated proteins and to stimulate cytodifferentiation in numerous cells, including type II pneumocytes.\textsuperscript{6} However, the effects of a single course of ACS on lung maturation may be a temporary phenomenon.\textsuperscript{6} In the Cochrane meta-analysis, the benefits of a single course of ACS in terms of RDS have been demonstrated to be most significant 24 hours following treatment and to continue up to 7-10 days.\textsuperscript{2} This raises the question as to whether repeated courses of ACS will further decrease the risk of RDS, death or IVH in infants born to women who remain at increased risk of
preterm birth 7 or more days after treatment with ACS. Based on this hypothesis, multiple courses of ACS has become a routine practice in some centers.9-10

Animal data suggest multiple courses of ACS induce progressive improvement in postnatal lung function.43-47 However, other studies suggest adverse effects on fetal growth and neuronal development.43,48,49,58 Human studies suggest that multiple courses of ACS may be associated with a decreased risk of RDS and pulmonary disease.35,54 In addition, babies born after multiple courses of ACS, but within 7 days of the last treatment, demonstrated improved respiratory compliance compared with those babies born more than 7 days after a single course of ACS.59 However other studies suggest the potential for adverse maternal and neonatal effects after exposure to multiple courses of ACS.14,38,39,57 In a recent randomized trial of early dexamethasone vs. placebo treatment in very low birth weight infants to reduce the risk of BPD, early-dexamethasone treated infants had a higher risk of NEC compared to those treated with placebo (13% vs. 4%, P = 0.02). In this study the early treatment with dexamethasone had no effect on death or BPD (OR 0.9, 95% CI 0.8, 1.1).60

As the evidence to support multiple courses of ACS is limited, we believed it was important to systematically review these studies in order to assess potential risks and benefits.

Compared to meta-analyses of RCTs, meta-analyses of observational studies present particular difficulties due to the effects of confounding factors. Because gestational age at birth is an important potential confounder, we limited our review to studies in which gestational age at birth was documented to be similar between groups or was controlled for in the analysis. The principles for meta-analysis of observational data are similar to those for
randomized data. However, greater care is needed in the interpretation of the results because of the potential biases.

One of the biggest limitations of the cohort studies in this review was selection bias. Essentially, women who received multiple courses of ACS were different from those who received only a single course of ACS. Either the women presented at an earlier gestational age with a risk factor for preterm birth and thus may have had a higher likelihood of adverse outcomes and/or they delivered at a later gestational age, which was why they had time to receive repeated courses of ACS and thus may have had a lower likelihood of adverse outcome. In addition, there were differences in other potential confounding variables, e.g. multiple pregnancies, PPROM, preeclampsia and no study controlled for all of these factors. All the studies in this review were retrospective with respect to looking at the association between multiple vs single course of ACS for immediate neonatal outcomes. That is, in no study was it clear that the research questions and study protocol were finalized prior to the exposure of women to ACS. In some studies it was hospital policy to give multiple courses of ACS and in these studies, selection bias would have been strongest. However, in other studies use of multiple courses of ACS was at the discretion of the treating physician and in these studies selection bias may have been less. Hence it is likely that in these studies, the population which received multiple courses of ACS differed from the population that received a single course.

Our aim in this review was to determine the strength of the evidence for or against the use of multiple courses of ACS. Considering all of the potential confounding variables, it is difficult to interpret the results and draw conclusions. It is impossible to know whether the findings are due to differences in the population (selection bias) or due to the effect of
multiple courses of ACS. It is interesting that there was no significant difference in the rate of composite neonatal morbidity (defined as RDS, BPD, sepsis, NEC or neonatal death) between multiple courses of ACS and a single course of ACS in a recently reported randomized clinical trial of 502 pregnant women (in abstract form).\textsuperscript{31} This may suggest that the findings from the observational studies are more likely due to the effects of confounding variables.

There is some evidence, published only in abstract form, suggesting that multiple exposure to corticosteroids may have long-term adverse consequences for the fetus. French et al\textsuperscript{11} reported an increased risk of problem child behavior in children at 3 years of age who were exposed to 3 or more courses of ACS. Esplin et al\textsuperscript{61} found an abnormal Psychomotor Developmental Index in children at a mean of 21.5 months of age who were exposed to multiple courses of ACS. However, Rotmensch et al\textsuperscript{49} showed no impairment in long term growth and no elevation in blood pressure following multiple courses of ACS compared to single course in children at 2.2-6.4 years of age. Follow up studies of infants enrolled in the randomized trials of a single course of ACS have not demonstrated any long-term adverse effects.\textsuperscript{23-25,62} In a 12-year follow up study, there were no significant differences between the children who were exposed to a single course of ACS compared to those who were exposed to placebo, in terms of growth, or in terms of lung, neurologic or ophthalmologic function.\textsuperscript{34} In a 20-year follow up of young adults whose mothers had participated in a randomized trial to receive one course of ACS or placebo, no differences were found between the corticosteroid treated and placebo groups as to medical or psychological variables and systolic blood pressure was actually significantly lower in the corticosteroid group.\textsuperscript{62}
This systematic review and meta-analysis of observational studies in humans, while limited by the retrospective nature of the studies and presence of selection bias in all studies, reported a decreased risk of RDS and PDA, but an increased risk of endometritis following multiple courses of ACS. Multiple courses of ACS had no significant effect on other neonatal and maternal outcomes. It is not possible to establish the true effects of multiple courses of ACS by reviewing the results of observational studies due to the effect of confounding variables. We believe that the practice of repeating courses of ACS should be addressed in large multicenter randomized trials with an emphasis on assessing long-term effects on growth and neurodevelopment.
Table 2.1 Characteristics of Included Studies in Humans

<table>
<thead>
<tr>
<th>Author Year</th>
<th>Participants*</th>
<th>ACS</th>
<th>MC Mothers (N)</th>
<th>SC Mothers (N)</th>
<th>SC Infants (N)</th>
<th>SC Infants (N)</th>
<th>Number of MC (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbasi 2000</td>
<td>≥ 24 w. at birth (with most outcomes reported only for those delivered at 24-34 w)</td>
<td>B or D</td>
<td>192</td>
<td>255</td>
<td>177</td>
<td>248</td>
<td>2-12</td>
</tr>
<tr>
<td>Banks 1999</td>
<td>25-32 w</td>
<td>B or D</td>
<td>NA</td>
<td>300</td>
<td>NA</td>
<td>410</td>
<td>2-8</td>
</tr>
<tr>
<td>Elimian 1999</td>
<td>Neonates weighing &lt; 1750 g</td>
<td>B</td>
<td>NA</td>
<td>93</td>
<td>NA</td>
<td>261</td>
<td>NA</td>
</tr>
<tr>
<td>French 1999</td>
<td>20-32 w. survivors at 3 years of age</td>
<td>B</td>
<td>43</td>
<td>43</td>
<td>123</td>
<td>123</td>
<td>2-&gt;3</td>
</tr>
<tr>
<td>Ghidini 1997</td>
<td>PPROM &lt; 32 w</td>
<td>B</td>
<td>89</td>
<td>89</td>
<td>47</td>
<td>47</td>
<td>NA</td>
</tr>
<tr>
<td>Pratt 1999</td>
<td>24-34 w</td>
<td>B</td>
<td>136</td>
<td>NA</td>
<td>273</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Smith 2000</td>
<td>24-30 w</td>
<td>D</td>
<td>14</td>
<td>14</td>
<td>53</td>
<td>53</td>
<td>2-3</td>
</tr>
<tr>
<td>Vermillion 2000</td>
<td>24-36 w</td>
<td>B</td>
<td>186</td>
<td>186</td>
<td>267</td>
<td>267</td>
<td>3.6±0.5†</td>
</tr>
</tbody>
</table>

*all participants had exposure to ≥ 1 courses of ACS; † mean number of doses of steroids; ACS: antenatal corticosteroids; B: betamethasone; D: dexamethasone; w: weeks; PPROM: preterm prelabour rupture of membranes; NA: not available; MC: multiple courses of ACS; SC: single course of AC
Table 2.2 Handling of Potential Confounders for Size at Birth & Clinical Outcomes

<table>
<thead>
<tr>
<th>Author Year</th>
<th>GA at birth</th>
<th>GA at 1st course of ACS</th>
<th>Time from last course of ACS to delivery</th>
<th>PPROM</th>
<th>Pre-eclampsia/HT</th>
<th>Multiple pregnancy</th>
<th>Policy of steroid administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbasi 2000</td>
<td>ND</td>
<td>NA</td>
<td>Data reported by subgroups</td>
<td>D, higher in SC</td>
<td>D, higher in SC</td>
<td>Data reported by subgroup</td>
<td>At discretion of treating physician</td>
</tr>
<tr>
<td>Banks 1999</td>
<td>Data reported by subgroup, regression analysis</td>
<td>NA</td>
<td>Data reported by subgroup, regression analysis</td>
<td>NA</td>
<td>Excluded</td>
<td>D, higher in MC, not controlled for in the analysis</td>
<td>At discretion of treating physician</td>
</tr>
<tr>
<td>Elimian 1999</td>
<td>D, regression analysis</td>
<td>NA</td>
<td>NA</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
<td>Hospital policy</td>
</tr>
<tr>
<td>French 1999</td>
<td>ND</td>
<td>D, lower in group receiving ≥3 courses</td>
<td>Data reported by subgroup</td>
<td>NA</td>
<td>Excluded</td>
<td>Excluded</td>
<td>At discretion of treating physician</td>
</tr>
<tr>
<td>Ghidini 1997</td>
<td>ND</td>
<td>NA</td>
<td>NA</td>
<td>All patients</td>
<td>Excluded</td>
<td>Excluded</td>
<td>Hospital policy</td>
</tr>
<tr>
<td>Pratt 1999</td>
<td>Data reported by subgroup</td>
<td>NA</td>
<td>NA</td>
<td>D, higher in SC</td>
<td>NA</td>
<td>ND, first baby considered in the analysis</td>
<td>Hospital policy</td>
</tr>
<tr>
<td>Smith 2000</td>
<td>ND</td>
<td>NA</td>
<td>NA</td>
<td>D, higher in MC</td>
<td>ND</td>
<td>Excluded</td>
<td>At discretion of treating physician</td>
</tr>
<tr>
<td>Vermillion 2000</td>
<td>ND</td>
<td>NA</td>
<td>D, regression analysis</td>
<td>Excluded at the time of enrollment</td>
<td>NA</td>
<td>Excluded</td>
<td>Hospital policy *</td>
</tr>
</tbody>
</table>

GA: gestational age; PPROM: preterm premature rupture of membranes; HT: hypertension; ACS: antenatal corticosteroids; NS: not available; D: difference in incidence of confounder between groups; ND: no difference in incidence of confounder between groups; MC: multiple courses of ACS; SC: single course of ACS; *weekly doses during the first 2 years, then rescue therapy, then single course therapy during the last year of the study period.
Table 2.3 Multiple (vs. single) Courses of Antenatal Corticosteroids and Neonatal Outcomes

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Multiple Courses</th>
<th>Single Course</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RDS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abbasi, 2000</td>
<td>67/192 (34.9%)</td>
<td>80/177 (45.2%)</td>
<td>0.65 (0.43, 0.99)</td>
</tr>
<tr>
<td>Banks I, 1999</td>
<td>88/107 (82.2%)</td>
<td>182/228 (79.9%)</td>
<td>1.17 (0.65, 2.12)</td>
</tr>
<tr>
<td>Banks II, 1999</td>
<td>72/193 (37.3%)</td>
<td>90/181 (49.7%)</td>
<td>0.60 (0.40, 0.91)</td>
</tr>
<tr>
<td>French, 1999</td>
<td>9/40 (22.5%)</td>
<td>41/121 (33.9%)</td>
<td>0.57 (0.25, 1.30)</td>
</tr>
<tr>
<td>Ghidini, 1997</td>
<td>51/89 (57.3%)</td>
<td>30/47 (63.8%)</td>
<td>0.76 (0.37, 1.58)</td>
</tr>
<tr>
<td>Pratt I, 1999</td>
<td>12/33 (36.4%)</td>
<td>44/86 (51.2%)</td>
<td>0.55 (0.24, 1.25)</td>
</tr>
<tr>
<td>Pratt II, 1999</td>
<td>4/25 (16%)</td>
<td>10/56 (17.9%)</td>
<td>0.88 (0.25, 3.12)</td>
</tr>
<tr>
<td>Pratt III, 1999</td>
<td>2/78 (2.6%)</td>
<td>5/131 (3.8%)</td>
<td>0.66 (0.13, 3.50)</td>
</tr>
<tr>
<td>Smith, 2000</td>
<td>9/14 (64.3%)</td>
<td>26/53 (49.1%)</td>
<td>1.87 (0.55, 6.32)</td>
</tr>
<tr>
<td>Vermillion, 2000</td>
<td>76/186 (40.9%)</td>
<td>104/267 (38.9%)</td>
<td>1.08 (0.74, 1.59)</td>
</tr>
<tr>
<td>Total</td>
<td>390/957 (40.8%)</td>
<td>612/1347 (44.5%)</td>
<td>0.79 (0.66, 0.96)</td>
</tr>
<tr>
<td><strong>Mortality</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abbasi, 2000</td>
<td>6/192 (3.1%)</td>
<td>4/177 (2.3%)</td>
<td>1.40 (0.39, 5.03)</td>
</tr>
<tr>
<td>Banks I, 1999</td>
<td>17/107 (15.9%)</td>
<td>30/229 (13.1%)</td>
<td>1.25 (0.66, 2.39)</td>
</tr>
<tr>
<td>Banks II, 1999</td>
<td>8/193 (4.1%)</td>
<td>5/181 (2.8%)</td>
<td>1.52 (0.49, 4.74)</td>
</tr>
<tr>
<td>French, 1999</td>
<td>3/43 (7%)</td>
<td>14/123 (11.4%)</td>
<td>0.58 (0.16, 2.14)</td>
</tr>
<tr>
<td>Pratt I, 1999</td>
<td>2/33 (6.1%)</td>
<td>13/86 (15.1%)</td>
<td>0.36 (0.08, 1.70)</td>
</tr>
<tr>
<td>Pratt II, 1999</td>
<td>0/25</td>
<td>2/56 (3.6%)</td>
<td>0.43 (0.02, 9.26)</td>
</tr>
<tr>
<td>Pratt III, 1999</td>
<td>0/78</td>
<td>0/131</td>
<td></td>
</tr>
<tr>
<td>Smith, 2000</td>
<td>0/14</td>
<td>9/53 (17%)</td>
<td>0.16 (0.01, 2.95)</td>
</tr>
<tr>
<td>Vermillion, 2000</td>
<td>19/186 (10.2%)</td>
<td>10/267 (3.7%)</td>
<td>2.92 (1.33, 6.44)</td>
</tr>
<tr>
<td>Total</td>
<td>55/871 (6.3%)</td>
<td>87/1303 (6.7%)</td>
<td>1.16 (0.67, 1.98)</td>
</tr>
<tr>
<td><strong>IVH</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abbasi, 2000</td>
<td>4/192 (2.1%)</td>
<td>2/177 (1.1%)</td>
<td>1.86 (0.34, 10.29)</td>
</tr>
<tr>
<td>Ghidini, 1997</td>
<td>1/89 (1.1%)</td>
<td>4/47 (8.3%)</td>
<td>0.12 (0.01, 1.13)</td>
</tr>
<tr>
<td>Pratt I, 1999</td>
<td>1/33 (3%)</td>
<td>6/86 (7%)</td>
<td>0.42 (0.05, 3.60)</td>
</tr>
<tr>
<td>Pratt II, 1999</td>
<td>0/25</td>
<td>1/56 (1.8%)</td>
<td>0.73 (0.03, 18.43)</td>
</tr>
<tr>
<td>Pratt III, 1999</td>
<td>0/78</td>
<td>0/131</td>
<td></td>
</tr>
<tr>
<td>Smith, 2000</td>
<td>0/14</td>
<td>5/53 (9.4%)</td>
<td>0.30 (0.02, 5.83)</td>
</tr>
<tr>
<td>Vermillion, 2000</td>
<td>8/186 (4.3%)</td>
<td>12/267 (4.5%)</td>
<td>0.96 (0.38, 2.38)</td>
</tr>
<tr>
<td>Total</td>
<td>14/617 (2.3%)</td>
<td>30/817 (3.7%)</td>
<td>0.75 (0.38, 1.48)</td>
</tr>
<tr>
<td><strong>BPD</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abbasi, 2000</td>
<td>11/192 (5.7%)</td>
<td>6/177 (3.4%)</td>
<td>1.73 (0.63, 4.79)</td>
</tr>
<tr>
<td>Banks I, 1999</td>
<td>31/107 (29%)</td>
<td>62/229 (27.1%)</td>
<td>1.10 (0.66, 1.83)</td>
</tr>
<tr>
<td>Banks II, 1999</td>
<td>31/193 (16.1%)</td>
<td>20/181 (11%)</td>
<td>1.54 (0.84, 2.82)</td>
</tr>
<tr>
<td>French, 1999</td>
<td>8/43 (18.6%)</td>
<td>13/123 (10.6%)</td>
<td>1.93 (0.74, 5.05)</td>
</tr>
<tr>
<td>Pratt I, 1999</td>
<td>6/33 (18.2%)</td>
<td>21/86 (24.4%)</td>
<td>0.69 (0.25, 1.89)</td>
</tr>
<tr>
<td>Pratt II, 1999</td>
<td>0/25</td>
<td>0/56</td>
<td></td>
</tr>
<tr>
<td>Pratt III, 1999</td>
<td>1/78 (1.3%)</td>
<td>1/131 (0.8%)</td>
<td>1.69 (0.10, 27.38)</td>
</tr>
<tr>
<td>Smith, 2000</td>
<td>4/14 (28.6%)</td>
<td>11/53 (20.8%)</td>
<td>1.53 (0.40, 5.81)</td>
</tr>
<tr>
<td>Total</td>
<td>92/685 (13.4%)</td>
<td>134/1036 (12.9%)</td>
<td>1.30 (0.95, 1.78)</td>
</tr>
<tr>
<td><strong>Sepsis</strong>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abbasi, 2000</td>
<td>19/192 (9.9%)</td>
<td>11/177 (6.2%)</td>
<td>1.66 (0.77, 3.59)</td>
</tr>
<tr>
<td>Ghidini, 1997</td>
<td>13/89 (14.6%)</td>
<td>9/47 (19.1%)</td>
<td>0.72 (0.28, 1.84)</td>
</tr>
<tr>
<td>Pratt I, 1999</td>
<td>13/27 (48.1%)</td>
<td>43/66 (65.2%)</td>
<td>0.50 (0.20, 1.23)</td>
</tr>
<tr>
<td>Pratt II, 1999</td>
<td>3/22 (13.6%)</td>
<td>17/49 (34.7%)</td>
<td>0.30 (0.08, 1.15)</td>
</tr>
<tr>
<td>Pratt III, 1999</td>
<td>5/74 (6.8%)</td>
<td>11/126 (8.7%)</td>
<td>0.76 (0.25, 2.27)</td>
</tr>
<tr>
<td>Study</td>
<td>Incidence</td>
<td>95% CI</td>
<td>p Value</td>
</tr>
<tr>
<td>---------------</td>
<td>-----------</td>
<td>--------</td>
<td>---------</td>
</tr>
<tr>
<td>Smith, 2000</td>
<td>1/14 (7.1%)</td>
<td>2/53 (3.8%)</td>
<td>1.96 (0.16, 23.34)</td>
</tr>
<tr>
<td>Vermillion, 2000</td>
<td>10/186 (5.4%)</td>
<td>3/267 (1.1%)</td>
<td>5.00 (1.36, 18.42)</td>
</tr>
<tr>
<td>Total</td>
<td>64/604 (10.6%)</td>
<td>96/785 (12.2%)</td>
<td>0.97 (0.51, 1.85)</td>
</tr>
</tbody>
</table>

**PDA**

<table>
<thead>
<tr>
<th>Study</th>
<th>Incidence</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbasi, 2000</td>
<td>24/192 (12.5%)</td>
<td>37/177 (20.9%)</td>
<td>0.54 (0.31, 0.95)</td>
</tr>
<tr>
<td>Pratt I, 1999</td>
<td>3/33 (9.1%)</td>
<td>15/86 (17.4%)</td>
<td>0.47 (0.13, 1.76)</td>
</tr>
<tr>
<td>Pratt II, 1999</td>
<td>3/25 (12%)</td>
<td>4/56 (7.1%)</td>
<td>1.77 (0.37, 8.59)</td>
</tr>
<tr>
<td>Pratt III, 1999</td>
<td>0/78</td>
<td>3/131 (2.3%)</td>
<td>0.23 (0.01, 4.59)</td>
</tr>
<tr>
<td>Smith, 2000</td>
<td>0/14</td>
<td>11/53 (20.8%)</td>
<td>0.13 (0.01, 3.30)</td>
</tr>
<tr>
<td>Total</td>
<td>30/342 (8.8%)</td>
<td>70/503 (13.9%)</td>
<td>0.56 (0.35, 0.90)</td>
</tr>
</tbody>
</table>

**NEC**

<table>
<thead>
<tr>
<th>Study</th>
<th>Incidence</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbasi, 2000</td>
<td>12/192 (6.3%)</td>
<td>9/177 (5.1%)</td>
<td>1.24 (0.51, 3.03)</td>
</tr>
<tr>
<td>Ghidini, 1997</td>
<td>6/89 (6.7%)</td>
<td>4/47 (8.5%)</td>
<td>0.78 (0.21, 2.90)</td>
</tr>
<tr>
<td>Pratt I, 1999</td>
<td>1/33 (3%)</td>
<td>2/86 (2.3%)</td>
<td>1.31 (0.11, 14.98)</td>
</tr>
<tr>
<td>Pratt II, 1999</td>
<td>2/25 (8%)</td>
<td>1/56 (1.8%)</td>
<td>4.78 (0.41, 55.39)</td>
</tr>
<tr>
<td>Pratt III, 1999</td>
<td>0/78</td>
<td>0/131</td>
<td></td>
</tr>
<tr>
<td>Smith, 2000</td>
<td>1/14 (7.1%)</td>
<td>0/53</td>
<td>11.89 (0.46, 308.42)</td>
</tr>
<tr>
<td>Vermillion, 2000</td>
<td>10/186 (5.4%)</td>
<td>12/267 (4.5%)</td>
<td>1.21 (0.51, 2.86)</td>
</tr>
<tr>
<td>Total</td>
<td>32/617 (5.2%)</td>
<td>12/267 (4.5%)</td>
<td>1.29 (0.76, 2.18)</td>
</tr>
</tbody>
</table>

**Birth weight <10%**

<table>
<thead>
<tr>
<th>Study</th>
<th>Incidence</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>French,1999</td>
<td>11/43 (25.6%)</td>
<td>21/123 (17%)</td>
<td>1.67 (0.73, 3.83)</td>
</tr>
<tr>
<td>Ghidini, 1997</td>
<td>11/89 (12.4%)</td>
<td>34/47 (6.4%)</td>
<td>2.07 (0.55, 7.81)</td>
</tr>
<tr>
<td>Vermillion, 2000</td>
<td>7/186 (3.8%)</td>
<td>16/267 (6%)</td>
<td>0.61 (0.25, 1.52)</td>
</tr>
<tr>
<td>Total</td>
<td>29/518 (9.1%)</td>
<td>40/437 (9.2%)</td>
<td>1.21 (0.58, 2.54)</td>
</tr>
</tbody>
</table>

*for sepsis there was statistically significant heterogeneity (P = 0.03); OR: odds ratio; CI: confidence interval; RDS: respiratory distress syndrome; IVH: intraventricular hemorrhage; BPD: broncho pulmonary dysplasia; NEC: necrotizing enterocolitis; PDA: patent ductus arteriosus. Banks I: 25-27 weeks; Banks II: 28-32 weeks; Pratt I: < 32 weeks; Pratt II: 32-34 weeks; Pratt III: > 34 weeks.
Table 2.4 Multiple (vs. single) Courses of Antenatal Corticosteroids and Maternal Outcomes

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Multiple Courses</th>
<th>Single Courses</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chorioamnionitis</strong>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abbasi, 2000</td>
<td>61/192 (32%)</td>
<td>52/177 (29%)</td>
<td>1.12 (0.72, 1.74)</td>
</tr>
<tr>
<td>Ghidini, 1997</td>
<td>22/89 (25%)</td>
<td>18/47 (38%)</td>
<td>0.53 (0.25, 1.13)</td>
</tr>
<tr>
<td>Vermillion, 2000</td>
<td>13/186 (7%)</td>
<td>2/267 (0.7%)</td>
<td>9.96 (2.22, 44.67)</td>
</tr>
<tr>
<td>Total</td>
<td>96/467 (21%)</td>
<td>72/491 (15%)</td>
<td>1.46 (0.47, 4.59)</td>
</tr>
<tr>
<td><strong>Endometritis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abbasi, 2000</td>
<td>19/192 (10%)</td>
<td>6/177 (3%)</td>
<td>2.79 (1.24, 6.29)</td>
</tr>
<tr>
<td>Vermillion, 2000</td>
<td>25/186 (13%)</td>
<td>11/267 (4%)</td>
<td>3.57 (1.79, 7.12)</td>
</tr>
<tr>
<td>Total</td>
<td>44/378 (12%)</td>
<td>17/444 (4%)</td>
<td>3.22 (1.90, 5.45)</td>
</tr>
<tr>
<td><strong>Maternal Infection</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pratt, 1999</td>
<td>45/101 (45%)</td>
<td>85/218 (39%)</td>
<td>1.26 (0.78, 2.03)</td>
</tr>
</tbody>
</table>

*for chorioamnionitis there was statistically significant heterogeneity ($P = 0.002$)
Systematic Review of Published Literature in Animals

A. Objective

The aim of this study was to systematically review the available evidence relating to the effects of repeated vs. single doses of antenatal corticosteroids (ACS) on lung maturation, nervous system and growth in animals.

B. Sources

We searched MEDLINE (1966-2001), and Embase (1980-2001) for studies involving animals, published in English, using the search terms adrenal cortex hormones, glucocorticoids, betamethasone, dexamethasone, lung, fetal organ maturity, maturation, respiratory distress syndrome, brain, development, growth, pregnancy and pregnancy complications.

C. Methods

Articles were included in the review if they reported on randomized controlled studies which compared repeated doses of ACS with a single dose, with or without placebo given to pregnant animals. Articles were included if they investigated the clinical effects of the multiple doses of ACS rather than investigating the pathway of the effects of the multiple doses of ACS.
D. Results

A total of 19 studies (Table 3.1) met the inclusion criteria, of which 8 reported on the effects of corticosteroids on the fetal lung, 7 on the fetal nervous system, and 11 on fetal growth.

D.1 Lung Function

D.1.1 Sheep

Ikegami et al.\textsuperscript{13} randomized pregnant ewes to receive 1 to 4 doses of 0.5 mg/kg betamethasone or saline placebo at 7 day intervals from 104 to 124 days gestation. All lambs were delivered preterm at 125 days gestation (normal term pregnancy in sheep is 147 days). Total thoracic compliance increased by 28% after one dose, by 77% after two doses, by 100% after three doses, and by 150% after 4 doses of betamethasone. The ventilatory efficiency index (an integrated measurement of gas exchange) increased following two and three doses of betamethasone and increased more than three-fold from the control value after four doses of betamethasone. Polk et al.\textsuperscript{14} randomized fetal sheep at 121 days of gestation to receive saline solution or betamethasone (0.5 mg/kg) as a single injection and six days later they received saline or betamethasone again. Lung compliance significantly increased after betamethasone exposure. However, the duration of steroid exposure or a second dose of steroid induced no further increase in lung compliance. The ventilation efficiency index also improved nearly 50% after betamethasone exposure but no significant effect of either dosing interval (1 day vs. 7 days before delivery) or number of doses (one vs. two) was noted.

Repeated doses of ACS have been associated with increased production and secretion of surfactant components. In the study by Polk et al.\textsuperscript{14} surfactant pool sizes increased in animals delivered 7 days after ACS. However, no additional effect of a second dose of
steroids was noted. The level of surfactant protein B mRNA was significantly augmented in animals receiving two doses of betamethasone but this effect was not shown for surfactant protein A mRNA nor for surfactant protein C mRNA. Ballard et al.\textsuperscript{65} in a study with similar design as Ikegami et al.\textsuperscript{42} found that preterm lambs who were exposed to more than 2 doses of antenatal betamethasone had a dose dependent increase in concentration of saturated phosphatidylcholine and surfactant protein A and B (a maximal increase of 2 to 3 -fold in tissue and 10 to 15 -fold in lavage fluid). Walther et al.\textsuperscript{66} randomized pregnant sheep to receive one, two, three, or four doses of 0.5 mg/kg betamethasone or saline placebo on 104, 111, 118, 124 days of gestation. They assessed the preterm lamb lung tissue for antioxidant enzymes (the primary defense against free radicals), lipid hydroperoxide, and carbonyl proteins (the latter two are free radicals which mediate oxygen-induced cellular damage to immature lung). The activity of all antioxidant enzymes further increased with additional doses of betamethasone and was maximal after 4 doses. The levels of lipid hydroperoxide and carbonyl protein content decreased stepwise after each dose of betamethasone and were lowest after four doses.

D.1.2 Monkeys

Engle et al\textsuperscript{44} randomized pregnant monkeys at 132 days of gestation to receive varying doses of dexamethasone or placebo as a single dose injection or 4 repeated injections at 12 hour intervals. Preterm monkeys who were exposed to 4 doses of antenatal dexamethasone (0.5 mg/kg) had a significantly higher level of total lung phosphatidylcholine, surfactant phosphatidylcholine and the surfactant-phosphatidylcholine to total phosphatidylcholine ratio compared to a single injection, suggesting that repeat
dosing of ACS produce a much greater enhancement of fetal pulmonary maturation than a single injection of the same total dose.

D.1.3 Rabbits

Pratt et al\textsuperscript{45} assigned pregnant rabbits to receive saline or 1, 2, or 3 doses of betamethasone (0.1 mg/kg) at day 19 of gestation. A significant increase was observed for both surfactant protein A and surfactant protein B in preterm rabbits who were exposed to 3 doses of betamethasone. In their study, histologic sections of fetal lung demonstrated significant maturation after 3 doses of betamethasone. Sun et al\textsuperscript{46} assigned pregnant rabbits at 27 days of gestational age to receive betamethasone, 0.1 mg/kg, as one dose or two divided doses, or 0.2 mg/kg as a single injection. They noted improvement in ventilation and lung compliance in rabbits who received two doses of ACS compared to a single dose, despite lower alveolar saturated phosphatidylcholine pool size in the two doses group. This suggests that the maturational effects of ACS in rabbits are not primarily on the surfactant system, rather on the fetal lung structure. The higher dose of betamethasone (0.2 x 1) was not associated with improvement in lung maturation, making the point that total dose is not critical variable. Rather the timing and spacing of the dose is more important for lung maturation.

D.1.4 Mice

Stewart et al\textsuperscript{47} randomly assigned mice to 1 of 3 groups to receive either a placebo or betamethasone (0.1 mg/kg) as a single dose on gestational day 14 or as a multidose twice daily on day 14 and 15. They showed that fetuses exposed to repeated doses of ACS had a higher breathing score (scale 0 to 5: 0, no breathing; 5, unlabored breathing) at gestational day 16 than either to a single dose or placebo (mean score 4.6 vs. 3.8 or 1.3: P<0.001).
Alveolar development (0, pseudoglandular; 1, canalicular; 2, canalicular/terminal sac; 3, terminal sac; 4, terminal sac/alveolar; 5, alveolar budding) was also greater in the multiple steroid group than in the single group or placebo group in their study (mean score of 4.4 vs. 3.5 or 1.6; P<0.001).

**D.2 Nervous System**

**D.2.1 Sheep**

Dunlop et al\(^9\) randomly allocated pregnant ewes to receive betamethasone or saline on day 104, 111, 118, and 124 of gestation to examine the optic nerve in preterm fetal sheep (day 125). The brain weight and the cross-sectional area of the optic nerves showed a strong trend (P = 0.07) toward lower values in the steroid group. The nerves appeared less mature with a significantly lower percentage of axons having become fully myelinated in the steroid group with values of 32.7% vs. 52.4% (P< 0.0001) in the control group. Analysis of the sequence of myelination showed that axons at an initial stage of myelination were rare in both groups. However axons at an intermediate stage of myelination were more frequent in the steroid group (11.1%) than in the control group (7.2%), indicating that the time course of myelination may be protracted by steroid treatment.

In a series of studies, Quinlivan et al\(^6\) randomized pregnant ewes into one of three treatment groups: control, single, and repeated corticosteroid treatment. Ewes received an injection of betamethasone (0.5mg/kg) on day 104 in the single steroids group, and on days 104, 111, 118, and 123 in the repeated steroid group. Controls received normal saline at those times.

First, Quinlivan et al\(^6\) looked at the optic nerve in the fetal lamb on day 125 (preterm) and in day 145 (term). They did not find any differences in the length of the optic
nerve or its total cross sectional area between the three treatment groups at either gestational age. There was a significant reduction in the mean optic axon fascicle area following repeated ACS treatment compared to controls at day 125 (1.42 mm² vs. 1.74 mm², \( P=0.048 \)). However, no differences in the mean optic axon fascicle area were observed at 145 days for either treatment. Axon density was significantly increased following repeated ACS treatment compared to controls when examined at 125 days, but not at 145 days. There were less mature nerves with a higher percentage of unmyelinated axons (65% vs. 34%, \( P=0.0001 \)) and a lower percentage of fully myelinated axons (24% vs. 51%, \( P=0.0001 \)) following repeated steroids compared to controls at day 125. They found no significant differences in the percentage of unmyelinated and fully myelinated nerves between the three treatment groups at 145 days, suggesting a catch up of the delay in nerve myelination at term.

Next, Quinlivan et al.\(^6\) investigated retinal maturation on day 125 (preterm) and on day 145 (term). They reported a significant delay in retinal maturation following repeated injections of ACS in sheep. Repeated ACS significantly altered patterns of eye growth, with all layers of retina being significantly thinner in the repeated steroid group at 125 days (11%-18% thinner, \( P=0.0001 \)). At 145 days, eye diameters were significantly reduced (2.23 cm vs. 3.02 cm, \( P=0.03 \)) and all layers of retina were significantly thickened (10%-12% increase, \( P=0.0001 \)) following repeated doses of ACS. Thus, repeated doses of steroids were associated with a decrease in eye growth, but also with a significant increase in retinal thickening.

Lastly, Quinlivan et al.\(^6\) evaluated the growth of the sciatic nerve on day 125 (preterm) and on day 145 (term). The growth of the fascicle-containing area of the sciatic nerve was significantly reduced following repeated steroid therapy compared to controls at
day 125 (2.58 mm$^2$ vs. 3.19 mm$^2$, $P=0.01$). However, they did not find any significant differences in the total cross-sectional area of the sciatic nerve between treatment groups at the same gestational age. At 145 days (term), there was a significant reduction in the total cross-sectional and fascicle-containing areas of the sciatic nerve following repeated steroid treatment vs. controls. (5.19 mm$^2$ vs. 8.74 mm$^2$, $P=0.0002$ and 4.23 mm$^2$ vs. 2.81 mm$^2$, $P=0.0002$) respectively. A single dose of steroid did not have any effect on the growth of the sciatic nerve at term and preterm. Neither single nor repeated steroid treatments significantly altered the proportion of fully myelinated axons in the sciatic nerve at 125 or 145 days, suggesting steroid therapy did not exert a significant effect upon sciatic nerve myelination.

Huang et al$^{70}$ in a similar study, found a decrease in fetal brain growth following repeated corticosteroid treatment. For those receiving a single dose at 125 days, there were no significant differences in whole-brain weight between the steroid-treated animals and controls (38.0 ± 1.81 g vs. 42.5 ± 1.65 g, $P=0.07$). However, maximum anterior-posterior cerebral length and maximum cerebral depth were significantly reduced in the steroid treatment group compared to controls. At 145 days, whole-brain weight was significantly lower in the steroid group compared to controls (47.5 ± 1.70 g vs. 53.4 ± 1.73 g), as was the whole-brain volume, cerebral volume, cerebellar weight, maximum cerebral width and depth. For those receiving repeated doses of steroid, the whole brain weight was significantly reduced at 125 and 145 days compared to controls (35.5 ±1.65 g vs. 42.5 ± 1.65 g $P=0.005$ and 42.4 ± 1.52 g vs. 53.4 ± 1.73 g $P= 0.001$ respectively). The whole brain volume, cerebral weight, cerebral volume, maximum anterior-posterior cerebral length and maximum cerebral width and depth all were reduced following repeated steroid treatment compared to controls at day 125. All of these measurements plus cerebellar weight and brain-stem weight were
also significantly reduced following repeated steroid treatment compared to controls at 145 days.

D.2.2 Monkeys

Uno et al\textsuperscript{71} randomly assigned pregnant monkeys to receive a single dose of dexamethasone on day 132 (with doses of 0.5, 5, or 10 mg/kg) or multiple doses of dexamethasone on days 132 and 133 (with doses of 0.125 mg/kg × 4, 1.25 mg/kg × 4, or 2.5 mg/kg × 4 at 12 hour intervals) or placebo. The fetuses were delivered at day 135 (preterm). Two additional monkeys received dexamethasone (1.25 mg/kg × 4) on day 132, and their fetuses delivered at day 162 (term). The investigators showed that multiple doses of dexamethasone were associated with a decreased number of neurons and with degeneration in neurons in the hippocampus. Degeneration was dose dependent and repeated injections induced more severe damage than single injections at the same total dose. Degenerative changes induced by repeated doses of dexamethasone were still clearly evident in fetuses studied at 162 days.

D.2.3 Mice

Rayburn et al\textsuperscript{72} randomized pregnant mice to receive single or repeated doses of betamethasone or saline. They reported that repeated administration of ACS was not associated with different responses to motivation/anxiety testing (separation vocalization, homing, auditory response, elevated plus maze, exploratory activity, and male aggression) by the offspring at any period in the life-span. Separation vocalization at postnatal day 5 was less after 4 and 8 doses of betamethasone compared to a double doses of betamethasone or placebo. However by postnatal day 7, separation vocalization was uncommon and no differences were found between the betamethasone-exposed and the placebo-exposed
offspring. In exploratory activity (using the radial eight arm maze), the only statistically significant differences was the shorter time required to make the initial eight choices for adult female offspring exposed previously to 8 doses of betamethasone rather than to the placebo.

D.3 Growth

D.3.1 Sheep

Ikegami et al.\(^4^3\) found a decrease in birth weight following ACS (a 15% reduction after one dose of ACS, 19% after 2 doses, and 27% after 3 and 4 doses). There were no significant changes in lung to body weight ratio, lung dry weight to lung wet weight ratio, or lung protein to body weight ratio. However, Polk et al.\(^6^4\) did not show any differences in birth weight between animals receiving single vs. repeated doses of ACS. Jobe et al.\(^4^8\) randomly assigned pregnant sheep to receive one dose of 0.5 mg/kg betamethasone at 104 days gestational age or three doses of betamethasone at 104, 111, and 118 days gestational age or saline for controls. They examined lambs for birth weight and body measurement at 125 days (preterm) and 145 days (term) gestational age. They reported a 11% reduction in birth weight for preterm lambs and a 14% reduction in birth weight at term for those who were exposed to a single dose of steroids compared to controls. Also, they reported a 25% reduction in birth weight for preterm lambs and 19% reduction in birth weight at term for those who were exposed to 3 doses of ACS compared to those who were exposed to a single dose of steroids. At 125 days, the weight of the liver and brain was decreased \((P=0.06)\) after one dose and all organ weights decreased after 3 doses of ACS \((P<0.05)\). However, the organ weight/body weight ratios did not change as a result of prenatal ACS exposure except for the liver which was smaller. At 145 days, kidney, brain, and liver weights decreased after one dose, and all organ weights except the adrenal glands decreased after 3 doses of ACS \((P<0.05)\). However,
organ weight/body weight ratios were not changed after delivery at term. Quinlivan et al\textsuperscript{73} reported a significant reduction in body and organ weight and biometry for preterm and term lambs with repeated dosing of steroids compared to controls [birth weight at preterm (mean ± SD): 2558 ± 201 g vs. 3177 ± 216 g; birth weight at term (mean ± SD): 3924 ±519 g vs. 5856 ± 330 g respectively]. In their study, thymus, spleen and liver were the organs mostly severely affected. Dunlop et al\textsuperscript{49} showed lower biometric measures of growth (weight, femur-length, occipito-snout, brain volume, brain weight and nerve cross-sectional area) following repeated doses of ACS compared to controls although the differences were not statistically significant.

Newnham et al\textsuperscript{74} randomized pregnant ewes to receive betamethasone or saline, which were given to either the mother or fetus as a single dose on day 104 or multiple doses on days 104-118 at a 7-day intervals. A random 50% of the ewes were delivered at 125 days and the remainder were delivered at 145 days. They found reductions in birth weight, placental weight and the weight of major organs following repeated maternal doses of betamethasone, but direct fetal injections did not show the same effect. This finding indicates that in sheep the effect of betamethasone on fetal growth is dependent on the route of administration while administration by either route enhance lung maturation.

D.3.2 Monkeys

In a study by Engle et al\textsuperscript{44} in the monkey, body weight was generally not significantly affected at any steroid dose given either as a single or as a repeated-dose injection compared to no ACS. Following a single injection of ACS the weight (mean ± SD) of the liver was increased and this increase was even greater following repeated injections of ACS (10.54 ±
1.69 g vs. 12.17 ± 1.89 g vs. 16.22 ± 4.13 g, \( P=0.048 \) for 0.5 mg/kg of dexamethasone)

respectively.

**D.3.3 Rabbits**

Pratt et al\(^45\) showed a significant reduction in birth weight with increasing numbers of doses of antenatal betamethasone. Late treatment resulted in a greater decline in birth weight than did the same number of doses given at an earlier gestational age. Repeated doses of betamethasone were found to result in a greater proportional decrease in fetal lung weight.

Sun et al\(^46\) found a single dose of 0.2 mg/kg betamethasone given 48 hours before delivery had the same effect as divided doses of 0.1 mg/kg betamethasone on birth weight. Both caused a 20% reduction in birth weight compared to controls. A single dose of 0.1 mg/kg betamethasone caused a 9.4% reduction in birth weight compared to controls but had no effect on lung maturation indicators.

**D.3.4 Mice**

Stewart et al\(^47\) reported a significant reduction in fetal lung weight (mean ± SD) after exposure to repeated doses of betamethasone than after a single dose or after placebo (18.3 ± 1.0 g vs. 21.4 ± 1.3 g vs. 23.3 ± 1.3 g, \( P=0.02 \)). The ratio of lung to body weight was similarly affected. The reduced lung weight persisted into adulthood in mice who were exposed to repeated dosing of ACS. Stewart et al\(^75\) randomized mice to receive 2, 4, or 8 doses of 0.1 mg betamethasone or placebo. They found that the steroid treated group had fewer live pups with fewer male survivors and lower birth weights which were dose related. The group exposed to 8 doses of betamethasone, had significantly fewer survivors (mean ± SD) (7.3 ± 1.1 vs. 11.9 ± 0.8, \( P<0.01 \)), lower birth weight (mean ± SD) (1.46 ± 0.04 gm vs. 3.32 ± 0.04 gm, \( P<0.01 \)), shorter body length (mean ± SD) (3.13 ± 0.04 cm vs. 3.32 ± 0.04 cm).
and narrower head width (mean ± SD) (7.3 ± 0.6 mm vs. 7.8 ± 0.3 mm, P<0.001) compared with the placebo-exposed group. However, there were no differences in head size and width and in body length and weight of the offspring by postnatal day 7. There were not any significant differences in functional development and physical maturation in the offspring who were exposed to multiple doses of ACS compared to the placebo group. They found that reproductive capability, perinatal outcomes, and growth and development of the second-generation offspring were not different following multiple doses of ACS compared to placebo.

E. Discussion

This review provides evidence that repeated doses of corticosteroids have both beneficial and adverse effects in animals. Studies in sheep and mice found that repetitive exposure to maternal antenatal corticosteroids results in a progressive improvement in postnatal lung function. However repeated doses of antenatal corticosteroids have been found to cause growth restriction in different animal species. Repeated doses of antenatal corticosteroids delayed development of the nervous system and restricted brain growth, but the long term effect of this is unknown. Repeated doses of steroids were not associated with any effect on long term behavior among mice offspring.

Prenatal glucocorticoids can cause hypertension and glucose intolerance in adult offspring in the rat. Prenatal glucocorticoids may program specific effects in the brain, particularly upon the hypothalamic-pituitary-adrenal (HPA) axis. Prenatal programming of the HPA axis is mediated, at least in part, via alteration in glucocorticoid receptor gene expression in the hippocampus, which is an important locus of feedback control on the HPA axis.
The timing of maturation of the HPA axis relative to birth is specific for species and closely related to brain development. Much of the neuroendocrine development takes place in utero for animals who give birth to mature young (sheep, guinea pigs, and primates), while it occurs in the postnatal period for species who give birth to immature young (rats, rabbits, and mice). Therefore, prenatal glucocorticoid treatment in late gestation will impact on different stages of brain and HPA development depending on the species. Receptor sensitivity is another important consideration when extrapolating among different studies and species. Mice and rats are considered corticosensitive compared to other species, such as guinea pigs and primates which are considered corticoreistant.

Evidence from studies which had addressed multiple doses of ACS and their long term effects in animals are concerning. The differences in species in brain development during pregnancy, sensitivity to the glucocorticoid receptors, and the differences in dosing of glucocorticoids and stages of pregnancy among studies, make it difficult to extrapolate directly the results of these studies to humans.

However, this evidence of potential benefits (improved lung function) and potential risks (adverse effect on nervous system and growth restriction) raises the possibility that there may be a tradeoff between increasing the likelihood that babies will survive but also increasing the risk of long term neurodevelopmental problem.

Therefore, until there is clear evidence that benefits outweigh risks in humans, repeat courses of ACS should not be given to women unless this is done in the context of a randomized controlled trial.
Table 3.1 Characteristics of Included Studies in Animals

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>ACS</th>
<th>Outcomes</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ikegami, 1997</td>
<td>sheep*, n=55</td>
<td>B (1)</td>
<td>lung function, growth</td>
<td>↑ lung function, ↓ birth weight</td>
</tr>
<tr>
<td>Ballard, 1997</td>
<td>sheep, n=55</td>
<td>B (1)</td>
<td>lung function</td>
<td>↑ surfactant</td>
</tr>
<tr>
<td>Walther, 1998</td>
<td>sheep, n=55</td>
<td>B (1)</td>
<td>lung function</td>
<td>↑ antioxidant enzymes, ↓ free radicals</td>
</tr>
<tr>
<td>Polk, 1997</td>
<td>sheep, n=30</td>
<td>B (2)</td>
<td>lung function, growth</td>
<td>no effect on lung function and birth weight</td>
</tr>
<tr>
<td>Pratt, 1999</td>
<td>rabbits**, n=35</td>
<td>B (3)</td>
<td>lung function, growth</td>
<td>↑ lung function, ↓ birth and lung weight</td>
</tr>
<tr>
<td>Sun, 1993</td>
<td>rabbits, n=17</td>
<td>B (4)</td>
<td>lung function</td>
<td>↑ lung function, ↓ birth weight</td>
</tr>
<tr>
<td>Stewart, 1998</td>
<td>mice†, n=90</td>
<td>B (5)</td>
<td>lung function, growth</td>
<td>↑ breathing score and alveolar development, ↓ birth and lung weight</td>
</tr>
<tr>
<td>Engle, 1996</td>
<td>monkey‡, n=63</td>
<td>D (6)</td>
<td>lung function, growth</td>
<td>↑ surfactant, on effect on birth weight</td>
</tr>
<tr>
<td>Uno, 1990</td>
<td>monkey, n=48</td>
<td>D (7)</td>
<td>nervous system</td>
<td>↓ neurons, degeneration</td>
</tr>
<tr>
<td>Dunlop, 1997</td>
<td>sheep, n=6</td>
<td>B (8)</td>
<td>nervous system, growth</td>
<td>delay in optic nerve myelination, no effect on birth weight</td>
</tr>
<tr>
<td>Huang, 1999</td>
<td>sheep, n=24</td>
<td>B (1)</td>
<td>nervous system</td>
<td>↓ fetal brain growth</td>
</tr>
<tr>
<td>Quinlivan, 1999</td>
<td>sheep, n=36</td>
<td>B (1)</td>
<td>nervous system</td>
<td>delay in myelination in brain</td>
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<td>Quinlivan, 1999</td>
<td>sheep, n=30</td>
<td>B (1)</td>
<td>nervous system</td>
<td>delay in sciatic nerve growth</td>
</tr>
<tr>
<td>Quinlivan, 2000</td>
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<td>B (1)</td>
<td>nervous system</td>
<td>delay in retinal maturation</td>
</tr>
<tr>
<td>Rayburn, 1998</td>
<td>mice, n=60</td>
<td>B (9)</td>
<td>nervous system‡</td>
<td>no effect on behavioral outcomes</td>
</tr>
<tr>
<td>Jobe, 1998</td>
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<td>B (1)</td>
<td>growth</td>
<td>↓ birth weight</td>
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<tr>
<td>Stewart, 1997</td>
<td>mice, n=60</td>
<td>B (10)</td>
<td>growth</td>
<td>↓ birth weight, body length, head width</td>
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<td>Newnham, 1999</td>
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<td>B (1)</td>
<td>growth</td>
<td>↓ birth and organ weight</td>
</tr>
<tr>
<td>Quinlivan, 1998</td>
<td>sheep, n=46</td>
<td>B (1)</td>
<td>growth</td>
<td>↓ birth and organ weight</td>
</tr>
</tbody>
</table>

*term pregnancy in sheep = 147 days; ** term pregnancy in rabbits = 31 days; † term pregnancy in monkey = 165 days; ‡ term pregnancy in mice = 19 days; § Behavioral testing, separation vocalization, homing, auditory response, elevated plus maze, radial eight arm maze, male aggression; B: betamethasone; D: dexamethasone; ↑: increased; ↓: decreased

(1) B (0.5 mg/kg) on day 104 as a single dose or on days 104, 111, 118, and 124 as multiple doses, control group received placebo; (2) Four treatment groups: 1) saline + saline, 2) B + saline, 3) saline + B, 4) B + B, first dose at 121 days and second dose at 127 days of gestation; (3) Saline solution or 1, 2, or 3 courses of B (early treatment beginning day 19), 2 additional groups received 1 or 2 late courses; (4) B (0.1 mg/kg) at 48
and 24 hours before delivery, two groups with a single dose (0.1 mg, 0.2 mg B); (5) B (0.1 mg) as a single dose on day 14 or B (0.1 mg) as a multi-dose twice daily on day 14 and day 15. control group received placebo; (7) D (0.5, 5, or 10 mg/kg) as a single injection or multiple injections (4 doses of 0.125, 1.25, 2.5 mg/kg at 12 h interval) on day 132, control group received placebo; (8) B (0.5 mg/kg) on day 104, 111, 118, and 124, control group received saline; (9) B (0.2 mg) on day 14, B (0.1 mg) on days 13 to 16. B (0.1 mg) bid on day 14 and 15. B (0.1 mg) bid on day 13-16. control groups received placebo; (10) 2, 4, or 8 doses of B (0.1 mg/kg) on day 13 to 16. control groups received placebo.
The Multiple Courses of Antenatal Corticosteroids for Preterm Birth Study Design

A. Research Questions

A.1 Primary Research Question

For women at 24-30 weeks of gestation who remain at increased risk of preterm birth 7 or more days following a single course of ACS, are multiple courses of ACS every 7 days until 33 weeks effective in reducing the risk of perinatal or neonatal mortality or significant neonatal morbidity (defined as one or more of the following: RDS, BPD, IVH, PVL, and NEC) compared to placebo?

A.2 Other Research Questions

1. For women at 24-30 weeks who remain at increased risk of preterm birth 7 or more days following a single course of ACS do multiple courses of ACS every 7 days until 33 weeks increase or decrease the risk of a) neonatal sepsis, b) retinopathy of prematurity (ROP), and c) patent ductus arteriosus (PDA) compared to placebo?

2. Are multiple courses of ACS associated with higher or lower: a) birth weight, b) birth length, c) birth head circumference, d) birth abdominal circumference, e) length of stay in a neonatal intensive care unit and, f) number of days of assisted ventilation and intubation compared to placebo?
3. Do multiple courses of ACS increase or decrease the risk of: a) clinical chorioamnionitis and, b) antepartum or postpartum maternal infection (pneumonia, endometritis, wound infection, sepsis, pyelonephritis), compared to placebo?

4. How do women view their care if they receive multiple courses of ACS, compared to placebo?

B. Methods

B.1 Research Design

The proposed study is a multi-center, double-blind, randomized placebo controlled trial with prognostic stratification for gestational age (24-27 weeks: 28-30 weeks) and center. Randomization will be centrally controlled using a telephone computerized randomization service. Eligible and consenting women will be randomized within center and by gestational age groups to receive additional courses of ACS or placebo, using random block sizes of 2 and 4.

B.2 Selection Criteria for Participants

The study population consists of pregnant women at increased risk of preterm birth, at 24-30 weeks of gestation, 7 or more days following an initial course of ACS.

B.2.1 Inclusion Criteria

1. Women who have previously received one completed course of ACS, at least 7 days ago and continue to be at increased risk of preterm birth

2. Gestational age greater than or equal to 24 weeks of gestation and less than 31 completed weeks of gestation [gestational age will be determined by the clinician using menstrual history and early ultrasound if available]

To be eligible for trial entry women will have received one completed course of ACS defined
as: 2 doses of intramuscular betamethasone. 12 mg/dose, given at 12 or 24 hour intervals: or
4 doses of intramuscular dexamethasone. 5-6 mg/dose, given at 12 hour intervals.

To be considered at increased risk of preterm birth women will have to have had one or more
of the following: regular uterine contractions, a shortened cervical length or cervical dilation.
PPROM, antepartum bleeding secondary to placental separation or placenta previa, history of
preterm birth, maternal hypertension or other medical condition requiring preterm delivery.
intrauterine growth restriction or other fetal condition requiring preterm delivery.

B.2.2. Exclusion Criteria

1. Women requiring chronic doses of corticosteroids secondary to medical conditions (e.g.
   systemic lupus erythematosus, congenital adrenal hyperplasia)

2. Women with a contraindication to corticosteroids

3. Women with clinical evidence of chorioamnionitis (temperature ≥ 38°C. as defined in
   B.4.2.6)

4. Known lethal congenital anomaly (e.g. anencephaly)

Note that PPROM is not an exclusion to participation. This is because there is no evidence
that the benefits of multiple courses of ACS would not apply to women with PPROM, and
because there is no substantive evidence for an increased risk of maternal or fetal infection
following ACS for women with PPROM.21

B.3 Maneuver

B.3.1 Schema
B.3.2 Prior to Randomization

Within the 24 hours preceding enrollment in the study, women will have a non-stress test to rule out fetal compromise, and their temperature taken to rule out clinical evidence of chorioamnionitis (as defined in B.4.2.6). An obstetrical ultrasound will be performed within the 2 weeks prior to randomization to determine the number of fetuses, the estimated weight of the fetus(es), the presence of lethal or other congenital anomalies, the presence of placental anomalies, and the adequacy of the amniotic fluid. When women are identified as being at increased risk of preterm birth, and are given their initial course of ACS, they will be informed about the study in the event they remain undelivered after 7 days and continue to be at increased risk of preterm birth. A participant information sheet, outlining the possible benefits and risks of additional courses of ACS and the details of the study, will be provided. Eligible women will be invited to participate in the study. Women who agree to participate will sign a consent form, and baseline information will be collected prior to randomization.

B.3.3. Randomization
Patients will be randomized using the centralized telephone randomization service at the Maternal, Infants & Reproductive Health Research Unit at The Center for Research in Women's Health at the University of Toronto. Women will be assigned to receive either additional courses of ACS or placebo. Randomization will be stratified by participant center and gestational age at randomization (24-27 and 28-30 weeks). The randomization service will allocate a study number, which will conceal the patient's actual group assignment, to each participant (corresponding to treatment or control). The study number will then be given to the center pharmacist who will provide the study drug corresponding to the study number. If the study number indicates treatment, the study drug will consist of two syringes of 12 mg betamethasone/syringe. If the study number indicates control, the study drug will consist of two syringes of placebo (saline). The study drug will be given 24 hours apart for two doses. If it is not possible to obtain betamethasone in solution such that it appears similar to saline, the syringes will be coated so that the appearance of the study drug is not visible. If coding the syringes is not acceptable in a center, the drug will be administered by an individual not involved in the patient's care to remain blinding of care givers and patients.

B.3.4 ACS Group

Women allocated to a study number corresponding to ACS will receive a course of ACS, which will consist of two doses, 12 mg per dose, of betamethasone given intramuscular 24 hours apart. Following this, if the woman remains at increased risk of preterm birth, she will continue to receive weekly courses of ACS until 33 weeks gestation. All patients and care-givers will remain blind to the actual treatment given.

B.3.5 Placebo Group
Women allocated to a study number corresponding to placebo will receive a course of placebo, which will consist of two doses of placebo (saline), given intramuscular 24 hours apart. Following this, if the woman remains at increased risk of preterm birth she will receive weekly courses of placebo until 33 weeks gestation. All patients and care-givers will remain blind to the actual treatment given.

**B.3.6 Both Groups**

Centers will be encouraged to treat women with antibiotics if they develop clinical signs of chorioamnionitis, or if they are in labor and either they are known to be colonized with group B streptococcus (GBS) or their GBS status is unknown. If infants are born less than 1500 grams, centers will be encouraged to perform ophthalmologic exams to look for retinopathy of prematurity (ROP) and to perform a cranial ultrasound between day 1 and 3, and on day 7 and day 21 to look for evidence of IVH and periventricular leukomalacia (PVL).

All babies will undergo a cranial ultrasound to look for evidence of IVH and PVL at hospital discharge or at 40 weeks of gestation, which ever comes first. All other aspects of medical care for mothers and their infants will be determined by the treating physician, according to local hospital policies. Data will be collected to describe other maternal/fetal treatments, such as the use of tocolytics prior to delivery, and the use of neonatal treatments, such as surfactant, antibiotics, corticosteroids, and indomethacin.

All mothers will be asked to complete a structured questionnaire following birth to indicate their likes and dislikes regarding their care and their participation in the study.

**B.4 Outcomes**

**B.4.1 Primary Outcome**
Perinatal or neonatal mortality or significant neonatal morbidity is the primary Composite outcome. *Perinatal or neonatal mortality* is defined as stillbirth, or neonatal death during the first 28 days of life or prior to hospital discharge, whichever is later. *Significant neonatal morbidity* is defined as one or more of the following:

1. **RDS** (defined as: PaO2 < 50 mmHg in room air, central cyanosis in room air, or a requirement for supplement oxygen to maintain PaO2 > 50 mmHg and X-ray compatible with RDS [low lung volumes and reticulograndular appearance to lung fields with or without air bronchograms])

2. **Bronchopulmonary dysplasia (BPD)** (defined as requiring oxygen at corrected postnatal gestational age of 36 weeks and X-ray compatible with BPD)

3. **IVH grade III or IV**, diagnosed by cranial ultrasound using categorization of Papile et al\(^{82}\) or at autopsy

4. **PVL** (including periventricular cyst) diagnosed by cranial ultrasound

5. **NEC** (defined as either perforation of intestine, pneumatosis intestinalis or air in the portal vein, diagnosed by X-ray, surgery, or at autopsy)\(^{83}\)

**B.4.2 Additional Outcomes:**

1. **Neonatal infection** (clinical signs of infection and one or more of the following: a positive culture of blood, cerebrospinal fluid [CSF], urine, tracheal aspirate, or lung tissue at autopsy; a positive Gram’s stain of CSF; a chest X-ray compatible with pneumonia; or a histological diagnosis of pneumonia at autopsy)

2. **Retinopathy of prematurity (ROP)**, diagnosed in one or both eyes

3. **Birth weight, birth length, birth abdominal circumference and birth head circumference**

4. **Length of stay in neonatal intensive care and number of days on assisted ventilation with**
intubation

5. PDA (patent ductus arteriosus) requiring treatment or surgery

6. Clinical chorioamnionitis (defined as maternal temperature $\geq 38^\circ$C prior to delivery and one or more of the following: maternal tachycardia $\geq 120$ bpm, white blood cell count $\geq 20,000/mm^3$, fetal tachycardia $>160$ bpm, uterine tenderness, or foul smelling amniotic fluid)

7. Maternal infection (defined as one or more of the following: endometritis [postpartum maternal temperature $\geq 38^\circ$C and tender fundus without other source of infection], pneumonia [maternal temperature $\geq 38^\circ$C and signs of pneumonia on X-ray], wound infection [drainage of purulent material or wound breakdown], pyelonephritis [maternal temperature $\geq 38^\circ$C, positive urine culture and costal vertebral angle tenderness], or sepsis [positive maternal blood culture]

8. Women’s likes and dislikes regarding their care and participation in the study

B.5 Methodological Issues

B.5.1 Compliance

Women in the study will be given a booklet and will be instructed to carry it with them. The booklet will contain the details of the study protocol, their study number, the dates and times of courses of study drug received and when future courses are required. If a participant is discharged from hospital, the women will be given her remaining study drugs to take with her to give to the referring physician. Therefore, if women are discharged from a referral hospital, compliance with the protocol can be assured. Referring physicians in each center will be informed about the study, generally, and specifically before any of their
Chapter 4. The Multiple Courses of Antenatal Corticosteroids for Preterm Birth Study Design

patients are randomized. The study coordinator in each center will maintain ongoing contact with each patient and will track patients weekly, to assure that they receive the study drug, or that there is a valid reason why the study drug is not given.

B.5.2 Contamination

Contamination is unlikely to be a problem because physicians and patients will be blinded to the group allocation. To prevent control patients accidentally receive the treatment, women in the study will be given a booklet to carry with them, which will identify their involvement in the study. Copies of this information will be sent to the referring physician and will be available to the physician treating the patient.

B.5.3 Co-intervention

Information regarding co-interventions will be collected on the data forms. Co-interventions include maternal antibiotics, tocolytic agents, and neonatal treatments such as surfactant, antibiotics, corticosteroids, and indomethacin. Co-interventions will likely be similar between groups as the physicians and patients will not be aware of the group allocation, unless the co-interventions are a result of the effect of the multiple courses of ACS on the mother or infant.

B.5.4 Blinding

Physicians and patients will be blind to group of allocation. However, we recognize that ACS may cause maternal hyperglycemia, which may unblind caregivers, particularly if a woman has diabetes. Centers will be counseled to assume that all women are receiving ACS if they are concerned about side effects and monitor women in the study accordingly. If adverse effects occur such that continuation of ACS is contraindicated, the study drug will be discontinued, regardless of the group the woman has been allocated to. Thus blinding in this
situation, will be maintained. If a complication develops, in which the subsequent care of the patient is dependent on knowing the treatment assignment, arrangements will be made to allow for unblinding. The study research coordinator (or her designate) in the Data Center will be available by pager, 24 hours/day, 7 days/week, to provide the details regarding an individual’s allocation group, should this be necessary.

**B.5.5 Non-Randomized Patients**

Information regarding the number of patients approached and their decisions and the reasons for non-participation will be collected by the research nurse to compare non-randomized and randomized patients to determine if there are differences and thus to assess the generalizability of the trial results.

**B.5.6 Losses to Follow up**

When the condition resulting in the woman being at increased risk of preterm birth improves and she is referred back to her local physician for continuing care, centers will be asked to have a system in place to continue to track participants through to delivery to ensure that the study drugs are administered, if appropriate, the data are collected at the relevant times, the cranial ultrasound is taken, and the maternal questionnaire is completed before discharge from the hospital. There is no follow up for mothers and babies after discharge from the hospital.

**B.6 Sample Size & Feasibility**

**B.6.1 Sample Size**

The sample size has been calculated to be 1032 women (516/group). A sample size of 1032 women will have 80% power of finding a 30% reduction in risk of RDS from 20% to
14% with a 1-tailed \( \alpha \) error of 0.05 (Epi Info 6). We felt that a reduction risk from 20% to 14% would represent a clinically important difference and that a reduction in risk less than this would not justify the use of multiple courses of ACS because of the potential for possible greater risk of neurodevelopmental problems long term.

In calculating the sample size we have assumed the following:

1. The rate of RDS would be a reasonable surrogate measure for estimating the rate of perinatal or neonatal mortality or serious neonatal morbidity in this study.

2. A 1-tailed test is appropriate as the primary research question is whether multiple courses of ACS will lead to a reduction in perinatal or neonatal mortality or serious neonatal morbidity. Furthermore, the health care policy implication of (1) ACS having no benefit or (2) ACS being detrimental are the same, namely: ACS should not be used. Consequently, type I error does not need to be used to limit the probability of concluding detriment when, in truth, there is no benefit.

3. The rate of RDS is approximately 20% for infants in the placebo group. This is based on the information from the Ballard trial, in which women at risk of preterm birth were enrolled at <30 weeks gestation to receive thyrotropin releasing hormone (TRH) plus ACS versus placebo plus ACS (1 course). For those infants in the ACS and placebo group, who were born >10 days following entry to the study, the rate of RDS was 19%.

**B.6.2 Feasibility**

If a centre has 4000 deliveries/year, 16 women will be recruited each year. To recruit 1032 women in 2 years, we will need centers to provide 129,000 deliveries/year or
approximately 32 centers with 4000 deliveries/year. Prior to beginning this trial we would need to determine the interest among 32 or more centres in Canada and other countries.

Feasibility is based on the following assumptions: we estimate that approximately 1.6% of births at participating centers will present between 24 and 29 weeks gestation at increased risk of preterm birth. This is based on the actual recruitment rate into the Liggins and Howie study of ACS. In that study, the rate of recruitment at 24-36 weeks was 3.91%. Of these women, 24% delivered prior to 32 weeks. Assuming that 20% of the recruited mothers would have presented prior to 30 weeks gestation, we estimate that 0.78% (3.91% x 0.2) of all births would have presented as at increased risk of preterm birth prior to 30 weeks gestation. Assuming that only 50% of eligible patients were recruited to the study, 1.6% (0.78% x 2) of all births would have presented at increased risk of preterm birth.

In previous studies, 35%-55% of women were undelivered 7 days or more following a single course of ACS. Assuming that 40% of women will be undelivered after 7 days, 0.6% (1.6% x 0.4) of births at a center should be eligible to participate in this study. In the Ballard trial, 66% of eligible women agreed to participate in the study. Assuming that 66% of eligible women will agree to participate in the study, 0.4% (0.6% x 0.66) of all births in a center will be recruited to the study.

**B.7 Analysis Plan**

**B.7.1 Interim Analysis**

One interim analysis will be carried once complete data have been received on the first 400 patients enrolled. All data about the primary outcome will be received by the data monitoring committee. The data will be presented using an intention to treat approach, blinded to the actual group. If there is proof that the rate of the primary outcome is higher in
one group, then allocation group will be revealed. If the rate of the primary outcome is higher in ACS group vs. placebo group at $P<0.002$ (1-tailed) then the trial will be stopped. A one-sided test will be performed as we intend to stop the study early only if we can conclude that ACS is detrimental. Otherwise the trial will continue until the sample size has been achieved and the results will remain confidential and unknown to the participant centers.

**B.7.2 Final Analyses**

The final analysis will be based on the intention to treat principle, which will include all patients as randomized. Baseline characteristics of all patients will be collected just prior to randomization and will be compared to demonstrate any major dissimilarity between the two groups.

Logistic regression will be used to calculate the adjusted odds ratios and 95% confidence intervals for the comparison of the two study groups with respect to the primary outcome, perinatal or neonatal mortality, or significant neonatal morbidity, controlling for possible prognostic factors (gestational age at enrollment, singleton vs. multiple pregnancy, PROM, etc.). The level of statistical significance for the analysis of the primary outcome will be $P<0.05$. Outcome rates will be reported as the proportion of infants affected. However, because outcomes between infants in a multiple birth are correlated, the statistical analysis will be based on the pregnancy as the unit of analysis. Therefore, if any infant in a multiple birth has the outcome, then the pregnancy will be deemed to have the outcome. In addition, rate differences will be used to determine the number needed to treat.

Logistic regression will also be used to calculate the adjusted odds ratios and 95% confidence intervals for the comparison of the two study groups with respect to the other neonatal and maternal binary outcomes. Analysis of covariance will be used to compare the
group's continuous outcome measures, controlling for baseline demographic and prognostic characteristics. The level of statistical significance for these analyses will be \( P<0.01 \) (the lower \( P \) value for the analysis of secondary outcomes is chosen to minimize the likelihood of type I error). Continuous outcome measures that are skewed will undergo the appropriate transformation (most likely log or square root) prior to analysis. Descriptive statistics will be used to compare the participants' responses to questions about what they liked and disliked about their care and about participation in the study.

**B.8 Ethics**

Ethical approval will be obtained from every participant center. Women will be informed about the study after they receive their first course of corticosteroids. Women will be advised that they will have weekly injections, which will be slightly painful. All obstetricians will be informed of the trial and asked to offer participation in the trial to their patients. Women will be asked to sign a consent form before they are entered into the trial and women will be included in the study only if they have been adequately informed and have agreed to take part. Women will be able to withdraw without prejudice at any stage of the trial. Patients' names will not be linked to the data collected.

**B.9 Data Management**

Baseline characteristics will be transmitted to the data center during the randomization telephone call. The research nurse at each participating center will collect the other data on previously provided paper forms. They will be sent to the data center and will be scanned into a Teleform data management system. Logic and range checks will be used to verify the accuracy of the data. Any incomplete or inaccurate data will be rechecked with the
centers. The data manager will be responsible for checking the data before the analysis is started.

B.10 Time Schedule and Duties of Research Personnel

First year

Contact to the centers

Developing manuals of operations

Developing data forms and distributing them to the centers

Providing pharmacy packages for the centers

Randomization service and data base development

Recruitment into trial

Data collection

Second year

Recruitment into trial

Data collection

Interim analysis

Third year

Recruitment into trial

Data collection

Fourth year

Recruitment into trial

Data collection

Final analysis
Duties of research personnel: *The Steering Committee* is responsible for the decisions for the trial and for assisting with recruitment strategies. *The Data Monitoring Committee* is responsible for reviewing the interim analysis and making recommendations for early termination of the trial. *The Research Coordinator* has the overall responsibility for day to day management of the trial, regular liaison with center personnel, prepare and development of procedure manuals and data forms, organization and administration of center funding, organization and scheduling of meetings and site visits.

**B.11 Budget and Justification**

**Personnel:**

a) **Trial co-ordinator**: a full time position with overall responsibility for day to day management of the trial. Specific tasks include: regular liaison with center personnel: organization and administration of center funding: organization and scheduling of site visit and meetings.

b) **Data manager**: 0.5 time position with responsibility for all data management activities. Specific tasks include: some computer programming: maintenance of the computer randomization service: data forms design.

c) **Data entry clerk/ secretary**: a full time position with responsibility for data entry. correspondence with centers regarding incomplete or inaccurate data, production of a monthly newsletter and other secretarial tasks.

d) **Statistician**: part time position with the responsibility for statistical support and analysis and data management support. Payment is on a per hour basis and includes 3 weeks programmer time (40 hrs /wk, $70/hr), one week for the interim analysis and 4 weeks during the analysis phase.
Services and supplies:

a) Pharmacy costs: based on the Canadian tocolytic study it is estimated that the average time from enrolment to delivery will be around 3 weeks. So, we will need 3 courses (6 syringes) per patient on average.

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<th>Third week</th>
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<tr>
<td>4 courses</td>
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↑ Start of the trial

b) Office supplies and services: including printing forms, manuals, brochures and monthly newsletters, postal and courier services for the returning of data forms from the centers. telephone and fax charges including use of the computerized randomization system. A pager will be required as a backup for the computerized randomization service. Two computers and one printer will be needed for the data entry and software will be renewed or upgrade during the trial.

b) Travel: include the costs for attending at workshops (one person from each participating center), site visits and investigators meetings and attending at the meetings for the presentation of results.
### Chapter 4. The Multiple Courses of Antenatal Corticosteroids for Preterm Birth Study Design

#### Research Staff

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#### Services & supplies

| Pharmacy | $12600 | $34380 | $34380 | $21780 | $0 | $103140 |
| Office supplies | $2400 | $2400 | $2400 | $2400 | $600 | $10200 |
| Printing, forms, newsletter | $3000 | $3000 | $3000 | $1500 | $0 | $10500 |
| Postage & courier | $8500 | $9800 | $9800 | $7000 | $0 | $35100 |
| Telephone & Fax | $4000 | $5000 | $5000 | $3000 | $0 | $17000 |
| Bellboy | $200 | $200 | $200 | $200 | $0 | $800 |
| Computer hard & software | $6000 | $300 | $300 | $300 | $100 | $7000 |
| Randomization service | $1600 | $600 | $600 | $300 | $0 | $3100 |
| Service subtotal | $38300 | $55680 | $55680 | $36480 | $700 | $186840 |

#### Trial management

| Start up@250/center | $16250 | $0 | $0 | $0 | $0 | $16250 |
| Recruitment@250/patient | $25000 | $125000 | $125000 | $48500 | $0 | $323500 |
| Subtotal | $41250 | $125000 | $125000 | $48500 | $0 | $339750 |

#### Travel

| Workshops | $66000 | $66000 | $66000 | $0 | $0 | $198000 |
| Site visits | $30000 | $15000 | $15000 | $0 | $0 | $60000 |
| Presentation | $0 | $0 | $0 | $6000 | $0 | $6000 |
| Travel subtotal | $96000 | $81000 | $81000 | $6000 | $0 | $264000 |

### Total budget

- $319,728
- $401,617
- $400,189
- $230,874
- $46,897
- $1,399,305
The Pilot Study

A. Objective

To determine the feasibility of a multi-center randomized double blind placebo controlled trial (the proposed trial) to investigate the effects of multiple courses of antenatal corticosteoids (ACS) on perinatal or neonatal mortality and on neonatal and maternal morbidity when preterm delivery occurs more than 7 days following the initial course of ACS therapy.

A. Research Questions

1) What is the rate of recruitment of eligible pregnant women who remain undelivered and continue to be at increased risk of preterm birth 7 or more days following a single course of ACS?

2) What is the risk of complications among women who remain undelivered and continue to be at increased risk of preterm birth 7 or more days following a single course and receive weekly courses of ACS until 33 weeks that require discontinuation of the study treatment?

3) What is the level of cortisol, adreno corticotropin hormone (ACTH) and cortisol binding globulin (CBG) in cord blood and in maternal blood immediately following delivery for women who remain undelivered and continue to be at increased risk of preterm birth 7 or more days following a single course and receive weekly courses of ACS until 33 weeks?
C. Methods

Pregnant women were eligible for the trial if they remained undelivered 7 or more days following a single course of ACS, were 24 to 30 weeks of gestation and continued to be at increased risk of preterm birth. A single course of ACS was defined as 2 doses of intramuscular betamethasone, 12 mg/dose, given at 12 or 24 hour intervals; or 4 doses of intramuscular dexamethasone, 5-6 mg/dose, given at 12 hour intervals. To be considered at increased risk of preterm birth, women had one or more of the following: regular uterine contractions; a shortened cervical length or cervical dilation; preterm prelabour rupture of the membranes (PPROM); antepartum bleeding secondary to placental separation or placenta praevia; history of preterm birth; maternal hypertension or other medical condition increasing the risk of preterm delivery or intrauterine growth restriction or other fetal condition increasing the risk of preterm delivery. Women were excluded if they required chronic doses of corticosteroids secondary to medical conditions, had a contraindication to corticosteroids, had clinical evidence of chorioamnionitis or if their fetus(es) had a known lethal congenital anomalies.

The study was approved by the research ethics committees at the University of Toronto and Sunnybrook and Women’s College Health Sciences Center in Toronto. Patients were recruited from the Women’s College Campus of Sunnybrook & Women’s College Health Sciences Center and from Mount Sinai Hospital.

Within the 24 hours preceding enrolment in the study, women had a non-stress test to rule out fetal compromise, and their temperature was taken to rule out clinical evidence of chorioamnionitis. An obstetrical ultrasound was performed within 2 weeks prior to randomization to determine the number of fetuses, the estimated weight of the fetus(es), the
presence of lethal or other congenital anomalies, the presence of placental anomalies, and the adequacy of the amniotic fluid. When women were identified as being at increased risk of preterm birth, and were given their initial course of ACS, they were informed about the study in the event they remain undelivered 7 or more days following the first course and continued to be at increased risk of preterm birth. Eligible women were invited to participate in the study. Women who agreed to participate signed a consent form, and baseline information was collected prior to randomization (Appendix A).

Randomization was stratified by gestational age (24-27 weeks: 28-30 weeks) and by center, using block sizes of 2, and was centrally controlled by the local pharmacist at each center, who kept the randomization code. When the pharmacy was called to randomize a patient, they assigned the next study number to the patient, which corresponded to either betamethasone or saline placebo. In order to maintain blinding, the pharmacist prepared the study treatments in a syringe covered with a yellow tape. The study number also corresponded to a study package at the pharmacy which contained data forms and test tubes for blood samples which were given to the patient after randomization. The pharmacist at each center was not masked to the treatment allocated to the patient.

A course of ACS consisted of two doses, 12 mg per dose, of betamethasone (Celestone Soluspan; Schering Canada Inc.) given intramuscularly 24 hours apart. A course of placebo consisted of two doses of normal saline, given intramuscularly 24 hours apart. Following this, if the woman remained at increased risk of preterm birth, she continued to receive weekly courses of ACS or placebo until 33 weeks gestation. The injection of the study treatment was given by a designated research nurse in each hospital who was unblinded to the study treatment but was not caring for the patient to minimize unblinding of patients.
Although physicians and patients were blinded to group of allocation, we recognized that ACS may cause maternal hyperglycemia, which may unblind caregivers, particularly if a woman had diabetes. Centers were counseled to assume that all women were receiving ACS, if they were concerned about side effects and to monitor women in the study accordingly. If adverse effects occurred such that continuation of ACS was contraindicated, the study drug was discontinued, regardless of the group the woman had been allocated to. Thus blinding in this situation, was maintained. If a complication developed, in which the subsequent care of the patient was dependent on knowing the treatment assignment, arrangements were made to allow for unblinding. The project director (FA) in the Data Center was available by pager, 24 hours/day, 7 days/week, to provide the details regarding an individual’s allocation group, should this be necessary.

Women in the study were given a card and were instructed to carry it with them (Appendix B). The card contained the study title and logo, the study number and the contact information for the project director (FA). If a participant was discharged from hospital, she was given a letter plus a copy of the study protocol and working protocol to take with her to give to her physician (Appendix C.D). The letter identified the patient’s study number, the dates and times of courses of study drug already received, when future courses were required, and the instructions for blood samples. Therefore, if women were discharged from the hospital, compliance with the protocol was assured. Referring physicians in each center were informed about the study before any of their patients were randomized. The project director maintained ongoing contact with each patient and tracked patients weekly, to assure that they received the study drug, or that there was a valid reason why the study drug was not given. Data on compliance regarding treatment were collected by the project director.
Centers were encouraged to treat women with antibiotics if they developed clinical signs of chorioamnionitis, or if they were in labor and either they were known to be colonized with group B streptococcus (GBS) or their GBS status was unknown. A sample of cord blood was taken from babies to measure plasma cortisol, CBG and ACTH. Mothers had a sample of blood taken immediately following delivery to measure plasma cortisol, CBG and ACTH. Blood samples were centrifuged at 760 rpm for 15 minutes at 4°C. The plasma was collected and stored at -70°C until assay. Cord and maternal blood tests were run in batch at the end of the study. If infants were born less than 1500 grams, centers were encouraged to perform ophthalmologic exams to check for retinopathy of prematurity (ROP) and to perform a cranial ultrasound between day 1 and 3, and on day 7 and day 21 to check for evidence of IVH and periventricular leukomalacia (PVL). Babies also underwent a cranial ultrasound to check for evidence of IVH and PVL at hospital discharge or at 40 weeks of gestation, whichever came first. All other aspects of medical care for mothers and their infants were determined by the treating physician, according to local hospital policies. Data were collected to describe other maternal/fetal treatments, such as the use of tocolytics prior to delivery, and the use of neonatal treatments, such as surfactant, antibiotics, corticosteroids, and indomethacin (Appendix E).

All mothers were asked to complete a structured questionnaire following birth to indicate their health situation and their likes and dislikes regarding their care and their participation in the study (Appendix E).

Plasma cortisol and ACTH concentrations were measured by radioimmunoassay methods (ImmunoChem™ CORTISOL and RSL 125I ACTH. ICN Biomedicals. Inc. Costa Mesa, California) respectively. The manufacturer states that the cortisol assay kit does not
react with either betamethasone or dexamethasone. Assays for plasma ACTH that were off the standard curve (we assumed that high concentrations in ACTH were due to the stress of the labor), were repeated using diluted plasma samples. For each patient, we provided 4 smaller test tubes to store the plasma, 2 for maternal plasma and 2 for cord plasma in order to have a duplicate stored sample for the assay.

The outcomes of the pilot study were rate of recruitment, risk of complications that required discontinuation of study treatment in the two groups and levels of plasma cortisol, CBG, ACTH in cord blood and in maternal blood immediately following delivery in the two groups. The other outcomes were perinatal or neonatal mortality or significant neonatal morbidity which is the primary outcome for the proposed main trial. Significant neonatal morbidity is defined as one or more of the following: RDS, BPD, IVH, PVL and NEC (as defined in the proposed main trial, see Chapter 4).

Other outcomes included birth weight, birth length, birth abdominal circumference and birth head circumference, neonatal infection, ROP, PDA, length of stay in neonatal intensive care and number of days on assisted ventilation with intubation (as defined in the proposed main trial, see Chapter 4).

Additional maternal outcomes included clinical chorioamnionitis, maternal infection (endometritis, pneumonia, wound infection, or sepsis), maternal complications, women’s likes and dislikes regarding their care and participation in the study (as defined in the proposed main trial, see Chapter 4).

Baseline information plus the reasons for non-participation in the study were obtained from all non-randomized patients who received one course of ACS in the two participating hospitals.
D. Analysis Plan

The analysis of the outcome data was based on an “intention to treat” approach, which included all patients as randomized. Descriptive statistics were calculated to check for any major dissimilarities in the study groups with regard to patient demographics, prognostic and other baseline information. Differences between groups in plasma cortisol and ACTH were compared between the two groups using Student’s t test \( P<0.05 \) if the results were normally distributed, otherwise using the Wilcoxon rank sum test. Correlation between the level of hormones was calculated using Spearman’s Ranks correlation coefficient test \( P<0.05 \). Descriptive statistics were used for the analysis of other outcomes. Because outcomes between infants in multiple births are correlated, the statistical analysis was based on the pregnancy as the unit of analysis. Therefore, if any infant in a multiple birth had the outcome, then the pregnancy was deemed to have the outcome (for binary outcomes), or the mean of the two outcomes was calculated (for continuous outcomes). The perinatal/neonatal outcomes were also presented with the baby as the unit of analysis. The analysis was undertaken blinded to the group of allocation.

E. Results

E.1 Recruitment

113 patients were identified as being potentially eligible for the study between September 1, 1999 to August 31, 2000 from Sunnybrook & Women’s College Health Sciences Center and Mount Sinai Hospital in Toronto. Of the 113 patients, 87 (77%) were eligible for the study and 26 (23%) were ineligible. Twelve patients were recruited. Thus the overall recruitment rate was \( 12/87 \) 14%. (Figure 5.1)
Of the 26 who were not eligible for the study, 2 (8%) patients needed chronic doses of steroids due to a medical condition (asthma, rheumatoid arthritis) and 24 (92%) patients delivered within 7 days following the first course of ACS.

Of the 87 who were eligible for the study, 12 (14%) were recruited. 66 (76%) refused to participate, and 9 (10%) were not approached. These latter patients were mostly discharged from hospital before the correct contact information could be established.

Of the 66 who were eligible for the study and refused to participate, the reasons for not participating in the study were: 1) the patient did not want to participate: 52 (79%). Of these: 4 (6%) patients wanted to receive more steroids based on advice received from their physician and 34 (52%) patients did not want to receive more steroids also based on advice received from their physician. Ten (15%) patients did not want to be randomized and 4 (6%) patients refused for other reasons. These reasons included: being afraid of needles, participation in other studies, husbands disagreed, or no wish to participate in any kind of research. 2) Physicians did not wish their patients to participate: 14 (21%). Of these: 1 (2%) physician wanted the patient to receive more steroids. 11 (17%) physicians did not want their patients to receive more steroids and 2 (3%) physicians did not wish their patients to participate for medical reasons such as gestational diabetes.

E.2 Baseline characteristics

The baseline characteristics of participants in the two groups and non-participants are shown in Table 5.1.

E.3 Compliance with the Protocol

Five patients in the ACS group and 3 patients in the placebo group delivered before 33 weeks gestation. One patient in each group discontinued study treatment after they
received the first course. The patient in the ACS group had gestational diabetes and was concerned that the study treatment had made her blood glucose more difficult to control. She received one more course of study treatment 2 weeks later. The other patient was discharged from hospital with bed rest instruction and it was inconvenient for her to go to her obstetrician’s office weekly to receive the study treatments. After she delivered, she mentioned that the real reason for her decision was lack of support from medical staff at the participating hospital. One patient in each group was discharged from hospital before 33 weeks gestation but they still were at increased risk of preterm birth. They both received their weekly study treatments at their obstetrician’s office. The study treatment was prepared at the hospital pharmacy every week and was sent to the obstetrician’s office on the same day. All patients except one delivered at the same hospital where they have been enrolled to the study. The patient who delivered at another hospital was at term and blood samples plus the other data were obtained. The compliance with the study treatment is shown in Table 5.2.

The mean number of courses for all participants was $3 \pm 2$ [median ($5^{th},95^{th}$ centile): $2 (1.8)$]. The mean number of courses was $3 \pm 2$ for the ACS group and $4 \pm 3$ for the placebo group [median ($5^{th},95^{th}$ centile): $2 (1.7)$ vs. $3.5 (1.8)$]. Two (33%) women in each group received one course of study treatment [overall: 4 (33%)]. Two (33%) women in the ACS group and 1 (17%) woman in the placebo group received 2 courses of study treatment [overall: 3 (25%)]. Two (33%) women in the ACS group and 3 (50%) women in the placebo group received more than 2 courses of study treatment [overall: 5 (42%)] (Table 5.3).

The blood samples from the first 3 patients who enrolled into the study were missed. They all had an emergency cesarean section in the middle of the night. The head ultrasound
at the time of discharge from hospital and the measurement of the abdominal circumference were also missed for 2 of these babies, all of which were born at term.

There were no complications from the study treatment which required discontinuation of the study drugs.

E.4 Outcomes

Median (5th,95th centile) time from first course of the study treatment to delivery was shorter for the ACS group compared to the placebo group [23 (5.96) vs. 57 (1.89) days]. Overall, the median (5th,95th centile) time from first course of the study treatment to delivery was 30 (1.87) days for all participants. Mean gestational age at birth was also lower for the ACS group compared to the placebo group (31 ± 4 vs. 35 ± 5 weeks). Six (67%) babies in the ACS group and 4 (57%) babies in the placebo group were born before 34 weeks of gestation. No baby was born at term in the ACS group, but 3 babies were born at term in the placebo group. Overall 9 pregnancies were delivered before 37 weeks of gestation (75%). Median (5th,95th centile) birth weight, birth length, birth head circumference and birth abdominal circumference for babies in the ACS group compared to babies in the placebo group were: 1530 (828,2360) g vs. 2750 (1095,3914) g; 41 (33.47) cm vs. 49.5 (38.51) cm; 29 (24.33) cm vs. 32 (26.34) cm; 21 (20.30) cm vs. 29 (19.34) cm, respectively. There were 2 pregnancies with birth weight less than 10th in the ACS group compared to none in the placebo group. 87 Median (5th,95th centile) time of stay in NICU was 7 (0.54) days in ACS group compared to 4 (0.37) days in placebo group. The rate of composite outcome (death, RDS, BPD, IVH, PVL and NEC) was 2 (33%) for the ACS group compared to 3 (50%) for the placebo group (Table 5.5). The rates of death, RDS, BPD, IVH, PVL, NEC and the other neonatal outcomes are
shown in Table 5.4, which compares the neonatal outcomes with the pregnancy, as well as the baby as the unit of analysis.

Median (5th, 95th centile) duration of rupture of membranes for mother was 0.03 (0.431) hours in ACS group and 3.7 (0.508) hours in placebo group. The rate of cesarean section was 4 (67%) in the ACS group and 3 (50%) in the placebo group [overall: 7 (58%)]. There were no infections of any kind or other complications in the mothers in either group (Table 5.6).

E.4 Hormone Assay

Three maternal blood samples and 4 cord blood samples were obtained from the ACS group (3 women missed having their blood samples taken). Six maternal blood samples and 7 cord blood samples were obtained from the placebo group. The median (5th, 95th centile) time from the last course of study treatment to delivery was 11.5 (5.69) days for the ACS group compared to 11 (1.84) days for the placebo group. The results for cortisol and ACTH were not normally distributed, so we reported the median instead of the mean and we used the Wilcoxon rank sum test instead of the Student's t-test. There was a trend toward a lower level of maternal cortisol and neonatal cortisol in the ACS group compared to the placebo group. although the differences were not statistically significant (P = 0.09). There was no statistically significant difference in maternal ACTH or neonatal ACTH between the ACS group and the placebo group (Tables 5.7 & 5.8).

There was no statistically significant correlation between maternal cortisol and maternal ACTH (P =0.1), neonatal cortisol and neonatal ACTH (P = 0.4), maternal cortisol and neonatal cortisol (P = 0.5) and maternal ACTH and neonatal ACTH (P = 0.5).
We decided not to measure the plasma CBG concentrations because blood samples were originally missing for 3/12 mothers and 5/16 babies. After the ACTH and cortisol tests were completed, there remained an adequate sample for the measurement of CBG in only 7/12 mothers (of whom only 1 was in the ACS group) and 9/16 babies (of whom only 2 were in the ACS group).

E. 5 Questionnaire

Eleven completed questionnaires were received. The response rate was 11/12 (92%). One woman left the hospital before completing the questionnaire and her contact address was not correct. The mean weight gain during pregnancy was 35 ± 9 pounds in the ACS group, 31 ± 9 pounds in the placebo group and 33 ± 9 pounds for all participants.

Women were asked if they had headache, moon face, acne, excessive hair growth, swelling of their bodies, striae, sleep problems, muscle weakness, change in appetite, unusual bruising, memory problems and mood swings after randomization to the study. Their responses are shown in Table 5.9.

When women were asked what they thought they received as the study treatment, 2 (18%) stated corticosteroids, 3 (27%) stated placebo and 6 (55%) stated that they were not sure which treatment they had received (Table 5.9).

Five (45%) women stated that they liked the fact that they had a chance to get multiple courses of ACS. Four (36%) women stated that participation in the study caused them to feel reassured about their health. Eight (73%) women stated that participation in the study caused them to feel reassured about their babies’ health. Four (36%) women stated that they disliked being randomized. Two (18%) women stated that participation in the study caused them to feel worried about their health and also about their babies’ health (Table 5.9).
When women were asked if they would agree to participate in this research study, if time went backwards and they had to do it all over again, one woman in the ACS group said she would not want to participate. All the others stated that they would participate again.

The mother language and spoken language for all participants was English except for one in each group (Somali and Tagalog). Women in the ACS groups identified their ethnic/cultural backgrounds as Jamaican, Filipino, European and Canadian. Women in the placebo group identified their ethnic/cultural backgrounds as Moslem, Irish, English, Slovenian and Portuguese. Women commented that they participated in the study because of the potential benefits for the baby and their wish to help future mothers.

F. Discussion

This study had a low rate of recruitment due principally to poor physician support. The involvement of pregnant women in drug trials raises a number of ethical and practical issues, including the uncertain effect of drugs on the fetus, that historically have led to a general exclusion of women of childbearing years from research. Research into preterm labor is an area that potentially is affected by the reluctance of women and researchers to test experimental medications during pregnancy. The low recruitment rate in tocolytic trials confirms this problem. While pregnant women may be willing to accept risks to themselves, they may believe that they are not entitled to place the fetus at risk. The majority of participants in this study stated that participation in the study caused them to feel reassured about their babies health while a lower number of women stated that participation in the study caused them to feel reassured about their own health. This suggests that the baby’s health is a more important matter for pregnant women than their own health. Women must trust that the researchers truly are in equipoise, that there is no known difference between the
proposed treatments or interventions. Some women will prefer to assume that "my doctor knows best about me and my baby" and will not be happy to enter into a discussion of uncertainty regarding which treatment.\textsuperscript{88} Other trials have reported that the discussion of uncertainty regarding which treatment is best is one of the barriers to patient participation in RCTs.\textsuperscript{89} Another factor which may influence a patient's decision to participate in a RCT is additional procedures and appointments which may cause discomfort and/or inconvenience.\textsuperscript{89}

In our trial, some women did not want to receive weekly injections (painful needles) and wished to avoid the inconvenience of attending weekly appointments to receive study treatments. This was the reason why one of the participants who was discharged home was not compliant with the study intervention. The influence of family members is also a reason for poor participation.\textsuperscript{89}

The Canadian Preterm Labour Trial of ritodrine vs. placebo recruited 29% of eligible women to the trial. In that study, 69% of the time, physicians declined to have their patient participate in the trial.\textsuperscript{86} Participation in randomized controlled trials may alter the doctor-patient relationship and concern about this may be a barrier for clinicians to recruit their patients to the trial.\textsuperscript{89} Other barriers to clinician participation in RCTs include lack of clinical autonomy, that is loss of decision making power and independence.\textsuperscript{89} Another factor which has been reported in other trials as a barrier is the clinician's concerns about treatment toxicity or side effects.\textsuperscript{89} In this study, the majority of women refused to participate in the trial because their doctor had a preference for a single course of ACS followed by a rescue dose after 28 weeks of gestation. The physicians were concerned about the number of courses of ACS which women would potentially have received if they entered into the trial. Another factor which adversely affected recruitment was the fact that the pilot study was a
small non-funded study with no ability to answer the clinical question. One of the known barriers to clinician participation in clinical trials is not having an important research question. One obstetrician in one of the participating hospitals mentioned that because the pilot study was small and there was no financial support to follow mothers and babies longer term, he and his colleagues did not support it.

To minimize recruitment problems because of poor physician support, in the multi-center MACS, we have changed the study treatment interval from every 7 days to every 14 days. We anticipate this will increase physician support. Other strategies we propose to use in MACS to overcome the problem of low recruitment include having more participating centers, ensuring that there are dedicated remunerated research staff in all centers and increasing effective communication with clinicians through collaborative meetings and a well organized centralized Data Coordinating Center.

There were no complications in the pilot study which required discontinuing the study treatment, but the numbers enrolled into the study were small.

The compliance with the protocol overall was good. We missed blood samples from the first 3 patients who enrolled into the study. They all had an emergency cesarean section in the middle of the night. The head ultrasound at the time of discharge from hospital and the measurement of the abdominal circumference were also missed for 2 patients. So, these two outcome measures were deleted from the MACS protocol because of concerns of missing data.

Another issue in this study was unblinding. The betamethasone (celestone) which we used as ACS in this study was not a clear solution while normal saline (placebo) was clear. The study treatments were prepared in covered syringes and were injected by a research
nurse who was not involved in patient care. However, there were a few situations in which patients thought they were aware of their treatment assignment. There were 2 patients in each group who guessed correctly in the questionnaire when they were asked what they thought that they had received as the study treatment. This may not be a problem in MACS, as the placebo has been made to look almost the same as the betamethasone (celestone) and all the drug vials and syringes are covered to minimize unblinding.

One of the risks associated with multiple courses of ACS is potential adrenal insufficiency. One single course of ACS causes a transient decrease in cortisol level in both mother and the fetus. The cortisol level returns to the untreated level by 48 hours in mother and by 6 days in the fetus. But these neonates are able to respond normally to the stresses of birth asphyxia and RDS at that time.6 Terrone et al90 did not find any adrenal suppression in neonates who were exposed to multiple courses of ACS. however Banks et al38 found that adrenal suppression was greater and persisted longer after treatment in neonates receiving ≥ 3 courses of ACS versus a single course. Similarly, McKenna et al91 described suppression of the maternal pituitary adrenal axis after administration of ≥ 2 courses of ACS. In our pilot study, there was a trend toward a lower level of maternal cortisol and neonatal cortisol but not maternal ACTH and neonatal ACTH in the ACS group compared to the placebo group, although the differences were not statistically significant. The trend toward a lower level of maternal cortisol and neonatal cortisol in the ACS group may be due to the fact that babies in the ACS group were born at earlier gestational age at which their hypothalamic-pituitary-adrenal axis was not mature enough to react to the stress of delivery or because fewer babies in the ACS group had a vaginal delivery. It is possible that any adverse consequences of multiple courses of ACS would manifest only in those infants delivered closer to the time of
the most recent course. In our study this was not likely to be a factor, as the median time from the last course of study treatment to delivery was 11.5 vs. 11 days for the ACS group and the placebo group respectively. It is also possible that the lower maternal and fetal cortisol levels with multiple courses of ACS represent some degree of adrenal suppression. It is, however, difficult to interpret these results due to the small number of patients enrolled.

The fact that 75% of babies in this study were born less than 37 weeks of gestation shows that the inclusion criteria of the study could identify those patients who were really at increased risk of preterm birth. The high rate of cesarean section for mothers also confirms the high risk population enrolled in this study. The lower median birth weight, birth length, birth head circumference and birth abdominal circumference in the ACS group is concerning. This may have been due to chance (the gestational age at randomization was lower for the ACS group, or there were more twin pregnancies in the ACS group compared to the placebo group), due to a direct effect of ACS on fetal growth or gestational age at birth. There were more babies with a birth weight less than the 10th percentile in the ACS group. This also may be due to chance. The median time from the first course of study treatment to delivery was shorter for the ACS group compared to the placebo group. Whether multiple courses of steroid cause uterine contractions and shorten the duration of pregnancy is a question which will be addressed in the larger RCT (MACS). The primary outcome of MACS (death, RDS, PBD, IVH, PVL and NEC) was 2 (33%) pregnancies in the ACS group compared to 3 (50%) pregnancies in the placebo groups in this small pilot study.

The sample size for MACS was calculated to be 1900 pregnancies. This sample size is needed to find a significant reduction in the composite outcome from 12% to 8%. 80% power, 1 tailed $\alpha$ error of 0.025. This sample size was substantially larger than the sample
size for the proposed RCT. This was because we changed the study treatment interval from every 7 days to every 14 days and we decreased the likelihood of type I error (from 0.05 to 0.025).

Other changes to MACS were: 1) we deleted the head ultrasound at the time of discharge from hospital, 2) we deleted the measurement of the abdominal circumference and 3) we added a 2 year follow up for behavioral and neurodevelopmental outcomes to assess the effect of multiple courses of ACS on behavioral and neurologic outcome. A longer term follow up of these children is planned in the future.
113 women were identified as being potentially eligible

- 26 women were not eligible
  - 9 women were missed
  - 66 women refused to participate
    - 6 women assigned multiple course of ACS group
      - Outcomes
    - 6 women assigned placebo group
      - Outcomes
- 87 women were eligible
  - 12 women were randomized
    - Outcomes
Table 5.1 Baseline Information of Participants and Non-participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>ACS (N = 6)</th>
<th>Placebo (N = 6)</th>
<th>Participants (N = 12)</th>
<th>Non-participants (N = 75)</th>
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<td>No. (%)</td>
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<tr>
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<td>848 (564,1323)</td>
<td>955 (550,1624)</td>
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<tr>
<td>Regular uterine contractions</td>
<td>1 (17)</td>
<td>3 (50)</td>
<td>4 (33)</td>
<td>16 (21)</td>
</tr>
<tr>
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<td>2 (33)</td>
<td>2 (33)</td>
<td>4 (33)</td>
<td>20 (27)</td>
</tr>
<tr>
<td>Antepartum vaginal bleeding</td>
<td>1 (17)</td>
<td>1 (17)</td>
<td>2 (17)</td>
<td>16 (21)</td>
</tr>
<tr>
<td>Prelabour rupture of membranes</td>
<td>1 (17)</td>
<td>2 (33)</td>
<td>3 (25)</td>
<td>24 (32)</td>
</tr>
<tr>
<td>Medical condition</td>
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<td>0</td>
<td>1 (8)</td>
<td>2 (3)</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
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</tr>
<tr>
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<td>17 (23)</td>
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<td>2 (33)</td>
<td>4 (33)</td>
<td>21 (28)</td>
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<td>1 (17)</td>
<td>2 (17)</td>
<td>16 (21)</td>
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<td>3 (50)</td>
<td>4 (33)</td>
<td>24 (32)</td>
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<td>0</td>
<td>6 (8)</td>
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<tr>
<td>Other</td>
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<tr>
<td>Growth retardation</td>
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<td>0</td>
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<td>0</td>
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<td>3 (4)</td>
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<td><strong>Other treatments:</strong></td>
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</tr>
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<td>3 (25)</td>
<td>19 (25)</td>
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<td>Betamimetics</td>
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<td>0</td>
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<td>MgSO4</td>
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<td>0</td>
<td>0</td>
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<td>0</td>
<td>2 (17)</td>
<td>8 (11)</td>
</tr>
<tr>
<td>Nitroglycerin</td>
<td>3 (50)</td>
<td>0</td>
<td>3 (25)</td>
<td>12 (16)</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>2 (33)</td>
<td>4 (67)</td>
<td>6 (50)</td>
<td>19 (25)</td>
</tr>
<tr>
<td><strong>Referral (patient had originally planned to deliver in another hospital):</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Highest level of care provided in the original hospital:</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (NICU)</td>
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<td>0</td>
<td>0</td>
<td>2 (3)</td>
</tr>
<tr>
<td>2 (NICU, but only for a few days)</td>
<td>2 (33)</td>
<td>4 (33)</td>
<td>6 (50)</td>
<td>35 (47)</td>
</tr>
<tr>
<td>1 (no NICU)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3 (4)</td>
</tr>
</tbody>
</table>

NA: not applicable; US: ultrasound; * one baby had omphalocele, one of the twins had acardia/ live one had hypoplastic heart, one baby had cystic adenomatoid malformation of the right lung; † endometriosis, gestational diabetes, proteinuria plus thrombocytopenia; ‡ von Willebrand's disease, 2 cases of gestational diabetes, uterine fibroids, ulcerative colitis; ¶ twin twin transfusion syndrome, one of the twin had acardia/ live one had hypoplastic heart, cystic adenomatoid malformation of the right lung; PPROM: preterm prelabour rupture of membranes
Table 5.2 Compliance with The Study Treatment

<table>
<thead>
<tr>
<th>Patient</th>
<th>Drug</th>
<th>Gestational age (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 25 26 27 28 29 30 31 32 33 34-36 37+</td>
</tr>
<tr>
<td>1 ACS</td>
<td></td>
<td>R  2× 2× 2× 2× 2× 2×</td>
</tr>
<tr>
<td>2 P</td>
<td></td>
<td>R  2× 2× 2× 2× 2× 2×</td>
</tr>
<tr>
<td>3 ACS</td>
<td></td>
<td>R  2× 2× 2× 2× 2× 2×</td>
</tr>
<tr>
<td>4 P</td>
<td></td>
<td>R  2× 2× 2× 2× 2× 2×</td>
</tr>
<tr>
<td>5 ACS</td>
<td>R, T**</td>
<td>2× 2×</td>
</tr>
<tr>
<td>6† ACS</td>
<td></td>
<td>R  2× 2× 2× 2× 2× 2×</td>
</tr>
<tr>
<td>7 P</td>
<td></td>
<td>R  2× 2×</td>
</tr>
<tr>
<td>8 P</td>
<td></td>
<td>R  2× 2× 2× 2× 2× 2×</td>
</tr>
<tr>
<td>9 ACS</td>
<td></td>
<td>R  2×</td>
</tr>
<tr>
<td>10‡ P</td>
<td></td>
<td>R  2× - - -</td>
</tr>
<tr>
<td>11 ACS</td>
<td></td>
<td>R  2× 2× 2× 2× 2× 2×</td>
</tr>
<tr>
<td>12 P</td>
<td></td>
<td>R  2×</td>
</tr>
</tbody>
</table>

ACS: antenatal corticosteroids; P: placebo; 2×: received a complete course of study treatment; ×: received one dose of study treatment; -: did not receive any study treatment; D: delivery; R: randomization; T: tocolytics; * nitroglycerin patch; ** indomethacin; † patient had gestational diabetes and was concerned that the study treatment had made her blood glucose more difficult to control; ‡ patient was discharged from hospital with bed rest instruction and it was inconvenient for her to go to her obstetrician office weekly to receive the study treatments. After she delivered, she mentioned that the real reason for her decision was lack of the support from medical staff at the participating hospital.
Table 5.3 Treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ACS (N = 6)</th>
<th>Placebo (N = 6)</th>
<th>Overall (N=12)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>Mean number of courses</td>
<td>3 (± 2)</td>
<td>4 (± 3)</td>
<td>3 (± 2)</td>
</tr>
<tr>
<td>Median (5th, 95th centile)</td>
<td>2 (1.7)</td>
<td>3.5 (1.8)</td>
<td>2 (1.8)</td>
</tr>
<tr>
<td>Study treatment: 1 course received</td>
<td>2 (33)</td>
<td>2 (33)</td>
<td>4 (33)</td>
</tr>
<tr>
<td></td>
<td>2 (33)</td>
<td>1 (17)</td>
<td>3 (25)</td>
</tr>
<tr>
<td></td>
<td>2 (33)</td>
<td>3 (50)</td>
<td>5 (42)</td>
</tr>
<tr>
<td>Other treatments: Tocolytics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indomethacin</td>
<td>1 (17)</td>
<td>0</td>
<td>1 (8)</td>
</tr>
<tr>
<td>Nitroglycerin</td>
<td>2 (33)</td>
<td>1 (17)</td>
<td>3 (25)</td>
</tr>
<tr>
<td>Antibiotics:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before labor</td>
<td>1 (17)</td>
<td>2 (33)</td>
<td>3 (25)</td>
</tr>
<tr>
<td>During labor/delivery</td>
<td>3 (50)</td>
<td>1 (17)</td>
<td>4 (33)</td>
</tr>
<tr>
<td>After delivery of fetus</td>
<td>2 (33)</td>
<td>2 (33)</td>
<td>4 (33)</td>
</tr>
<tr>
<td>Corticosteroids other than study drug</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</table>
Table 5.4 Neonatal Outcomes

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>ACS (N = 6)</th>
<th>Placebo (N = 6)</th>
<th>ACS (N = 9)</th>
<th>Placebo (N = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>Sex: Female</td>
<td>NA</td>
<td>NA</td>
<td>4 (44)</td>
<td>2 (29)</td>
</tr>
<tr>
<td>Male</td>
<td>NA</td>
<td>NA</td>
<td>5 (56)</td>
<td>5 (71)</td>
</tr>
<tr>
<td>Median (5th,95th centile) time from first course to delivery (days)</td>
<td>23 (5.96)</td>
<td>57 (1.89)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Mean gestational age at birth</td>
<td>31 ± 4</td>
<td>35 ± 5</td>
<td>32 ± 4</td>
<td>34 ± 5</td>
</tr>
<tr>
<td>≤ 34</td>
<td>4 (67)</td>
<td>3 (50)</td>
<td>6 (67)</td>
<td>4 (57)</td>
</tr>
<tr>
<td>35-37</td>
<td>2 (33)</td>
<td>0</td>
<td>3 (33)</td>
<td>0</td>
</tr>
<tr>
<td>≥ 37</td>
<td>0</td>
<td>3 (50)</td>
<td>0</td>
<td>3 (43)</td>
</tr>
<tr>
<td>Median (5th,95th centile) birth weight (g)</td>
<td>1530 (828.2360)</td>
<td>2750 (1095.3914)</td>
<td>1840 (735.2360)</td>
<td>2420 (1005.3914)</td>
</tr>
<tr>
<td>Median (5th,95th centile) birth length (cm)</td>
<td>41 (33.47)</td>
<td>49.5 (38.51)</td>
<td>44 (32.47)</td>
<td>48 (36.51)</td>
</tr>
<tr>
<td>Median (5th,95th centile) birth head circumference (cm)</td>
<td>29 (24.33)</td>
<td>32 (26.34)</td>
<td>29 (24.33)</td>
<td>32 (24.34)</td>
</tr>
<tr>
<td>Median (5th,95th centile) birth abdomen circumference (cm)</td>
<td>21 (20.30)</td>
<td>21 (19.34)</td>
<td>21 (19.30)</td>
<td>28 (18.34)</td>
</tr>
<tr>
<td>Birth weight &lt; 10th percentile</td>
<td>2 (33)</td>
<td>0</td>
<td>3 (33)</td>
<td>1 (14)</td>
</tr>
<tr>
<td>1 min. Apgar score &lt; 7</td>
<td>3 (50)</td>
<td>1 (17)</td>
<td>4 (44)</td>
<td>1 (14)</td>
</tr>
<tr>
<td>5 min. Apgar score &lt; 7</td>
<td>1 (17)</td>
<td>1 (17)</td>
<td>1 (11)</td>
<td>1 (14)</td>
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<tr>
<td>Congenital anomaly</td>
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<td>1 (17)</td>
<td>0</td>
<td>1 (14)</td>
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<tr>
<td>Median (5th,95th centile) cord pH</td>
<td>7.3 (6.9,7.3)</td>
<td>7.3 (7.2,7.4)</td>
<td>7.3 (6.9,7.4)</td>
<td>7.3 (7.2,7.4)</td>
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<tr>
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<td>4 (67)</td>
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<td>5 (71)</td>
</tr>
<tr>
<td>Oxygen</td>
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<td>4 (67)</td>
<td>9 (100)</td>
<td>5 (71)</td>
</tr>
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<td>7 (78)</td>
<td>4 (57)</td>
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<td>Intubation or ventilation</td>
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<td>2 (33)</td>
<td>2 (22)</td>
<td>2 (29)</td>
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<tr>
<td>Neonatal respiratory support outside delivery room:</td>
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<td>2 (33)</td>
<td>4 (44)</td>
<td>3 (43)</td>
</tr>
<tr>
<td>Oxygen</td>
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<td>2 (33)</td>
<td>4 (44)</td>
<td>3 (43)</td>
</tr>
<tr>
<td>Intubation or ventilation</td>
<td>3 (50)</td>
<td>2 (33)</td>
<td>4 (44)</td>
<td>3 (43)</td>
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<td>18.28 (N=2)</td>
<td>2.412.44 (N=4)</td>
<td>18.2135 (N=3)</td>
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<td>2 (22)</td>
<td>1 (14)</td>
</tr>
<tr>
<td>Median (5th,95th centile) length of stay in hospital (days)</td>
<td>36 (4.91)</td>
<td>11 (2.83)</td>
<td>21 (4.99)</td>
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<td>Median (5th,95th centile) length of stay in NICU (days)</td>
<td>7 (0.54)</td>
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<td>Median (5th,95th centile) length of stay in intermediate care (days)</td>
<td>25 (0.63)</td>
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<td>13 (0.63)</td>
<td>11 (0.59)</td>
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<td>Surfactant</td>
<td>Antibiotics</td>
<td>Postnatal corticosteroids to prevent BPD</td>
<td>Postnatal corticosteroids to treat BPD</td>
</tr>
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<td>------------</td>
<td>-------------</td>
<td>------------------------------------------</td>
<td>----------------------------------------</td>
</tr>
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<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
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<table>
<thead>
<tr>
<th>Bronchopulmonary dysplasia</th>
<th>Need for oxygen at 36 weeks + X-ray</th>
<th>Need for oxygen at 28 days + X-ray</th>
<th>Need for oxygen at death or final discharge</th>
</tr>
</thead>
<tbody>
<tr>
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<td>2 (33)</td>
<td>1 (17)</td>
<td>1 (17)</td>
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<table>
<thead>
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<th>Patent ductus arteriosus</th>
<th>Indomethacin</th>
<th>PDA ligation</th>
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<td>2 (33)</td>
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<table>
<thead>
<tr>
<th>Retinopathy of prematurity</th>
<th>One eye</th>
<th>Both eyes [most severe stage]</th>
<th>Surgery</th>
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<table>
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<th></th>
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</thead>
<tbody>
<tr>
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<td>1 (17)</td>
<td>2 (33)</td>
<td>1 (11)</td>
</tr>
</tbody>
</table>

*comparison of outcomes with the pregnancy as the unit of analysis; ** comparison of outcomes with the baby as the unit of analysis, 3 sets of twins in the ACS group and 1 set of twins in the placebo group; NA: not applicable; † hypospadias plus no left kidney; ‡ for 3 pregnancies; †† for 2 pregnancies; †‡ for 4 babies; ††† for 3 babies; ‡‡ a set of twins on corticosteroids (mean days: 18.16 ± 2.82); ‡‡‡ the patient needed oxygen 25 days after discharge home; ‡‡‡‡ ACS group (positive culture of endotracheal tube), placebo group (positive culture of blood)
Table 5.5 Primary Outcome of the Proposed RCT

<table>
<thead>
<tr>
<th>Primary Outcome</th>
<th>Pregnancies*</th>
<th>Babies**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ACS (N = 6)</td>
<td>Placebo (N = 6)</td>
</tr>
<tr>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>Neonatal mortality or morbidity</td>
<td>2 (33)</td>
<td>3 (50)</td>
</tr>
<tr>
<td>Death</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Respiratory distress syndrome</td>
<td>1 (17)</td>
<td>1 (17)</td>
</tr>
<tr>
<td>Intraventricular haemorrhage</td>
<td>0</td>
<td>1 (17)</td>
</tr>
<tr>
<td>Periventricular leukomalacia</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bronchopulmonary dysplasia</td>
<td>1 (17)</td>
<td>1 (17)</td>
</tr>
<tr>
<td>Necrotising enterocolitis</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Comparison of outcomes with the pregnancy as the unit of analysis; ** comparison of outcomes with the baby as the unit of analysis. 3 sets of twins in the ACS group and 1 set of twins in the placebo group.
Table 5.6 Maternal Outcomes

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>ACS</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N = 6)</td>
<td>(N = 6)</td>
</tr>
<tr>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>Median (5th,95th centile) duration of ROM (hours)*</td>
<td>0.03 (0.43)</td>
<td>3.7 (0.508)</td>
</tr>
<tr>
<td>ROM &lt; 24 hours</td>
<td>5 (83)</td>
<td>4 (67)</td>
</tr>
<tr>
<td>ROM 24-72 hours</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ROM &gt; 72 hours</td>
<td>1 (17)</td>
<td>2 (33)</td>
</tr>
<tr>
<td>Labour induced</td>
<td>0</td>
<td>1 (17)</td>
</tr>
<tr>
<td>Spontaneous labour</td>
<td>2 (33)</td>
<td>2 (33)</td>
</tr>
<tr>
<td>No labour (Cesarean Section)</td>
<td>4 (67)</td>
<td>3 (50)</td>
</tr>
<tr>
<td>Diabetes after randomization</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hypertension after randomization</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chorioamnionitis</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Maternal Infection (endometritis, wound infection, urine infection, other infection)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Any other maternal complications</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Median (5th,95th centile) length of stay in the hospital (days)</td>
<td>4 (2.18)</td>
<td>3 (2.16)</td>
</tr>
</tbody>
</table>

ROM: rupture of membranes
Table 5.7 Maternal Hormones

<table>
<thead>
<tr>
<th>Hormones</th>
<th>ACS (n = 3)</th>
<th>Placebo (n = 6)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (5th,95th centile) cortisol (μg/dl)</td>
<td>13.8 (5.2,31.3)</td>
<td>58.2 (10.9,77.3)</td>
<td>0.09</td>
</tr>
<tr>
<td>Median (5th,95th centile) ACTH (pg/ml)</td>
<td>79.1 (70.1,84.3)</td>
<td>70.6 (35.4,1100)</td>
<td>0.89</td>
</tr>
</tbody>
</table>
Table 5.8 Cord Blood Hormones

<table>
<thead>
<tr>
<th>Hormones</th>
<th>ACS (n = 3)</th>
<th>Placebo (n = 6)</th>
<th>P value</th>
<th>ACS (n = 4)</th>
<th>Placebo (n = 7)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median (5th,95th centile)</strong></td>
<td>3.3 (2.2,9.4)</td>
<td>20.6 (5.72.8)</td>
<td>0.09</td>
<td>2.6 (2.9.4)</td>
<td>19.4 (4.8,72.8)</td>
<td>0.047</td>
</tr>
<tr>
<td>cortisol (μg/dl)</td>
<td>79.3 (44,1100)</td>
<td>124.7 (60,1100)</td>
<td>0.60</td>
<td>65.8 (35.7,1100)</td>
<td>152.3 (59.9,1100)</td>
<td>0.22</td>
</tr>
</tbody>
</table>

*comparison of outcomes with the pregnancy as the unit of analysis; ** comparison of outcomes with the baby as the unit of analysis
<table>
<thead>
<tr>
<th>Responses</th>
<th>ACS (n = 5)</th>
<th>Placebo (n = 6)</th>
<th>Total (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean weight gain</td>
<td>35 ± 9</td>
<td>31 ± 9</td>
<td>33 ± 9</td>
</tr>
<tr>
<td>Headache</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>4 (80)</td>
<td>1 (17)</td>
<td>5 (45)</td>
</tr>
<tr>
<td>Moderate</td>
<td>1 (20)</td>
<td>0</td>
<td>1 (9)</td>
</tr>
<tr>
<td>Severe</td>
<td>2 (40)</td>
<td>1 (17)</td>
<td>3 (27)</td>
</tr>
<tr>
<td>Frequency compared to before randomization:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased</td>
<td>2 (40)</td>
<td>1 (17)</td>
<td>3 (27)</td>
</tr>
<tr>
<td>Decreased</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No difference</td>
<td>2 (40)</td>
<td>0</td>
<td>2 (18)</td>
</tr>
<tr>
<td>No headache</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Taking medication for the headache</td>
<td>4 (80)</td>
<td>1 (17)</td>
<td>5 (45)</td>
</tr>
<tr>
<td>Moon face</td>
<td>3 (60)</td>
<td>4 (67)</td>
<td>7 (64)</td>
</tr>
<tr>
<td>Acne</td>
<td>2 (40)</td>
<td>1 (17)</td>
<td>3 (27)</td>
</tr>
<tr>
<td>Excessive hair growth</td>
<td>1 (20)</td>
<td>1 (17)</td>
<td>2 (18)</td>
</tr>
<tr>
<td>Swelling</td>
<td>0</td>
<td>3 (50) [1]</td>
<td>3 (27)</td>
</tr>
<tr>
<td>Striae</td>
<td>0</td>
<td>3 (50)</td>
<td>3 (27)</td>
</tr>
<tr>
<td>Difficulty sleeping</td>
<td>3 (60)</td>
<td>1 (17) [1]</td>
<td>4 (36)</td>
</tr>
<tr>
<td>Muscle weakness</td>
<td>2 (40)</td>
<td>1 (17)</td>
<td>3 (27)</td>
</tr>
<tr>
<td>Increase in appetite</td>
<td>2 (40)</td>
<td>2 (33)</td>
<td>4 (36)</td>
</tr>
<tr>
<td>Decrease in appetite</td>
<td>1 (20)</td>
<td>1 (17)</td>
<td>2 (18)</td>
</tr>
<tr>
<td>Unusual bruising</td>
<td>0</td>
<td>2 (33)</td>
<td>2 (18)</td>
</tr>
<tr>
<td>Memory problem</td>
<td>1 (20)</td>
<td>2 (33)</td>
<td>3 (27)</td>
</tr>
<tr>
<td>Mood change noticed by the patient</td>
<td>2 (40)</td>
<td>1 (17)</td>
<td>3 (27)</td>
</tr>
<tr>
<td>Mood change noticed by the family member</td>
<td>1 (20)</td>
<td>1 (17)</td>
<td>2 (18)</td>
</tr>
<tr>
<td>What women thought that they received:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACS</td>
<td>2 (40)</td>
<td>0</td>
<td>2 (18)</td>
</tr>
<tr>
<td>Placebo</td>
<td>1 (20)</td>
<td>2 (33)</td>
<td>3 (27)</td>
</tr>
<tr>
<td>Not sure</td>
<td>2 (40)</td>
<td>4 (67)</td>
<td>6 (55)</td>
</tr>
<tr>
<td>The things that women liked about the study:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>liked contacts with the research staff</td>
<td>4 (80)</td>
<td>4 (67)</td>
<td>8 (73)</td>
</tr>
<tr>
<td>liked being randomized</td>
<td>0</td>
<td>2 (33)</td>
<td>2 (18)</td>
</tr>
<tr>
<td>liked the fact that there were few extra demands upon time, finances, etc.</td>
<td>3 (60)</td>
<td>3 (50)</td>
<td>6 (55)</td>
</tr>
<tr>
<td>liked the fact that they had a chance to get multiple courses of ACS</td>
<td>4 (80)</td>
<td>1 (17)</td>
<td>5 (45)</td>
</tr>
<tr>
<td>liked the fact that they had a chance to</td>
<td>5 (100)</td>
<td>5 (83)</td>
<td>10 (91)</td>
</tr>
</tbody>
</table>
assist with research to help others  
<table>
<thead>
<tr>
<th></th>
<th>3 (60)</th>
<th>1 (17)</th>
<th>4 (36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>felt reassured about their health</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>felt reassured about their baby’s health</td>
<td>4 (80)</td>
<td>4 (67)</td>
<td>8 (73)</td>
</tr>
<tr>
<td>The things that women disliked about the study:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>disliked contacts with the research staff</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>disliked being randomized</td>
<td>2 (40)</td>
<td>2 (40)</td>
<td>4 (36)</td>
</tr>
<tr>
<td>disliked the fact that there were few extra demands upon time, finances, etc.</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>felt worried about their health</td>
<td>0</td>
<td>2 (33)</td>
<td>2 (18)</td>
</tr>
<tr>
<td>felt worried about their baby’s health</td>
<td>0</td>
<td>2 (33)</td>
<td>2 (18)</td>
</tr>
<tr>
<td>Participation in study again:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Definitely not</td>
<td>1 (20)</td>
<td>0</td>
<td>1 (9)</td>
</tr>
<tr>
<td>Probably not</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Probably yes</td>
<td>2 (40)</td>
<td>4 (67)</td>
<td>6 (55)</td>
</tr>
<tr>
<td>Definitely yes</td>
<td>2 (40)</td>
<td>2 (33)</td>
<td>4 (36)</td>
</tr>
<tr>
<td>Living arrangement:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alone</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>With a husband</td>
<td>5 (100)</td>
<td>6 (100)</td>
<td>11 (100)</td>
</tr>
<tr>
<td>With a family member</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>With a friend</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Help answering the questionnaire</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A husband</td>
<td>0</td>
<td>1 (17)</td>
<td>1 (9)</td>
</tr>
<tr>
<td>Member of the family</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Friend</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Doctor or nurse</td>
<td>1 (20)</td>
<td>0</td>
<td>1 (9)</td>
</tr>
<tr>
<td>Another person</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Comments*</td>
<td>1 (20)</td>
<td>1 (17)</td>
<td>2 (18)</td>
</tr>
</tbody>
</table>

[No.] for those reported the outcome as a big problem; *women commented that they participated in the study because of the potential benefits for the baby and their wish to help future mothers
It has been shown in randomized controlled trials that a single course of ACS decreases the risk of death, RDS and possibly IVH in preterm babies.\textsuperscript{2} Corticosteroids are known to accelerate maturation of developmentally regulated proteins and to stimulate cytodifferentiation in numerous cells, including type II pneumocytes. However, the effects of a single course of ACS on lung maturation may be a temporary phenomenon.\textsuperscript{6} In the Cochrane meta-analysis, the benefits of a single course of ACS in terms of RDS have been demonstrated to be most significant 24 hours following treatment and to continue up to 7-10 days.\textsuperscript{2} This raises the question as to whether repeated courses of ACS will further decrease the risk of RDS, death or IVH in infants born to women who remain at increased risk of preterm birth 7 or more days after treatment with ACS. Based on this hypothesis, multiple courses of ACS has become a routine practice in some centers.\textsuperscript{8-10}

A systematic review of randomized trials in animals provides evidence that repeated doses of ACS have some beneficial and also some adverse effects. Studies in different species of animals have found that repetitive exposure to maternal ACS resulted in a progressive improvement in postnatal lung function. However repeated doses of ACS were found to cause growth restriction in different animal species. Repeated doses of ACS delayed development of the nervous system and retarded brain growth, but the long term effect of this
is unknown. Repeated doses of steroids were not associated with any effect on long term behavior among mice offspring.

A systematic review and meta-analysis of observational studies in humans, while limited by the retrospective nature of the studies and presence of selection bias in all studies, reported a decreased risk of RDS and PDA, but an increased risk of endometritis following multiple courses of ACS. Multiple courses of ACS had no significant effect on other neonatal and maternal outcomes. It is not possible to establish the true effects of multiple courses of ACS by reviewing the results of observational studies due to the effect of confounding variables.92

There are no data available from published randomized trials as to the benefits and risks of multiple courses of ACS in humans. The data from a recently reported (in abstract form) randomized clinical trial of 502 pregnant women who were randomized to single vs. multiple courses of ACS showed no significant differences in composite neonatal morbidity (defined as severe RDS, BPD, severe IVH, PVL, sepsis, NEC, neonatal death) between the two groups. This study, although under-powered, is perhaps the best evidence to date of the effects of multiple vs. single courses of ACS.31

Therefore, well-designed randomized controlled trials of sufficient size are needed to assess the effects of repeated courses of ACS for pregnant women at increased risk of preterm birth in terms of important perinatal, neonatal and maternal outcomes.

The design of such a trial suggests that a sample size of approximately 2000 women is needed to assess a clinically important effect of multiple vs. single courses of ACS on neonatal outcomes. The pilot study showed that such a trial is feasible, but care should be given to the strategy for recruiting pregnant women. The data showed that the main reason
for the low recruitment rate was physician preference for fewer courses of ACS. Hopefully, the protocol for MACS which has been revised to provide ACS or placebo every 14 days instead of every 7 days, will be associated with higher rates of recruitment.

Data from the pilot study also raises the concern for maternal and neonatal adrenal suppression following multiple courses of ACS.

The NIH consensus conference in August 2000 concluded that a single course of ACS remained an important treatment in improving the outcome of preterm infants. However, information on the need for and safety of repeat dosing was confusing and unclear. While there was evidence that neonates exposed to multiple courses of ACS may have a decrease in the incidence of certain morbidity including RDS and PDA, there also were inconsistent concerns regarding the impact of multiple courses of ACS on fetal growth and long term neurodevelopment. Since the data in these studies were collected retrospectively and there was selection bias and numerous confounders not controlled for in the analyses, it was felt that the question of benefits and risks of multiple courses of ACS could not be answered by these study designs. To date, there are no published prospective, randomized trials of sufficient size to address this issue.

The final conclusion of the conference was that due to insufficient knowledge regarding the effect of repeat dosing for women remaining at increased risk of preterm birth more than 7 days following the initial dose, clinical practice should be restricted to a single dose, but that prospective trials should continue. These trials are either planned (UK) or are in progress (Australia, USA and Canada). Until the results of these trials are available, repeating courses of ACS, including rescue therapy, should not be given except within the context of these studies.
References


Appendix A
Multiple Courses of Antenatal Corticosteroids for Preterm Birth Study (MACS)

The Pilot Study

Participant Information Sheet

Preterm birth refers to infants born before 37 weeks of gestation. Preterm birth is a major cause of infant illness and even death. One of the most acute problems is called "respiratory distress syndrome" or RDS. Preterm infants get RDS because their lungs are not mature and do not produce a substance called "surfactant." Infants need surfactant in order to have normal lung function. When infants get RDS they have difficulties breathing and often require oxygen and the use of a machine to help them breathe. Another problem often experienced by preterm infants is "intraventricular hemorrhage" or IVH. Intraventricular hemorrhage refers to bleeding inside the baby's brain. RDS and IVH are two conditions that often lead to long term problems and can even contribute to an infant's death.

To help prevent these complications you have already received a course of antenatal corticosteroids. Most research studies show that the benefit of this treatment is between 24 hours and 7 days after the last injection. If you are still pregnant after 7 days of treatment and are at risk of preterm delivery, you may benefit from receiving multiple courses of antenatal corticosteroids.

The risks and benefits of multiple courses of antenatal corticosteroids are not clear. We know that a single course of antenatal corticosteroids reduces the risk of RDS from 45% to 20% in babies born before 32 weeks of gestation. We also know that a single course is associated with a decreased chance of death from 12% to 7%. Several small studies in humans and in animals have shown that multiple courses of antenatal corticosteroids are beneficial and lead to less RDS in babies born preterm. However, one small study in humans has shown a slight increased chance of necrotising enterocolitis, which is inflammation in the baby's intestine. Animal studies also caution that multiple courses of antenatal corticosteroids may have side effects such as decreased infant growth in the uterus. There also is a possibility of an increased chance of infection in mothers. Finally, there may be unknown adverse side effect for mothers and babies, which this study will identify.

We don't know which treatment is better for you and your baby. The only way for us to find this out is to perform a study like this one. The goal of this study is to compare a single treatment of antenatal corticosteroids to multiple courses of treatment. We will then learn which therapy is better and in the future be able to give women and their unborn infants the best treatment.

In order to take part in this study you should be at an increased risk of preterm birth, and be between 24-31 weeks of gestation. You should have already received one course of antenatal corticosteroids 7 or more days ago. If you agree to participate in this study, your treatment will be determined by random "as in the flip of a coin". This means that you have an equal, but random, chance of receiving antenatal corticosteroids or placebo. Placebo consists of a salt solution that is harmless to you and your baby. This study is double-blinded which means that neither you nor your physician will know which treatment you receive.
This is the only way to answer the question as to which treatment is better for you and your baby.

If you participate in this study, you will receive one treatment (2 injections 24 hours apart) each week as long as your doctor believes that you are at increased risk of preterm birth. If you are in the antenatal corticosteroid group, your injection will contain corticosteroids. If you are in the placebo group, your injection will contain a placebo. You will receive these treatments until 33 weeks gestation as long as your physician feels you are at increased risk of preterm birth.

Following your delivery, information about you and your baby will be collected from your and your baby’s medical records. The information collected will be about the presence of any problems of prematurity in your baby and whether there are any side effects for you or your baby. You will be have a sample of blood taken immediately following delivery. A sample of blood will be taken from the umbilical cord of your baby at delivery. We will also ask that your baby have a head ultrasound prior to leaving the hospital in order to identify any occurrence of IVH, which can be one of the problems of prematurity. In addition, we will ask you to fill out a questionnaire before discharge from hospital. The questionnaire will ask you some questions regarding any symptoms that you may experience during participation in the study plus your likes and dislikes about participation in the study. It will take about 20 minutes to complete this questionnaire.

Finally, you should know, that you are free at any time in the study to refuse to participate and that your participation or decision not to participate will not effect the usual care of you or your infant. If you decide not to participate in the study, you may not receive more courses of antenatal corticosteroids or you may receive more courses of antenatal corticosteroids according to your treating physician. Also, you should know, by signing this consent, the participants do not waive their legal rights, nor are the investigators, sponsors, or involved institution released from their legal and professional responsibilities. If there are any questions or concerns about your rights in the study you should contact the Women’s College Campus Research Ethics Board Coordinator at (416) 351-3733.
"Multiple Courses of Antenatal Corticosteroids for Preterm Birth Study (MACS)"

The pilot Study
Consent Form

INVESTIGATOR(S): {name}

I acknowledge that the research procedures described on the attached form, and of which I have a copy, have been explained to me. In addition, any questions that I have asked have been answered to my satisfaction. I have been informed of the alternatives to participation in this study. The possible risks and benefits of participation have been explained to me. I know that I may ask now, or in the future, any questions I have about the study or the research procedures. I have been assured that records relating to my and my baby's care will be kept confidential and that no information will be released or printed that would disclose my or my baby's personal identity without my permission.

I understand that I and my baby are free to withdraw from the study at any time. I further understand that if we choose not to complete the study, that the quality of medical care for me and my baby at {hospital name} will not be affected.

I hereby consent to participate in "MACS Pilot Study".

(Signature)  (Name)

(Witness)  (Date)
Appendix B
Multiple Courses of Antenatal Corticosteroids for Preterm Birth Study

I am a participant in the MACS Pilot Study
My Study Number is:

Patient’s Name: ____________________________

If you have any questions, please contact
Dr. Fariba Aghajafari (Project Director)
Tel: (416) 351-3800 # 2730
Pager: (416) 589-4296
Fax: (416) 351-3771
Appendix C
Date

Dear Ms. __________________________

Thank you for continuing to participate in the MACS Pilot Study (Multiple Courses of Antenatal Corticosteroids for Preterm Birth Study). The MACS Pilot Study is a randomized controlled trial comparing multiple courses of antenatal corticosteroids versus placebo for women at increased risk of preterm birth. You began receiving the study treatment on <date>. Attached is a summary of the dates on which the study drug was given to you. You should continue to receive courses of study drug (2 injections, 24 hours apart) until you reach 33 weeks gestation or until you deliver, whichever is sooner. as long as you continue to be at increased risk of preterm birth. Whether you are at increased risk of preterm birth or not, will be determined by your doctor. The dates on which you should continue to receive additional study drug are: <dates>. We will contact your hospital pharmacy to arrange to continue your study treatment.

Immediately following delivery, please ask your doctor or nurse to take a sample of blood from the baby’s umbilical cord and a sample of blood from you. These blood tests are an important part of the study. We have provided test tubes for this propose and we will arrange for them to be delivered to your doctor.

After your delivery, please complete the Postpartum Questionnaire and mail it to me in the self-addressed, self-stamped envelope that has been provided.

If you have any questions about this, please contact me directly at

Tel: (416) 351-3800 ext.#2730 / Pager: (416) 589-4296
Fax: (416) 351-3771
Email: fariba.aghajafari@utoronto.ca

Your contribution to the MACS Pilot Study is very much appreciated.

Sincerely yours,

Fariba Aghajafari, MD
MACS Pilot Study Project Director
Date

Dear Dr. ____________

Your patient Ms. ____________ is a participant in the MACS Pilot Study (Multiple Courses of Antenatal Corticosteroids for Preterm Birth Study). The MACS Pilot Study is a randomized controlled trial comparing multiple courses of antenatal corticosteroids versus placebo for women at increased risk of preterm birth. Ms. ____________ began receiving the study treatment on <date>. Attached you will find the summary of the dates and times on which the study drug was given. Ms. ____________ should continue to receive courses of study drug (2 injections, 24 hours apart) until she reaches 33 weeks gestation or until she delivers, whichever is sooner, as long as she continues to be at increased risk of preterm birth. The dates on which she should continue to receive additional study drug are: <dates>. We will contact your hospital pharmacy to arrange to continue Ms. ____________ study treatment.

In addition, please arrange to take a sample of cord blood and a sample of maternal blood immediately after delivery using the pre-labeled test tubes provided. The test tubes contain EDTA (lavender stopper tubes). One is for cord blood and one is for maternal blood.

If for some reason you do not have the pre-labeled test tubes, please arrange to take 10 ml of blood in a test tube containing EDTA (lavender stopper tubes) for both cord blood and maternal blood.

All test tubes should be labeled with the MACS Pilot Study, information as to whether the sample is maternal blood or cord blood, the maternal date of birth, the date and time the sample was taken, and the patient's study number.

Once the blood samples have been collected, put them back in the ziplock bag and put some ice inside the bag. It is very important to carry the blood samples on ice. Please send them to your hospital lab and ask them to process the samples according to the instructions for the processing of the samples on the next page. What we are asking the lab to do, is to spin down the blood samples and separate the plasma from the cells.

Please find enclosed a copy of the working protocol, which outlines the study requirements. Also, please find enclosed a copy of the data collection forms for Ms. ____________ (the treatment form, the maternal outcome form, and the neonatal outcome form).
form). We would be very grateful if you would complete the information on these forms for Ms ____________ or let us know how else we can obtain this information.

We have provided the postpartum questionnaire directly to Ms ____________ so that she can complete this after delivery and return it to us directly.

We will arrange to transfer the stored plasma (from the blood samples) to Dr. Matthews's lab at the University of Toronto.

If you have any questions about this, please contact me directly at

Tel: (416) 351-3800 ext.#2730 / Pager: (416) 589-4296
Fax: (416) 351-3771
Email: fariba.aghajafari@utoronto.ca

Your contribution to the MACS Pilot Study is very much appreciated.

Sincerely yours.

Fariba Aghajafari, MD
MACS Pilot Study Project Director

**Instructions for the preparation and storage of plasma**
*(for Cortisol, CBG, and ACTH assay)*

---

**SPECIMEN REQUIREMENT**
One hundred microliters in duplicate of EDTA plasma are required for the assay.

**Preparation of Plasma**
EDTA (7.2 mg /5 ml whole blood) is used as anticoagulant. Centrifuge the blood for 15 minutes at 760 x g to obtain hemolysis-free plasma. A short centrifugation at 20 °C will not be detrimental; however, refrigerated centrifugation is recommended. All plastics, glassware, or other material coming into contact with the specimen should be entirely free of any contamination.

**PRECAUTION:** Heparinized plasma yields falsely low values.

**Storage of Plasma**
Enzyme inhibitors are unnecessary at the sample collection phase; however, it is important to keep the sample cold (0 – 4 °C) after collection (for up to 8 hours). For long-term storage store the specimen at -70°C. It is very important to keep the specimen frozen, preferably in plastic tubes. A decrease in ACTH may be experienced with repeated freeze-thaw of the specimen.
Appendix D
Summary of Research..................................................................................................................2

Schema........................................................................................................................................3

Determine the patient’s eligibility...............................................................................................4
  Inclusion Criteria..........................................................................................................................4
  Exclusion Criteria........................................................................................................................4

1. Present the patient with the options for her care .................................................................5

2. Inform patient about the MACS Pilot Study.................................................................5

3. Pre-randomisation assessment and confirmation of eligibility........................................6

4. Non-randomized patient ......................................................................................................7

5. Obtain informed consent .......................................................................................................7

6. Complete the Entry Form up to question # 18.................................................................7

7. Randomize the patient............................................................................................................8

8. Start the treatment..................................................................................................................9

9. Continue the treatment .........................................................................................................10

10. When the patient goes into labor or delivery is planned call or page FA..................10

11. At delivery............................................................................................................................11

12. Instructions for the preparation and storage of plasma..................................................12

13. After delivery.......................................................................................................................13
Summary of research

**Introduction:** A single course of antenatal corticosteroids (ACS) reduces the risks of respiratory distress syndrome (RDS), intraventricular hemorrhage (IVH) and neonatal death in women at risk of preterm birth. Because the maximum benefit of therapy appears to occur when the infant is born between 24 hours and 7 days following the initial treatment, some physicians have suggested that repeated courses of corticosteroids be administered at weekly intervals. In some centers, this approach has become routine despite the fact that repeated courses of antenatal corticosteroids have not been evaluated in a randomized fashion. Retrospective human and animal studies have suggested that repeated courses of corticosteroids may be effective in reducing the risk of RDS, but there may be some adverse effects such as, intrauterine growth restriction, neonatal sepsis, necrotizing enterocolitis (NEC), and inhibition of maturation of the central nervous system. The question of the risks and benefits in human pregnancy of repetitive courses of antenatal corticosteroids can be evaluated properly only by a randomized controlled trial.

**Objective:** The proposed study is a pilot study to determine the feasibility of a multicentre randomized double blinded placebo controlled trial (MACS) to investigate the effects of repeated courses of maternal antenatal steroids on neonatal morbidity and mortality and maternal morbidity when preterm delivery occurs more than 7 days following the initial course of steroid therapy. This pilot study will look at the rate of recruitment, risk of complications that require discontinuation of the study treatment, and plasma cortisol, cortisol binding globulin (CBG), and adreno-corticotropin hormone (ACTH) in cord blood and maternal blood, immediately following delivery.

**Selection criteria:** Women at 24 –30 weeks of gestation, who remain at increased risk of preterm birth, 7 or more days following a single course of antenatal corticosteroids, are eligible to participate. Women requiring chronic corticosteroid treatment, women with contraindications to corticosteroids, women with clinical evidence of chorioamnionitis, and those with a fetus with a known lethal congenital anomaly are excluded.

**Randomization:** Eligible women will be randomly assigned using sequentially numbered sealed opaque envelopes held by the local pharmacy at each center, with stratification for gestational age and participating center, to receive repeated courses of either corticosteroids or placebo, until 33 weeks, as long as the patient remain at increased risk of preterm birth.

**Outcomes:** The primary outcome is perinatal or neonatal mortality or neonatal morbidity (one or more of: RDS, bronchopulmonary displasia (BPD), IVH, periventricular leucomalacia (PVL), or NEC. The secondary outcomes include neonatal sepsis, patent ductus arteriosus (PDA), length of stay in hospital and neonatal intensive care unit (NICU), birth weight, birth head circumference, birth length, birth abdominal circumference, and maternal infection.

**Trial management:** Recruitment will occur at Mount Sinai Hospital and Women’s College Campus, Sunnybrook & Women’s College Health Science Center for the period of one year.
Schema

Eligibility Assessment

- Not Eligible
  - Refusals
- Eligible
  - Informed Consent

Baseline Characteristics

Randomisation

- Courses of ACS q7days until 33 wks
  - Outcomes
- Courses of Placebo q7days until 33 wks
  - Outcomes
Determine the patient’s eligibility

**Inclusion Criteria**
1. Women who have previously received one completed course of ACS, at least 7 days ago and continue to be at increased risk of preterm birth.
2. Gestational age greater than or equal to 24 weeks of gestation and less than 31 completed weeks of gestation (24 0/7 weeks – 30 6/7 weeks) [gestational age will be determined by the clinician using menstrual history and early ultrasound if available]

To be **eligible for trial entry** women will have received one completed course of ACS defined as: 2 doses of intramuscular betamethasone, 12 mg/dose, given at 12 or 24 hour intervals; or 4 doses of intramuscular dexamethasone, 5-6 mg/dose, given at 12 hour intervals.

To be considered at increased risk of preterm birth women will have to have had one or more of the following: regular uterine contractions, a shortened cervical length or cervical dilation, preterm prelabour rupture of membranes (PPROM), antepartum bleeding secondary to placental separation or placenta previa, history of preterm birth, maternal hypertension or other medical condition increasing the risk of preterm delivery, intrauterine growth restriction or other fetal condition increasing the risk of preterm delivery.

**Exclusion Criteria**
1. Women requiring chronic doses of corticosteroids secondary to medical conditions (e.g. systemic lupus erythematosus, congenital adrenal hyperplasia)
2. Women with a contraindication to corticosteroids
3. Women with clinical evidence of chorioamnionitis (temperature ≥ 38°C)
4. Known lethal congenital anomaly (e.g. anencephaly)
1. **Present the patient with the options for her care**

For women at gestational age greater than or equal to 24 weeks and less than 31 completed weeks of gestation who have received one completed course of ACS, these situations may happen:

a. They may deliver within 7 days following the initial course of ACS, or

b. They may remain undelivered 7 days following the initial course of ACS. The options for these patients include:

1. not receiving any more courses of corticosteroids
2. participation in the *MACS Pilot Study*

2. **Inform the patient about the MACS Pilot Study**

a. Give the *Patient Information Sheet* to the patient to read and inform her about what is involved in the study.

b. Inform FA (Fariba Aghajafari, project director), who will also come to speak to the patient about what is involved in the study. **Call FA (project director) at 351-3800 ext. # 2730 and leave a message on her voice mail or page her at 589-4296**

**Note:** Inform the pharmacy of possibility of having a patient for randomization.
3. Pre-randomization assessment and confirmation of eligibility

If the patient is eligible for the study and she wishes to participate in the study, you should:

1. Check if the patient has had an obstetrical ultrasound within two weeks prior randomization, to determine:
   a. number of fetus(es)
   b. the estimated weight of the fetus(es)
   c. the presence of lethal or other congenital anomalies
   If there is a lethal congenital anomaly, the patient is not eligible for the study.
   d. the presence of placental anomalies
   e. the adequacy of the amniotic fluid

   If an ultrasound has not been done in the last 2 weeks, please do this or arrange for one.

2. Perform a non-stress test to rule out fetal compromise. This should be done within the 24 hours preceding enrollment.

3. Take the patient’s temperature to rule out clinical chorioamnionitis. This should be done within 24 hours preceding enrollment. If the patient’s temperature is $\geq 38^\circ C$, she is not eligible for the study.
4. Non-randomized patient

If the woman is eligible and does not wish to participate in the study, collect baseline information and the reasons for non-participation in the study using the Non-Randomized Patient Form. Leave this in (MSH-the MACS Pilot Study box in the nursing station in the labor and delivery unit) / (WCH- the MACS Pilot Study box in the nursing station on 3 East)

5. Obtain informed consent

If the woman is eligible and wishes to participate in the study, ask her to sign a consent form. Copies of the Consent Form can be found with this manual. Place the signed consent form in the woman’s medical record.

6. Complete the Entry Form up to question # 18

If a woman is eligible for the study and has signed a consent form, complete the baseline information on the Entry Form or call FA to do this.

Call FA (project director) at 351-3800 ext. # 2730 and leave a message on her voice mail or page her at 589-4296)
7. Randomize the patient

1. Call the hospital pharmacy (323-6400 # 4084 WCH / 586-8303 MSH); tell them the patient’s date of birth and the gestational age. The pharmacist will tell you the patient’s study number.
2. Record the study number on the top of each page of the Entry Form.

Call FA (project director) at 351-3800 ext. # 2730 and leave a message on her voice mail or page her at 589-4296 to let her know the name and study number of the patient that has been recruited.

3. Go to the pharmacy and get;
a) the Study Envelope compatible with the study number
b) the study treatment

*Pharmacy will need 1 day of advance notice before the treatment dose is ready to pick up!*

*Study Envelope contains:*

a. *Study Identification Card*
b. MATERNAL Pre-labeled test tube in a pre-labeled ziplock bag.
   1 lavender stopper test tube for MATERNAL blood
   2 pre-labeled plastic tubes for storage of prepared MATERNAL plasma
c. CORD Pre-labeled test tube in a pre-labeled ziplock bag.
   1 lavender stopper test tube for CORD blood
   CORD plasma
d. Sticker for the medical records
e. Data collection forms including *Treatment Form, Maternal Outcome Form, and Neonatal Outcome Form*
f. *Postpartum Questionnaire* plus self-addressed, self-stamped envelope
PLEASE:

1) Write the name of the patient on the Study Identification Card and give it to the patient
2) Put the test tubes packages, the data collection forms, and the postpartum questionnaire in (MSH-the MACS Pilot Study box in the nursing station in the labor and delivery unit) / (WCH- the MACS Pilot Study box in the nursing station on 3 East)
3) Place the enclosed sticker in the patient’s medical record

8. Start the treatment

1. Immediately after randomization give the patient the first dose (the prepared study drug in a coated syringe, intramuscularly). Twenty-four hours later give the second dose (the prepared study drug in a coated syringe, intramuscularly).

Pharmacy will need 1 day of advance notice before the treatment dose is ready to pick up!

After each dose write the date and time of the injection on the Treatment Form.

1. As long as the patient remains at increased risk of preterm birth, give 2 more doses of the study drug (the prepared syringe by the pharmacy, intramuscularly, 24 hours apart) to the patient every week until 33 weeks of gestation or until she delivers, which ever is sooner.

After each dose write the date and time of the injection on the Treatment Form.
9. Continue the treatment

1. If the patient is referred back to her referring hospital

Call FA (project director) at 351-3800 ext. # 2730 and leave a message on her voice mail or page her at 589-4296)
So that she can arrange for the continuation of the patient in the study.
She will:
   a. complete an information letter for the referring physician (see
generic version in the MACS Pilot Study Manual)
   b. complete an information letter for the patient (see generic version
in the MACS Pilot Study Manual)
   c. complete an information letter for the pharmacy (see generic
version in the MACS Pilot Study Manual)
   d. make copy of the treatment form for the patient and her physician

2. If the patient is discharged from hospital but she will continue to come to WCH or MSH

   Please arrange for the continuation of the study drug and recording of treatment injections.

10. When the patient goes into labour or delivery is planned
Call FA (project director) at 351-3800 ext. # 2730 and leave a message on her voice mail or page her at 589-4296 to let her know
11. At delivery

Take a sample of cord blood and a sample of maternal blood immediately after delivery using the pre-labeled test tubes in the ziplock bags in the Study Envelope. The test tubes contain EDTA (lavender stopper tubes). One is for cord blood and one is for maternal blood.

If you can not find the test tubes, please arrange to take 10 ml of blood in a test tube containing EDTA (lavender stopper tubes) for both cord blood and maternal blood.

All test tubes should be labeled with the MACS Pilot Study, information as to whether the sample is maternal blood or cord blood, the date and time the sample was taken, maternal date of birth, and the patient’s study number.

Once the blood samples have been collected, put them back in the ziplock bags and put some ice inside the bags. It is very important to carry the blood samples on ice. Leave the pre-labeled plastic tubes inside the bags for lab use. (WCH-Send them to the lab (main floor).) (MSH, If the time is Monday to Friday between 8 am to 4 pm, send the bags to Christine Botsford Lab at prenatal unit. If it is after these hours or weekends send them to the Specimen Accessioning 6th floor, Room 601, Lab.)
12. Instruction for the preparation and storage of plasma (for Cortisol, CBG, and ACTH assay)

SPECIMEN REQUIREMENT
One hundred microliters in duplicate of EDTA plasma are required for the assay.

Preparation of Plasma
EDTA (7.2 mg/5 ml whole blood) is used as anticoagulant. Centrifuge the blood for 15 minutes at 760 × g to obtain hemolysis-free plasma. A short centrifugation at 20 °C will not be detrimental; however, refrigerated centrifugation is recommended. All plastics, glassware, or other material coming into contact with the specimen should be entirely free of any contamination.

PRECAUTION: Heparinized plasma yields falsely low values.

Storage of Plasma
Enzyme inhibitors are unnecessary at the sample collection phase; however, it is important to keep the sample cold (0 – 4 °C) after collection (for up to 8 hours). For long-term storage store the specimen at -70 °C. It is very important to keep the specimen frozen, preferably in plastic tubes. A decrease in ACTH may be experienced with repeated freeze-thaw of the specimen.

Call FA (project director) at 351-3800 ext. # 2730 and leave a message on her voice mail or page her at 589-4296 to let her when plasma is ready for pick-up.
13. After delivery

1. Give the mother the *Postpartum Questionnaire* and ask her to complete it and return it in the provided self-addressed, self-stamped envelope.

2. Measure the head circumference, abdominal circumference, length and weight of the baby. Record this information on the *Neonatal Form*.

   **If this is a multiple birth, record the information for twin A on the *Neonatal Form* provided and obtain forms for twin B, C, or D from FA.**

3. If the birth weight is less than 1500 grams, arrange an ophthalmologic exam to look for ROP (retinopathy of prematurity).

4. If the birth weight is less than 1500 grams, arrange for a cranial ultrasound between day 1 and 3, and on day 7 and 21 of age to look for IVH (intraventricular hemorrhage) and PVL (periventricular leukomalacia).

5. Arrange for a cranial ultrasound to look for IVH and PVL at hospital discharge or at 40 weeks of gestation, whichever comes first. **All babies should have this done.**

6. Complete the Treatment Form, Maternal Outcome Form and Neonatal Outcome Form and leave these in (MSH-the MACS Pilot Study box in the nursing station in the labor and delivery unit) / (WCH- the MACS Pilot Study box in the nursing station on 3 east)

Call FA (project director) at 351-3800 ext. #2730 and leave a message on her voice mail or page her at 589-4296 to let her know that these forms have been completed.
Thank you for your help with the MACS Pilot Study. If you have any questions, please don’t hesitate to call Dr. Fariba Aghajafari, the project director!
Appendix E
ENTRY FORM

Return to:
MACS Pilot Study
University of Toronto
Maternal, Infant and Reproductive Health Research Unit
at the Centre for Research in Women's Health
Suite 751, 790 Bay Street, Toronto, Ontario, Canada M5G 1N8
Tel: (416) 351-3800 # 2730
Fax: (416) 351-3771
ENTRY FORM

1. Maternal parity: (previous births ≥ 20 weeks)

2. Number of fetuses:

3. Racial background: (mark ONE ONLY)
   - asian
   - black
   - white/ caucasian
   - other

4. Gestational age:  
   - weeks
   - days

5. Methods of gestational age estimation: (mark ONE ONLY)
   - clinical only
   - ultrasound ± clinical

6. Date of the most recent ultrasound (US): (must be within TWO weeks of randomization)
   - year
   - month
   - day

7. What was the estimated fetal weight (all fetuses) on most recent US?
   a. singleton or twin A
   b. twin B
   c. fetus C
   d. fetus D

8. Amniotic fluid volume on most recent US? (in 1 or more sacs) (mark ALL that apply)
   - normal
   - increased
   - decreased

9. Evidence of any congenital anomalies? (in any fetus)
   - no
   - yes → if yes, specify ________________________________
   (if lethal congenital anomaly, exclude patient)

10. Evidence of any placental anomalies?
    - no
    - yes → if yes, specify ________________________________

11. Which drug was given as the first course of antenatal corticosteroids: (mark ONE ONLY)
    - betamethasone
    - dexamethasone

Timing of injections and dose of first course of antenatal corticosteroids:

a. year  month  day  24 hour clock  mg
b. year  month  day  24 hour clock  mg
c. year  month  day  24 hour clock  mg
d. year  month  day  24 hour clock  mg

CONTINUE TO PAGE 2
ENTRY FORM

12. Medical conditions:
   - no
   - yes → if yes, which one? (mark ALL that apply)
     - hypertension
     - diabetes
     - other (specify) ____________________________

13. Fetal conditions:
   - no
   - yes → if yes, which one? (mark ALL that apply)
     - growth retardation
     - other (specify) ____________________________

14. Reasons for increased risk of preterm birth? (mark ALL that apply)
   - regular uterine contractions (mark ALL that apply)
     → if yes, prior to first course
     → at randomization
   - a shortened cervical length or cervical dilatation (mark ALL that apply)
     → if yes, prior to first course
     → at randomization
   - antepartum vaginal bleeding secondary to placental separation or placenta previa
     (mark ALL that apply)
     → if yes, prior to first course
     → at randomization
   - prelabour rupture of membranes (mark ALL that apply)
     → if yes, prior to first course
     → at randomization
   - medical condition increasing risk of preterm delivery (mark ALL that apply)
     → if yes, prior to first course
     → at randomization
     → if yes, specify ____________________________
   - fetal condition increasing risk of preterm delivery (mark ALL that apply)
     → if yes, prior to first course
     → at randomization
     → if yes, specify ____________________________
   - past history of preterm birth
     → if yes, how many previous preterm births
     - yes
     - no

15. Antibiotics: (during the last TWO weeks)
   - no
   - yes

CONTINUE TO PAGE 3
ENTRY FORM

16. Tocolytics: (during the last TWO weeks)
   O no   O yes → if yes, (mark ALL that apply)
   O betamimetics (IV) → if yes, date and time of the last dose:
     [ ] [ ] [ ] [ ] [ ]
     year  month  day  24 hour clock
   O MgSO4 (IV) → if yes, date and time of the last dose:
     [ ] [ ] [ ] [ ] [ ]
     year  month  day  24 hour clock
   O indomethacin (PO or PR) → if yes, date and time of the last dose:
     [ ] [ ] [ ] [ ] [ ]
     year  month  day  24 hour clock
   O nitroglycerin patch → if yes, date and time of the last dose:
     [ ] [ ] [ ] [ ] [ ]
     year  month  day  24 hour clock
   O other (specify) ____________________________________________

17. Is this a referral? (That is, had the patient originally planned to deliver in another hospital?)
   O no   O yes → if yes, what is the highest level of care provided in that hospital?
   (mark ONE ONLY)
   O level 3 (MSH, WCH- NICU)
   O level 2 (NICU but for only few days)
   O level 1 (no NICU)

RANDOMIZATION PROCEDURE

Step 1: Complete Entry Form
Step 2: Call the hospital pharmacy (4084 WCH or 8303 MSH), tell them the maternal date of birth and the gestational age. Then the pharmacy will give you the study number
Step 3: Record the study number on the top of every page of this form
Step 4: Go to the pharmacy and get the study treatment compatible to the study number
Step 4: Give the injection

18. Date and time of randomization:
   [ ] [ ] [ ] [ ] [ ]
   year  month  day  24 hour clock
Patient's Study Number

Mother's date of birth

MACS
Pilot Study

TREATMENT FORM

Return to:
MACS Pilot Study
University of Toronto
Maternal, Infant and Reproductive Health Research Unit
at the Centre for Research in Women’s Health
Suite 751, 790 Bay Street, Toronto, Ontario, Canada M5G 1N8
Tel: (416) 351-3800 # 2730
Fax: (416) 351-3771
TREATMENT FORM

1. Was the study drug given after randomisation?
   - ○ no
   - ○ yes → if yes, give dates and times of injections:

   Week 1) a. ___________ ___________ ___________ ___________ ___________ 24 hour clock
   - b. ___________ ___________ ___________ ___________ ___________ 24 hour clock

   Week 2) a. ___________ ___________ ___________ ___________ ___________ 24 hour clock
   - b. ___________ ___________ ___________ ___________ ___________ 24 hour clock

   Week 3) a. ___________ ___________ ___________ ___________ ___________ 24 hour clock
   - b. ___________ ___________ ___________ ___________ ___________ 24 hour clock

   Week 4) a. ___________ ___________ ___________ ___________ ___________ 24 hour clock
   - b. ___________ ___________ ___________ ___________ ___________ 24 hour clock

   Week 5) a. ___________ ___________ ___________ ___________ ___________ 24 hour clock
   - b. ___________ ___________ ___________ ___________ ___________ 24 hour clock

   Week 6) a. ___________ ___________ ___________ ___________ ___________ 24 hour clock
   - b. ___________ ___________ ___________ ___________ ___________ 24 hour clock

   Week 7) a. ___________ ___________ ___________ ___________ ___________ 24 hour clock
   - b. ___________ ___________ ___________ ___________ ___________ 24 hour clock

   Week 8) a. ___________ ___________ ___________ ___________ ___________ 24 hour clock
   - b. ___________ ___________ ___________ ___________ ___________ 24 hour clock

   Week 9) a. ___________ ___________ ___________ ___________ ___________ 24 hour clock
   - b. ___________ ___________ ___________ ___________ ___________ 24 hour clock

   Week 10)a. ___________ ___________ ___________ ___________ ___________ 24 hour clock
   - b. ___________ ___________ ___________ ___________ ___________ 24 hour clock

2. Was the study treatment stopped before 33 weeks or never given?
   - ○ no
   - ○ yes → if yes, what was the reasons? (mark ALL that apply)
     - ○ risk for preterm birth disappeared
     - ○ complications developed (specify)
     - ○ patient delivered
     - ○ patient wanted to stop treatment (specify why)
     - ○ clinician wanted to stop treatment (specify why)
     - ○ other (specify)

CONTINUE TO PAGE 2 ➤
TREATMENT FORM

3. Did patient receive any of the following after randomisation: (mark ALL that apply)

- O tocolytics → if yes, (mark ALL that apply)
  - O betamimetics (IV) → if yes, date and time of the last dose given before delivery:
    year  month  day  24 hour clock

- O MgSO4 (IV) → if yes, date and time of the last dose given before delivery:
  year  month  day  24 hour clock

- O indomethacin (PO or PR) → if yes, date and time of the last dose given before delivery:
  year  month  day  24 hour clock

- O nitroglycerin patch (PO or PR) → if yes, date and time of the last dose given before delivery:
  year  month  day  24 hour clock

- O other (specify) __________________________

- O maternal antibiotics before labour
- O maternal antibiotics during labour/ prior to delivery of fetus(es)
- O maternal antibiotics after delivery of fetus(es)
- O corticosteroids given other than study drug
  → if yes, which ones were given? (mark ALL that apply)
    O betamethasone
    O dexamethasone
    O other (specify) __________________________
    → if yes, how many injections? □
    → if yes, dates and times of the last 4 injections:
    a. year  month  day  24 hour clock  mg
    b. year  month  day  24 hour clock  mg
    c. year  month  day  24 hour clock  mg
    d. year  month  day  24 hour clock  mg

- O other (specify) __________________________
Return to:
MACS Pilot Study
University of Toronto
Maternal, Infant and Reproductive Health Research Unit
at the Centre for Research in Women’s Health
Suite 751, 790 Bay Street, Toronto, Ontario, Canada M5G 1N8
Tel: (416) 351-3800 # 2730
Fax: (416) 351-3771
FETAL/NEONATAL OUTCOME FORM (for singleton or twin A)

1. Status of the baby at birth:
   - O alive
   - O stillborn → if stillborn, reason ____________________________

2. Sex:
   - O male
   - O female
   - O uncertain

3. Mode of delivery:
   - O caesarean section
   - O vaginal delivery

4. Date and time of delivery:
   - [ ] year
   - [ ] month
   - [ ] day
   - [ ] : [ ]

5. Gestational age at birth:
   - [ ] weeks
   - [ ] days

6. Birthweight:
   - [ ] gm

7. Birth length:
   - [ ] cm
   - O not measured

8. Birth head circumference:
   - [ ] cm
   - O not measured

9. Birth abdomen circumference:
   - [ ] cm
   - O not measured

10. Apgar score:
    - at 1 min [ ]
    - at 5 min [ ]
    - at 10 min [ ]
    (if done)

11. Did baby have any congenital anomalies?
    - O no
    - O yes → if yes, specify ____________________________

12. Did baby receive any of neonatal resuscitations in delivery /resuscitation room: (not including gastric suctioning)
    - O no
    - O yes → if yes, which one? (mark ALL that apply)
      - O oxygen
      - O bag and mask
      - O intubation and ventilation
      - O other resuscitation (specify) ____________________________

13. Was cord blood taken for blood gases?
    - O no
    - O yes → if yes, results (use arterial results if available, otherwise use venous; report all values or mark “not done”; if base measured and no base deficit, then report 8.8)
      - O arterial
      - O venous
      - pH [ ] [ ]
      - base deficit [ ] [ ]
      - HCO3 [ ] [ ]
      - O not done

14. Was cord blood taken for measuring of cortisol, ACTH, and CBG?
    - O no
    - O yes → if yes, date and time of blood sample taken:
      - [ ] year
      - [ ] month
      - [ ] day
      - [ ] : [ ]

CONTINUE TO PAGE 2 ➔
FETAL/NEONATAL OUTCOME FORM (for singleton or twin A)

15. Was head ultrasound done?
   - no
   - yes → if yes, dates of head ultrasounds:
     - a. ___ ___ ___
     - b. ___ ___ ___
     - c. ___ ___ ___
     - d. ___ ___ ___

16. Did baby have respiratory distress outside delivery/resuscitation room requiring treatment?
   - no
   - yes → if yes, which treatment did the baby receive? (mark ALL that apply)
     - oxygen
     - intubation and ventilation (not high frequency)
     - high frequency ventilation
     - nitric oxide
     - ECMO (extracorporeal membrane oxygenation)
   → if yes, reasons: (mark ALL that apply)
     - respiratory distress syndrome (RDS: PaO₂ < 50 mmHg in room air, central cyanosis in room air, or a requirement for supplemental oxygen to maintain PaO₂ > 50 mmHg and X-ray compatible with RDS (low lung volumes and reticulogranular appearance to lung fields with or without air bronchograms)
     - meconium aspiration syndrome (MAS: respiratory distress in the first 4 hours of life, oxygen requirement and X-ray compatible with MAS (coarse patchy infiltrates, focal areas of atelectasis, and emphysema)
     - infectious pneumonia (a chest X-ray compatible with pneumonia; or a histological diagnosis of pneumonia at autopsy)
     - pneumothorax or pneumomediastinum (extrapleural air diagnosed by chest radiograph or needle aspiration)
     - transient tachypnea of the newborn (TTN: mild respiratory distress that does not require surfactant)
     - other (specify)

17. Onset of assisted ventilation and intubation via intratracheal tube or naso pharyngeal / nasal cpap:
   ___ ___ ___ ___ ___ N/A

18. Final discontinuation of assisted ventilation and intubation via intratracheal tube or naso pharyngeal / nasal cpap:
   ___ ___ ___ ___ ___ N/A

CONTINUE TO PAGE 3
19. Did baby have necrotising enterocolitis (NEC)? (defined as either perforation of intestine, pneumatosis intestinalis or air in the portal vein, diagnosed by X-ray, surgery, or at autopsy)
   ○ no  ○ yes → if yes, how it was diagnosed? (mark ALL that apply)
   ○ surgery
   ○ post-mortem examination
   ○ clinically and X-ray

20. Did baby have neonatal infection? (clinical signs of infection and one or more of the following: a positive culture of blood, cerebrospinal fluid [CSF], urine, tracheal aspirate, or lung tissue at autopsy; a positive Gram’s stain of CSF; a chest X-ray compatible with pneumonia; or a histological diagnosis of pneumonia at autopsy)
   ○ no  ○ yes → if yes, specify______________________________

21. Did baby have bronchopulmonary dysplasia (BPD)?
   ○ no  ○ yes → if yes, (mark ALL that apply)
   ○ need for oxygen at 36 weeks adjusted gestational age
     (plus compatible X-ray)
   ○ need for oxygen at 28 days of life (plus compatible X-ray)

22. Did baby have periventricular- intraventricular haemorrhage (IVH)?
   ○ no  ○ yes → if yes, what was the most severe grade?  
   → if yes, how was IVH diagnosed? (mark ONE ONLY)
      ○ autopsy  ○ ultrasound
   → if yes, date diagnosed?
      year  month  day

23. Did baby have periventricular leucomalacia (PVL)?
   ○ no  ○ yes → if yes, date diagnosed?
      year  month  day

24. Did baby have patent ductus arteriosus (PDA) requiring treatment?
   ○ no  ○ yes → if yes, which kind of treatment?
      ○ indomethacin
      ○ PDA ligation
      ○ other treatment (specify)______________________________

CONTINUE TO PAGE 4
25. Was baby assessed for retinopathy of prematurity (ROP)?
   - no
   - yes → if yes, what was the most severe stage?
     - no
     - yes → if yes, was this in both eyes?
       - no
       - yes → if yes, what stage in each eye?
         - right
         - left

26. Did baby receive higher level of care than normal newborn?
   - no
   - yes → if yes, dates and times (complete all relevant dates and times, or mark not applicable, N/A)

27. Did baby receive any of the following treatments? (mark ALL that apply)
   - surfactant
   - antibiotics
   - postnatal corticosteroids to prevent BPD → if yes, date and time initiated?
   - postnatal corticosteroids to treat BPD → if yes, date and time initiated?
Patient’s Study Number

Mother’s date of birth

FETAL/NEONATAL OUTCOME FORM (for singleton or twin A)

28. Did baby die after birth?
   ○ no
   ○ yes → if yes, date and time?
     year
     month
     day
     24 hour clock

   → if yes, what was the most responsible cause of death: 

29. Did baby need oxygen at time of discharge home?
   ○ no
   ○ yes → if yes, date and time of discontinuation of supplemental O2?
     year
     month
     day
     24 hour clock

30. Date and time of discharge home?
   ○ N/A
MATERNFAL OUTCOME FORM

1. Date and time of rupture of membranes: [ ] [ ] [ ] [ ] 24 hour clock
   (if at delivery put date and time of delivery)
   (if a multiple pregnancy put date and time of first rupture of membranes)

2. Onset of labour: (mark ONE ONLY)
   O spontaneous   O no labour (caesarean before labour)   O induced

3. Onset of diabetes after randomisation:
   O no   O yes → if yes, (mark ONE ONLY)   O insulin given   O no insulin

4. Hypertension after randomisation:
   O no   O yes → if yes, (mark ONE ONLY)   O treatment given   O no treatment

5. Clinical chorioamnionitis: (defined as maternal temperature ≥ 38°C prior to delivery and one or more of the following: maternal tachycardia ≥120 bpm, white blood cell count ≥ 20,000/mm³, fetal tachycardia >160 bpm, uterine tenderness, or foul smelling amniotic fluid)
   O no   O yes

6. Maternal infection: O no → if yes, (mark ALL that apply)
   O endometritis (postpartum maternal temperature ≥38°C and tender fundus without other source of infection)
   O pneumonia (maternal temperature ≥38°C and signs of pneumonia on X-ray)
   O wound infection (drainage of purulent material or wound breakdown)
   O pyelonephritis (maternal temperature ≥38°C, positive urine culture and costal vertebral angle tenderness)
   O sepsis (positive maternal blood culture)
   O other (specify) ____________________________________________

7. Any other maternal complications: (specify) ____________________________________________

8. Was maternal blood taken for measuring cortisol, ACTH, and CBG?
   O no   O yes → if yes, date and time of blood sample taken:
   [ ] [ ] [ ] [ ] 24 hour clock

9. Date and time of discharge home:
   [ ] [ ] [ ] [ ] 24 hour clock
Thank you for your participation in the MACS Pilot Study. An important part of the answer to our research question is how participants are after the baby is born and how they felt about their care.

The feedback from the following questionnaire will be very helpful. All your answers will be kept strictly confidential.

Thank you very much for your help in answering an important question in obstetrical care today.
POSTPARTUM QUESTIONNAIRE

Part A: Your Health

1. Have you had any headaches since you began participation in the MACS Pilot Study?
   - O no
   - O yes → if yes, how severe were they? (mark ONE ONLY)
     - O mild
     - O moderate
     - O severe
   → if yes, did you take any medication for them?
     - O no
     - O yes
   → if yes, how would you describe the frequency of your headaches compared to during your pregnancy before your participation in the MACS Pilot Study?
     - O increased
     - O decreased
     - O no difference
     - O I had no headaches during my pregnancy before participation in the MACS Pilot Study

2. How much weight did you gain during your pregnancy?
   [ ] lb or [ ] kg

3. Have you noticed that your face has become “rounder” or “fuller” since you began participation in the MACS Pilot Study?
   - O no
   - O yes → if yes, how much of a problem has this been for you? (mark ONE ONLY)
     - O no problem at all
     - O a little problem
     - O a big problem

4. Have you noticed “acne” or “pimples” on your face since you began participation in the MACS Pilot Study?
   - O no
   - O yes → if yes, how much of a problem has this been for you? (mark ONE ONLY)
     - O no problem at all
     - O a little problem
     - O a big problem

CONTINUE TO PAGE 2
5. Have you noticed hair growing on your face or body since you began participation in the MACS Pilot Study?
   ○ no  ○ yes → if yes, how much of a problem has this been for you? (mark ONE ONLY)
   ○ no problem at all
   ○ a little problem
   ○ a big problem

6. Have you noticed any swelling of your body especially your legs, since you began participation in the MACS Pilot Study?
   ○ no  ○ yes → if yes, how much of a problem has this been for you? (mark ONE ONLY)
   ○ no problem at all
   ○ a little problem
   ○ a big problem

7. Have you noticed any stretch marks on your body that are purple in color since you began participation in the MACS Pilot Study?
   ○ no  ○ yes → if yes, how much of a problem has this been for you? (mark ONE ONLY)
   ○ no problem at all
   ○ a little problem
   ○ a big problem

8. Have you noticed any difficulty sleeping since you began participation in the MACS Pilot Study?
   ○ no  ○ yes → if yes, how much of a problem has this been for you? (mark ONE ONLY)
   ○ no problem at all
   ○ a little problem
   ○ a big problem

9. Have you noticed muscle weakness since you began participation in the MACS Pilot Study?
   ○ no  ○ yes → if yes, how much of a problem has this been for you? (mark ONE ONLY)
   ○ no problem at all
   ○ a little problem
   ○ a big problem

CONTINUE TO PAGE 3 ➔
10. Have you noticed any *increase* in your appetite since you began participation in the MACS Pilot Study?
   ○ no ○ yes ➔ *if yes*, how much of a problem has this been for you? (mark ONE ONLY)
      ○ no problem at all
      ○ a little problem
      ○ a big problem

11. Have you noticed any *decrease* in your appetite since you began participation in the MACS Pilot Study?
   ○ no ○ yes ➔ *if yes*, how much of a problem has this been for you? (mark ONE ONLY)
      ○ no problem at all
      ○ a little problem
      ○ a big problem

12. Have you noticed any unusual bruising on your body since you began participation in the MACS Pilot Study?
   ○ no ○ yes ➔ *if yes*, how much of a problem has this been for you? (mark ONE ONLY)
      ○ no problem at all
      ○ a little problem
      ○ a big problem

13. Have you noticed any unusual bleeding in your mouth or gums since you began participation in the MACS Pilot Study?
   ○ no ○ yes ➔ *if yes*, how much of a problem has this been for you? (mark ONE ONLY)
      ○ no problem at all
      ○ a little problem
      ○ a big problem

14. Have you noticed any difficulty remembering things since you began participation in the MACS Pilot Study?
   ○ no ○ yes ➔ *if yes*, how much of a problem has this been for you? (mark ONE ONLY)
      ○ no problem at all
      ○ a little problem
      ○ a big problem

CONTINUE TO PAGE 4 ➔
POSTPARTUM QUESTIONNAIRE

15. Have you noticed any change in your mood or any mood swings since you began participation in the MACS Pilot Study?
   - no
   - yes ➔ if yes, how much of a problem has this been for you? (mark ONE ONLY)
     - no problem at all
     - a little problem
     - a big problem
   ➔ if yes, please describe ________________________________

16. Have those close to you (family, friends) noticed any change in your mood or any mood swings since you began participation in the MACS Pilot Study?
   - no
   - yes ➔ if yes, how much of a problem has this been for you? (mark ONE ONLY)
     - no problem at all
     - a little problem
     - a big problem
   ➔ if yes, please describe ________________________________

17. Do you think the treatment that you received was corticosteroids or placebo?
   (mark ONE ONLY)
   - corticosteroids
   - placebo
   - not sure
POSTPARTUM QUESTIONNAIRE

Part B: Study Participation Evaluation

These questions are about your experience as a participant in the MACS Pilot Study. Section 1 is about what you may have LIKED about participating in this research study. Section 2 is about what you may have DISLIKED about participating in this research study. Place a mark in the circle beside each statement that applies to how you feel about being a participant in the MACS Pilot Study.

Section 1
Here is what I LIKED about participating in the MACS Pilot Study:
(mark ALL that apply)
1 O I liked my contacts with the research staff.
2 O I liked being randomized.
3 O I liked the fact that there were few extra demands upon my time, finances, etc.
4 O I liked the fact that I had a chance to get multiple courses of antenatal corticosteroids.
5 O I liked the fact that I had a chance to assist with research to help others like me.
6 O Participating in the MACS Pilot Study caused me to feel reassured about my health.
7 O Participating in the MACS Pilot Study caused me to feel reassured about my baby’s health.
8 O There was nothing I liked about being participant in the MACS Pilot Study.
9 O Other (please explain):

Section 2
Here is what I DISLIKED about participating in the MACS Pilot Study:
(mark ALL that apply)
1 O I disliked my contacts with the research staff.
2 O I disliked being randomized.
3 O I disliked the fact that there were extra demands upon my time, finances, etc.
4 O Participating in the MACS Pilot Study caused me to feel worried about my health.
5 O Participating in the MACS Pilot Study caused me to feel worried about my baby’s health.
6 O There was nothing I disliked about being participant in the MACS Pilot Study.
7 O Other (please explain):

Section 3
If time suddenly went backwards, and you had it to do all over again, would you agree to participate in this research study?
(mark ONE ONLY)
O Definitely not
O Probably not
O Probably yes
O Definitely yes
Please explain your answer to Section 3:
POSTPARTUM QUESTIONNAIRE

Part C: General Information

The following additional questions will provide us with a little more information about you.

1. When were YOU born? 
   
2. What is the date today? 
   
3. Most people have close ties to one ethnic group or nationality. Please state the one that best describes you. (Please print in block capitals)

4. What is the main Language that was spoken in your home when you were growing up? (Please print in block capitals – list one only)

5. What is the main language that is spoken in your home now? (Please print in block capitals – list one only)

6. Are you living with another adult person?
   O no  O yes ➔ if yes, O a husband/ partner  O another member of family  O a friend

7. Did anyone help you to answer any of the questions in this form?
   O no  O yes ➔ if yes, who?
   O a husband/ partner
   O another member of family
   O a friend
   O a doctor or nurse from the hospital
   O another person (please describe)__________________________

Please write any additional comments about your childbirth experience and your participation in this study that you think will be helpful for evaluation multiple courses of antenatal corticosteroids for preterm birth. Use the back of this page if necessary.

Please put the completed form in the envelope provided and return by mail.
Thank you for your help!
MACS
Pilot Study
NON-RANDOMIZED FORM

Return to:
MACS Pilot Study
University of Toronto
Maternal, Infant and Reproductive Health Research Unit
at the Centre for Research in Women’s Health
Suite 751, 790 Bay Street, Toronto, Ontario, Canada M5G 1N8
Tel: (416) 351-3800 # 2730
Fax: (416) 351-3771
### NON – RANDOMIZED PATIENT FORM

1. **Maternal parity:** (previous births ≥ 20 weeks)

2. **Number of fetuses:**

3. **Racial background:** (mark ONE ONLY)
   - ☐ Asian
   - ☐ Black
   - ☐ White/Caucasian
   - ☐ Other

4. **Gestational age:**

5. **Methods of gestational age estimation:** (mark ONE ONLY)
   - ☐ Clinical only
   - ☐ Ultrasound ± Clinical

6. **Date of the most recent ultrasound (US):** (must be within TWO weeks of randomization)

7. **What was the estimated fetal weight (all fetuses) on most recent US?**
   - a. Singleton or twin A
   - b. Twin B
   - c. Fetus C
   - d. Fetus D

8. **Amniotic fluid volume on most recent US?** (in 1 or more sacs) (mark ALL that apply)
   - ☐ Normal
   - ☐ Increased
   - ☐ Decreased

9. **Evidence of any congenital anomalies?** (in any fetus)
   - ☐ No
   - ☐ Yes → if yes, specify ________________________________
   - (if lethal congenital anomaly, exclude patient)

10. **Evidence of any placental anomalies?**
    - ☐ No
    - ☐ Yes → if yes, specify ________________________________

11. **Which drug was given as the first course of antenatal corticosteroids:** (mark ONE ONLY)
    - ☐ Betamethasone
    - ☐ Dexamethasone

    **Timing of injections and dose of first course of antenatal corticosteroids:**
    a. [Dates]
    b. [Dates]
    c. [Dates]
    d. [Dates]

    CONTINUE TO PAGE 2 →
Mother's date of birth

NON - RANDOMIZED PATIENT FORM

12. Medical conditions:
- no
- yes → if yes, which one? (mark ALL that apply)
  - hypertension
  - diabetes
  - other (specify) __________________________

13. Fetal conditions:
- no
- yes → if yes, which one? (mark ALL that apply)
  - growth retardation
  - other (specify) __________________________

14. Reasons for increased risk of preterm birth? (mark ALL that apply)
- regular uterine contractions (mark ALL that apply)
  → if yes, prior to first course
    - at randomization
  → a shortened cervical length or cervical dilatation (mark ALL that apply)
    → if yes, prior to first course
    - at randomization
  → antepartum vaginal bleeding secondary to placental separation or placenta previa
    (mark ALL that apply)
    → if yes, prior to first course
    - at randomization
  → prelabour rupture of membranes (mark ALL that apply)
    → if yes, prior to first course
    - at randomization
  → medical condition increasing risk of preterm delivery (mark ALL that apply)
    → if yes, prior to first course
    - at randomization
    → if yes, specify __________________________
  → fetal condition increasing risk of preterm delivery (mark ALL that apply)
    → if yes, prior to first course
    - at randomization
    → if yes, specify __________________________
  → past history of preterm birth
    → if yes, how many previous preterm births □
  → other (specify) __________________________

15. Antibiotics: (during the last TWO weeks)
- no
- yes

CONTINUE TO PAGE 3 ➔
NON – RANDOMIZED PATIENT FORM

16. Tocolytics: (during the last TWO weeks)
   O no  O yes → if yes, (mark ALL that apply)
   O betamimetics (IV) → if yes, date and time of the last dose:
     [year] [month] [day] [24 hour clock]
   O MgSO4 (IV) → if yes, date and time of the last dose:
     [year] [month] [day] [24 hour clock]
   O indomethacin (PO or PR) → if yes, date and time of the last dose:
     [year] [month] [day] [24 hour clock]
   O nitroglycerin patch → if yes, date and time of the last dose:
     [year] [month] [day] [24 hour clock]
   O other (specify) ____________________________

17. Is this a referral? (That is, had the patient originally planned to deliver in another hospital?)
   O no  O yes → if yes, what is the highest level of care provided in that hospital?
     (mark ONE ONLY)
     O level 3 (MSH, WCH- NICU)
     O level 2 (NICU but for only few days)
     O level 1 (no NICU)

18. Reason for not entering trial: (mark ONE ONLY)
   O not eligible → if yes, (mark ALL that apply)
   O contraindication to steroids
   O requiring chronic doses of steroids
   O clinical evidence of chorioamnionitis
   O known lethal congenital anomalies
   O other (specify) ____________________________

   O patient refused: → if yes, (mark ALL that apply)
   O wanted corticosteroids
   O did not want corticosteroids
   O did not want to be randomized
   O other (specify) ____________________________

   O clinician refused: → if yes, (mark ALL that apply)
   O preference for corticosteroids
   O preference for no corticosteroids
   O reasons not specified
   O other (specify) ____________________________

   O not approached
   O other