IMPACT OF INFANT SEX ON ANTENATAL STEROID EXPOSURE

THE IMPACT OF INFANT SEX ON PERINATAL OUTCOMES FOLLOWING EXPOSURE TO MULTIPLE COURSES VERSUS A SINGLE COURSE OF ANTENATAL CORTICOSTEROIDS: A SECONDARY ANALYSIS OF THE MACS RANDOMIZED CONTROLLED TRIAL

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A Thesis Submitted to the School of Graduate Studies in Partial Fulfilment of the Requirements for the Degree of Master of Science in Health Research Methodology

Master of Science (2020-2022), Health Research Methods, Evidence & Impact, McMaster University Hamilton, Ontario (Health Research Methodology)

Title: The Impact of Infant Sex on Perinatal Outcomes Following Exposure to Multiple Courses Versus a Single Course of Antenatal Corticosteroids: A Secondary Analysis of the MACS Randomized Controlled Trial

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NUMBER OF PAGES: ii, 32

Lay Abstract:

Antenatal corticosteroids (ACS) are given in pregnancies at risk of early birth. ACS help the lungs and other organs, such as the brain and kidneys to mature. ACS help improve babies' survival and reduce the risk of other health complications. Several animal studies suggest that infant sex can affect long-term outcomes after receiving a higher dose of ACS. The goal of our study was to look at the effect of infant sex on the relationship between the use of multiple courses (i.e., a higher dose) versus a single course of ACS and short-term outcomes. These outcomes include challenges with breathing, bleeding in the brain, problems in the bowel, and infant death. Our study found that infant sex did not significantly change the relationship between ACS and short-term infant outcomes, but further study is required on long-term outcomes as sex specific differences may emerge over time as reported in animal literature.

Abstract:

Objective:

Animal literature has suggested that the impact of antenatal corticosteroids (ACS) may vary by infant sex. Our objective was to assess the impact of infant sex on the use of multiple courses versus a single course of ACS and perinatal outcomes.

Study Design:

We conducted a secondary analysis of the Multiple Courses of Antenatal Corticosteroids (MACS) for Preterm Birth trial. Our primary outcome was a composite of perinatal mortality or clinically significant neonatal morbidity (including neonatal death, stillbirth, severe respiratory distress syndrome, intraventricular hemorrhage [grade III or IV], cystic periventricular leukomalacia, and necrotising enterocolitis [stage II or III]). Secondary outcomes included individual components of the primary outcome as well as anthropometric measures. Baseline characteristics were compared between participants who received multiple courses versus a single course of ACS. Multivariable regression analyses were conducted with adjustment for pre-defined covariates including an interaction between exposure to ACS and infant sex.

Results:

Data on 2304 infants were analyzed. The interaction term between treatment status (multiple courses versus a single course of ACS) and infant sex was not significant in the adjusted model for the primary outcome (p=0.86), nor for any of the secondary outcomes. Exposure to multiple courses versus a single course of ACS was not associated with the primary outcome either before or after adjustment (aOR 0.99, 95% CI 0.67 to 1.45, n=2292 infants). However, exposure to multiple courses versus a single course of ACS resulted in significantly

lower birth length (p=0.02) and head circumference at birth (p=0.04) although not birthweight (p=0.06).

Conclusions:

Infant sex did not modify the association between exposure to ACS and perinatal outcomes including perinatal mortality or neonatal morbidity or anthropometric outcomes. However, animal literature indicates that sex specific differences after exposure to ACS may emerge over time and thus investigating long-term sex-specific outcomes warrants further attention.

Acknowledgements:

First and foremost, I would like to thank my supervisor Dr. Sarah D McDonald for her guidance and support throughout the development of my thesis. This work would not have been possible without her excellent mentorship, patience, and encouragement. I am fortunate to have the opportunity to work with Dr. McDonald and will always be grateful for the opportunities she has provided for my professional growth. I would also like to thank my committee members, Dr. Pasqualina Santaguida, Dr. Amit Mukerji, Dr. Kellie E Murphy, and Dr. Elizabeth V Asztalos for their support and guidance in developing this project. I would also like to thank Dr. Stephen G Matthews and Dr. Ella Huszti for their guidance and support as co-authors of the manuscript associated with this thesis. A special thank you to Ms. Yidi Jiang for her data analysis and Dr. Gabriel Shapiro for his review of manuscript components. Last but not least, I would like to acknowledge and thank my family for their support and encouragement with my research endeavours.

Table of Contents

List of Tables and Figures

List of Abbreviations and Symbols:

ACS	Antenatal corticosteroids
CI	Confidence interval
MACS	Multiple Courses of Antenatal Corticosteroids for Preterm Birth Study
MD	Mean difference
OR	Odds ratio
RR	Risk Ratio

Declaration of Academic Achievement:

I, Kiran Ninan, declare this thesis work to be my own. I was part of the team that designed the study, prepared files for the thesis and wrote the thesis document including the manuscript.

My supervisor, Dr. McDonald, provided detailed guidance and feedback throughout the various stages of preparing this document as well as the journal submission. My thesis committee members, Dr. Santaguida, Dr. Mukerji, Dr. Murphy, and Dr. Asztalos, were involved in the study design and provided feedback concerning the design, journal manuscript and thesis document. Dr. Murphy led the creation of the research questions. Dr. Huszti and Dr. Matthews also provided feedback and support during the design, analysis and final review of the manuscript and met the authorship criteria. Ms. Jiang performed the statistical analysis under guidance of the study team and is also an essential co-authors of the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for the work.

Introduction:

Preterm birth (i.e., birth before 37 weeks of gestation) is a significant cause of morbidity and mortality and every year approximately 15 million babies are born preterm worldwide.[1] Use of antenatal corticosteroids (ACS) can help improve short-term outcomes for infants born preterm.[2,3] The use of a single course of ACS significantly reduces the risk of death and major morbidities such as respiratory distress syndrome and intraventricular hemorrhage.[4] The two commonly used ACS are: either two intramuscular injections of 12 mg of a betamethasone mixture (i.e., six mg of betamethasone phosphate and six mg of betamethasone acetate) given 24 hours apart or four intramuscular injections of dexamethasone phosphate given 12 hours apart to pregnant people at risk of preterm birth.[2,3] Betamethasone and dexamethasone are structurally similar with a difference in the configuration of one methyl group.[5] ACS readily cross the placenta (as they are not deactivated by 11β-hydroxysteroid dehydrogenase type 2 unlike maternal endogenous glucocorticoids),[6] bind to glucocorticoid receptors and alter gene expression of many target genes and can impact up to 20% of the transcriptome.[7] In the fetal lungs, ACS bind to glucocorticoid receptors and can induce cellular differentiation and alter cellular pathways to stimulate type II pneumocytes to produce and secrete surfactant to lower alveolar surface tension and aid in maintaining alveolar stability as part of lung maturation.[8,9] ACS also have effects on other organs, including cerebral vasoconstriction in the fetal brain[10] and stabilization of blood pressure,[11] which protects against intraventricular hemorrhage.[10]

In light of these short-term benefits, a single course of ACS (24 mg of betamethasone or dexamethasone) is recommended in pregnancies between 24 weeks and 34 weeks and 6 days of gestation at high risk of preterm birth within seven days.[3] However, preterm birth is difficult to predict, and data show that nearly 50% of infants exposed to ACS are born at or after 35 weeks

of gestation.[12] At this point, the benefits of ACS are diminished as fetal organs are better developed.[7] In pregnancies at risk of preterm birth where the optimal seven-day window between administration of ACS and birth is exceeded, the use of additional doses/courses of ACS has been considered.[2,13] Meta-analyses have shown that in the short-term the use of multiple courses versus a single course of ACS to be associated with significant reductions in respiratory distress syndrome (risk ratio [RR] 0.82, 95% confidence interval [CI] 0.74 to 0.90, nine trials, 3540 infants)[14] and use of respiratory support (RR 0.91, 95% CI 0.85 to 0.97, ten trials, 5,791 infants).[15] Despite reductions in respiratory distress syndrome (of any severity, of which mild respiratory distress syndrome would be the most common),[14] adverse effects of using multiple courses of ACS such as reduced fetal growth and neurodevelopmental harm have been reported in animal literature[16-19] and clinical trials and follow-up studies.[20-22] However, based on the short term neonatal benefits, some have advocated for the use of multiple courses of ACS (i.e., at least two total courses of ACS),[14,15] or a single repeat course[2] which requires further exploration given potential concerns.

The animal literature provides numerous examples of steroids exerting an impact on various organ development and function.[23-29] When compared to males, female lambs had significantly greater increases in lung volume, compliance and arterial oxygen partial pressure following prenatal steroid treatment[23] but the mechanisms underlying these differences remain poorly understood.[25] In male versus female sheep, no statistically significant differences were found in the structure of the lungs and the production/composition of surfactant[24] which led to the hypothesis that observed sex-based differences may be due to a difference in the lungs' adaptation to air-breathing.[24] Systematic reviews of animal literature have also reported sex-specific reductions in survival[30] as well as sex-specific adverse neurological effects such as

altered motor activity and neuropathology following repeated doses of ACS.[31] Animal studies have also shown other sex-specific effects with higher levels of glucocorticoids which can alter programming of the fetal hypothalamus pituitary adrenal (HPA) axis[26] which is essential for mechanisms controlling fetal development.[32,33] Multiple courses of synthetic glucocorticoids have been shown to reduce HPA responsiveness in juvenile[27,28] and adult male offspring[29] but increase responsiveness in juvenile female offspring.[27]

Given the interplay between ACS and fetal sex reported in animal literature,[23] we hypothesized that infant sex would be an effect modifier of treatment with multiple courses versus a single course of ACS and perinatal outcomes. Thus, our objective was to measure the impact of infant sex (as a potential effect modifier) on the association between the use of multiple courses versus a single course of ACS and perinatal outcomes.

Methods:

Participants:

This is a secondary analysis of data from the international, multi-center Multiple Courses of Antenatal Corticosteroids for Preterm Birth (MACS) randomized controlled trial (RCT). The initial MACS trial enrolled pregnant people between 25 and 32 weeks of gestation who had received an initial course of ACS (either betamethasone or dexamethasone) within the previous 14 to 21 days and continued to be viewed at risk of preterm birth based on clinical assessment. Enrolled participants were randomized to receive either a repeat course of ACS or a placebo every 14 days until birth or 33 weeks of gestation, whichever occurred first. Infants exposed to multiple courses of ACS or a standard single course (i.e., those who received placebo courses

following the initial course) and who had data on perinatal outcomes were included in the trial. Exclusion criteria for the trial were: contraindication(s) to ACS, need for chronic doses of ACS, evidence of chorioamnionitis, known lethal fetal congenital abnormality, or an initial course of ACS prior to 23 weeks of gestation.[20] Pregnant people with a single- or multiple-gestation pregnancy were eligible.[20] In addition, pregnant people with multiple pregnancy in which a fetus had died before 13 weeks of gestation were also eligible, but the dead fetus was not included in outcome assessment.[20]

Outcomes:

For the present analysis, our inclusion and exclusion criteria were consistent with the initial trial. Our primary outcome slightly differed but was also a composite of perinatal mortality or clinically significant neonatal morbidity. Perinatal mortality was defined as stillbirth or neonatal death during the first 28 days of life or before hospital discharge. Clinically significant neonatal morbidities included severe respiratory distress syndrome (i.e., defined as 1] needing assisted ventilation with an endotracheal tube and supplemental oxygen, both within the first 24 hours of life and for 24 hours or more, and 2] either a radiographic scan compatible with respiratory distress syndrome or surfactant given between the first 2–24 hours of life); intraventricular haemorrhage grade III or IV (as diagnosed by cranial ultrasound); cystic periventricular leukomalacia (i.e., periventricular cystic changes in the white matter, excluding subependymal and choroid plexus cysts, diagnosed by cranial ultrasound) and necrotising enterocolitis stage II or III (i.e., either perforation of intestine, pneumatosis intestinalis, or air in the portal vein, diagnosed by radiographic scan or at surgery). When compared to the original MACS trial, our composite outcome does not include bronchopulmonary dysplasia as this outcome is not as relevant to the effects of ACS based on more recent literature.[4] Other

components of the primary outcome were consistent with the initial MACS trial as they remain clinically/statistically relevant based on recent evidence.[4] Secondary outcomes included individual components of the primary composite outcome as well as anthropometric outcomes (birthweight [grams], length at birth [centimeters] and head circumference [centimeters]).

Statistical analysis:

Maternal baseline characteristics were compared between patients who received multiple courses versus a single course of ACS using the chi-square test, Student's t-test, or Mann-Whitney U test as appropriate. For the primary outcome, univariable and multivariable logistic regression models were used to estimate adjusted odds ratios (OR) and 95% confidence intervals (95% CIs). For secondary outcomes, univariable and multivariable logistic or linear regression models were used to estimate OR and mean differences (MD) respectively and were accompanied by 95% CIs. For continuous outcomes, skewed data were log transformed. For each outcome, based on clinical expertise and previous literature, biologically plausible covariates were considered for inclusion as part of the adjusted model (Table S1). Based on previous validation for multivariable logistic regression models,[34] we followed a permissive rule of thumb allowing a ratio of five outcome events per predictor variable (Table S1). Furthermore, to account for within-sibling co-linearity as 20% of pregnancies within each group were a multiple pregnancy, hierarchical models with generalized estimation equations (GEE) were used to calculate effect sizes. To prevent Type I error due to multiple analyses, p-values within adjusted models were determined using the Holm method, a modified Bonferroni correction which is more conservative than alternative options such as the False Discovery Rate.[35] To assess potential collinearity, variance inflation factors were determined within each adjusted

model. Analyses were performed using R version 4.0.3 (R Foundation for Statistical Computing).[36]

Principal and subgroup analyses:

Our main analyses involved an interaction term between treatment status (i.e., multiple courses versus a single course of ACS) and infant sex in adjusted models to assess the impact of infant sex as a potential effect modifier, with a statistically significant interaction defined as a p-value of <0.05. For comparing multiple courses versus a single course of ACS, we planned to stratify outcomes by infant sex if there was a significant interaction.

As part of a post-hoc analysis to further understand the impact of infant sex on perinatal outcomes, outcomes were also compared in male versus female infants within each randomization arm using unadjusted and adjusted regression analyses.

Details of ethics approval:

Our secondary analysis of RCT data was approved by the Hamilton Integrated Ethics Research Board (#14268).

Results:

Data were available on all 1853 pregnant people who participated in the MACS trial, while up to 2304 of their children were analyzed within regression models (Figure 1). When comparing multiple courses versus a single course of ACS, none of the baseline maternal/pregnancy characteristics varied significantly between the two randomized groups (Table 1).[20]

Multiple courses versus a single course of ACS (including an interaction term between infant sex and treatment status)

When comparing infants exposed to multiple courses versus a single course of ACS, the interaction term between treatment status (i.e., multiple courses versus a single course of ACS) and infant sex was not significant in the adjusted model for the primary composite perinatal outcome (p=0.86, Figure 2), nor for any of the secondary perinatal outcomes (e.g., p=0.93, p=0.62, p=0.48 for birthweight, birth length and head circumference respectively, Figure 2). The primary composite outcome of perinatal mortality or clinically significant neonatal morbidity did not differ between treatment groups, including after adjustment for biologically plausible confounding variables (adjusted odds ratio [aOR] 0.99, 95% CI 0.67 to 1.45, 2292 infants, Table 2). This adjusted analysis included an interaction term of treatment status*sex; gestational age at randomization; multiple-gestation pregnancy; preterm premature rupture of membranes at enrollment; antenatally suspected intrauterine growth restriction; pre-eclampsia; maternal diabetes; maternal smoking; maternal antibiotics prior to 2 weeks of enrollment; hypertension needing treatment; fetal anomalies; maternal substance abuse and time from first dose or pre-study corticosteroids to randomization. P-values for adjusted models were corrected using the Holm modified Bonferroni method (Table 2 and Tables S2-S4). No significant associations were observed in unadjusted or adjusted analyses between exposure to multiple courses versus a single course of ACS and any of the secondary neonatal morbidity outcomes (perinatal mortality, severe respiratory distress syndrome, intraventricular hemorrhage [grade III or IV], cystic periventricular leukomalacia or necrotizing enterocolitis [stage II or III]) in the short term. In adjusted analyses of infants, exposure to multiple courses of ACS was associated with lower

birth length (p=0.02) and head circumference (p=0.04), although not birthweight (p=0.06, Table 2).

Females versus males within each randomization arm (post-hoc analyses)

When comparing females versus males within each randomization arm (i.e., females versus males in the multiple course group [Table S2], and females versus males in the single course group [Table S3]), no significant adjusted associations were found, or the models did not converge due to a small number of outcome events/sample size.

Discussion:

Summary of results:

Infant sex was not a significant effect modifier of the relationship between exposure to ACS and perinatal outcomes. In infants exposed to multiple courses versus a single course of ACS, we found no significant differences in the risk of the clinically significant neonatal morbidity outcomes, even after adjusting for relevant potential confounding variables. Furthermore, there were no significant differences in outcomes between females and males within each randomization arm after adjusting for potential covariates.

Relation to other studies on the topic:

Some recent studies have found the impact of ACS versus non-exposure to vary based on infant sex.[37,38] One observational study of 710 infants born preterm found significantly improved odds of survival in males exposed to ACS but no significant association with mortality in females exposed to ACS.[37] A recent secondary analysis of an RCT of 2331 infants born late preterm found that infant sex was a significant effect modifier for antenatal betamethasone use

and a primary outcome of severe neonatal morbidity and when stratified, male sex was associated with a higher odds of severe neonatal morbidity when compared to female sex (aOR 1.95, 95% CI 1.25-3.05, 2331 infants).[38] However, a 2011 systematic review of eight RCTs and 2077 infants did not report any sex-based differences in short-term clinical outcomes such as neonatal mortality, respiratory distress syndrome, and intraventricular hemorrhage following exposure to ACS.[39] It should be noted that RCTs included in that review were conducted over two decades ago.[39] Given recent advances in neonatal care,[40] data from the MACS trial allowed for a more contemporary exploration of the impact of infant sex in a slightly larger population (2304 infants). While most studies compare ACS exposure to non-exposure,[4] our study did not find that infant sex was a significant effect modification when comparing multiple courses versus a single course of ACS. We also found no significant differences in our composite outcome between female and male infants within each randomization arm as part of our post-hoc analysis. However, we did find reductions in growth among infants exposed to multiple courses versus a single course of ACS, which in turn may lead to long-term consequences later in life.[41] This may be due to the fact that glucocorticoids inhibit hormones that are essential for fetal growth including insulin like growth factors I and II as well as placental lactogen.[42]

One previous study of MACS data considered the impact of infant sex, but on long-term outcomes instead of the short term ones examined here.[43] That study found that infant sex was significantly associated with neurocognitive/neurobehavioral disability in children at 5 years of age when included as a covariate in adjusted analyses.[43] However, exposure to multiple courses versus a single course of ACS was not significantly associated with neurocognitive/neurobehavioral disability after adjustment for infant sex and other covariates.[43]

Sex is an important factor to consider given that male and female fetuses differ in how they cope with complications/stress during pregnancy with female neonates shown to generally adapt better to ex utero life.[44] Previous animal literature reported sex-specific differences in respiratory outcomes[23,24] and adverse neurological outcomes including alteration in fetal programming and stress reactivity after exposure to prenatal glucocorticoids.[28,31,45] Sex-based differences in fetal development that result in adverse postnatal outcomes may be partly due to sex-based differences in the function and responsiveness of sex-linked placental genes and resulting protein structures.[44] It may also be possible that the sex-specific differences reported in the animal literature are seen earlier in life due to the use of much higher doses of glucocorticoids than what has been used clinically and in this trial. While our current analysis reported on outcomes measured at birth and within the neonatal period, based on this available animal literature we cannot rule out the possibility that sex specific effects may emerge later in life for those exposed to any dosing of ACS.

Strengths and limitations:

Our study has some important strengths. We were able to adjust our analyses for some biologically plausible covariates. Our analysis used data from a large double-blinded RCT that balances known and unknown confounders and reduces the risk of selection and information biases when comparing infants randomized to multiple courses versus a single course of ACS. For our main analysis (which tested for an interaction with infant sex) and each post-hoc analysis, there were no significant differences in the primary outcome or secondary outcomes involving neonatal morbidity between those exposed to multiple courses versus a single course of ACS. For our main analysis comparing multiple courses versus a single course of ACS anthropometric measurements were significantly lower in those exposed to multiple courses of

ACS versus a single course of ACS. However, when comparing males versus females in each randomization arm (i.e., our post-hoc analyses), most unadjusted anthropometric outcomes were no longer significant.

There are some limitations in our study. This was a post-hoc analysis of data from the original MACS trial, and all post-hoc analyses should be interpreted with caution, including ours. We were unable to account for all potentially important covariates (e.g., for our secondary outcomes of IVH, NEC and cPVL) as the majority of infants were born at or after 34 weeks of gestation, and hence had low rates of the pre-specified outcomes.

Areas for future research:

Future research should explore the impact of infant sex on the use of multiple courses versus a single course of ACS, as some outcomes may manifest later. The impact of the duration of steroid exposure (i.e., time from administration of ACS to birth) remains an important factor to consider when assessing perinatal and childhood outcomes.[46] Future research should explore this issue[47] along with infant sex, as animal literature suggests that: 1) the duration of exposure is important for an adequate physiological response to ACS[7] and 2) because ACS cause a sex-specific expression of gene profiles[48] that may depend on the gestational age at exposure.[49,50]

Conclusion:

In a secondary analysis of data from a robust multi-centre RCT, we found that infant sex was not a significant modifier of the relationship between exposure to multiple courses versus a single course of ACS and perinatal outcomes. Given sex-specific long-term effects reported in animal literature, further research is required to elucidate whether similar effects may emerge later in life for those exposed to ACS.

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Table 1: Baseline characteristics of pregnant people from the MACS trial

Maternal/pregnancy characteristics	Multiple course group (N=935 pregnant people)	Single course group (N=918 pregnant people)	P-value
Maternal age, years			
• Mean (SD)	29.05 (6.23)	29.14 (6.18)	0.78
• Median [Min, Max]	29.00 [14.30, 46.00]	28.70 [13.7, 46.80]	
Number of Fetuses, n (%)			0.98
• 1	737 (78.88%)	726 (79.08%)	
• 2	162 (17.33%)	158 (17.21%)	
• 3	36 (3.85%)	34 (3.70%)	
Number of previous pregnancies, n (%)			0.95
• 0	263 (28.13%)	252 (27.45%)	
• 1-4	577 (61.71%)	571 (62.20%)	
• >4	95 (10.16%)	91 (9.91%)	
• Missing	0 (0.00%)	4 (0.44%)	
Previous second-trimester abortion, 14-19 weeks, n (%)	65 (6.95%)	69 (7.52%)	0.70
Previous preterm birth, 20-30 weeks, n (%)	148 (15.83%)	162 (17.65%)	0.32
Previous preterm birth, 31-36 weeks, n (%)	174 (18.61%)	172 (18.74%)	0.99
History of a previous pregnancy with IUGR, n (%)	53 (5.67%)	60 (6.54%)	0.50
Method of gestational age calculation, n (%)			0.26
• Clinical only	31 (3.32%)	41 (4.47%)	
• Ultrasound + clinical	896 (95.83%)	874 (95.21%)	
• Missing	8 (0.86%)	3 (0.33%)	
Estimated fetal weight by ultrasound at randomisation, (g)			
• Mean (SD)	1202.40 (387.40)	1213.50 (389.10)	0.54
• Median [Min, Max]	1147.00 [264.00, 2930.00]	1174.00 [361.00, 2800.00]	
• Missing, n (%)	33 (3.53%)	18 (1.96%)	

Gestational age at randomization, weeks			
• Mean (SD)	29.30 (2.00)	29.38 (2.00)	0.34
• Median [Min, Max]	29.40 [21.00, 32.86]	29.29 [25.00, 33.86]	
Gestational age at randomization, n (%)			0.38
• <25 weeks	1 (0.11%)	0 (0.00%)	
• 25-27 weeks	256 (27.38%)	255 (27.77%)	
• 28-32 weeks	678 (72.51%)	661 (72.00%)	
• >32 weeks	0 (0.00%)	2 (0.22%)	
Fetal anomalies, n (%)	2 (0.21%)	5 (0.54%)	0.44
Pre-study course of ACS, n (%)			0.61
• Betamethasone	810 (86.63%)	786 (85.62%)	
• Dexamethasone	125 (13.37%)	131 (14.27%)	
• Missing	0 (0.00%)	1 (0.11%)	
Time from first dose of pre-study corticosteroids to randomization (days)			
• Mean (SD)	16.18 (2.57)	16.02 (2.93)	0.21
• Median [Min, Max]	15.00 [1.00, 34.00]	15.00 [0.00, 36.00]	
• Missing, n (%)	1 (0.11%)	0 (0.00%)	
Medical and obstetrical problems at enrolment, n (%)			
• Uterine contractions within previous week	520 (55.61%)	522 (56.86%)	0.62
• Short cervical length or cervical dilation	457 (48.88%)	450 (49.02%)	1.00
• Antepartum vaginal bleeding	129 (13.80%)	130 (14.16%)	0.87
• Preterm rupture of membranes	149 (15.94%)	142 (15.47%)	0.83
• Antenatal suspected intrauterine growth restriction	85 (9.09%)	74 (8.06%)	0.48
• Pre-eclampsia	43 (4.60%)	53 (5.77%)	0.30
• Smoking	108 (11.55%)	93 (10.13%)	0.36
• Substance abuse	7 (0.75%)	5 (0.54%)	0.80
• Hypertension needing treatment	66 (7.06%)	70 (7.63%)	0.71
• Maternal diabetes (Type I or II)	50 (5.35%)	39 (4.25%)	0.32
○ Controlled by diet only	32 (3.42%)	24 (2.61%)	0.38
○ Insulin dependent	19 (2.03%)	15 (1.63%)	0.64
○ Missing	1 (0.11%)	0 (0.00%)	

Maternal treatments during previous 2 weeks to enrollment, n (%)			
• Antibiotics	331 (35.40%)	292 (31.81%)	0.11
• Tocolytics	465 (49.73%)	439 (47.82%)	0.44
○ Betamimetics	266 (28.44%)	256 (27.89%)	0.90
○ Magnesium sulphate	81 (8.66%)	89 (9.69%)	0.33
○ Indomethacin	61 (6.52%)	50 (5.44%)	0.47
○ Calcium channel blocker	150 (16.04%)	135 (14.71%)	0.64
○ Other	98 (10.48%)	106 (11.55%)	0.76
Principal reason(s) for study participation, n (%)			
• Signs or symptoms of preterm labour	773 (82.67%)	777 (84.64%)	0.28
• Fetal anomalies or pathologies	115 (12.30%)	103 (11.22%)	0.51
• Maternal medical condition	199 (21.30%)	190 (20.70%)	0.79
• Multiple pregnancy	191 (20.43%)	179 (19.50%)	0.65
• History of obstetrical complications	287 (30.70%)	280 (30.50%)	0.96
National perinatal mortality rate, n (%)			0.94
• ≤10 in 1000	623 (66.63%)	612 (66.67%)	
• >10–20 in 1000	239 (25.56%)	238 (25.93%)	
• >20 in 1000	73 (7.81%)	68 (7.41%)	
Method of delivery, n (%)			0.46
• Vaginal delivery	396 (42.35%)	415 (45.21%)	
• Caesarean section	537 (57.43%)	501 (54.58%)	
• Missing	2 (0.21%)	2 (0.22%)	
Clinical chorioamnionitis, n (%)	22 (2.35%)	20 (2.18%)	0.92

Legend:

PPROM – Preterm Premature Rupture of Membranes; IUGR – Intrauterine Growth Restriction

Table 2: Perinatal outcomes for infants exposed to multiple courses versus a single course of ACS

Perinatal mortality or clinically significant neonatal morbidity	Multiple courses of ACS, n (%)	Single course of ACS, n (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI), N of infants*	Modified Bonferroni adjusted p-value
Total number of infants	N=1164	N=1140	-	-	-
Composite of perinatal mortality or clinically significant neonatal morbidity	150 (12.89%)	143 (12.54%)	1.04 (0.80, 1.35)	0.99 (0.67, 1.45)[a], N=2292*	1.00
Secondary components of the composite					
Perinatal mortality (i.e., stillbirth or neonatal death)	43 (3.69%)	40 (3.51%)	1.05 (0.67, 1.65)	1.15 (0.61, 2.16)[b], N=2295*	1.00
Surviving infants	N=1121	N=1100	-	-	-
• Severe RDS**	87 (7.76%)	77 (7.00%)	1.16 (0.82, 1.62)	1.05 (0.67, 1.65)[c], N=2211*	1.00
• IVH (grade III or IV)†	6 (0.54%)	9 (0.81%)	0.65 (0.23, 1.83)	N/A	N/A
• cPVL‡	9 (0.80%)	10 (0.91%)	0.87 (0.34, 2.24)	N/A	N/A
• NEC§	10 (0.89%)	12 (1.09%)	0.81 (0.32, 2.07)	N/A	N/A

Other secondary outcomes					
Anthropometry	Multiple courses of ACS, mean (robust SE), N of infants	Single course of ACS, mean (robust SE), N of infants	Unadjusted MD (95% CI)[d]	Adjusted MD (95% CI)[e], N of infants	Modified Bonferroni adjusted p-value
Birthweight (g)	2171.43 (26.41), N=1161	2282.60 (26.56), N=1139	-117.34 (-190.11, -44.56)	-104.82 (-188.50, -21.14), N=2296*	0.06
Length at birth (cm)	44.17 (0.19), N=1077	45.04 (0.18), N=1056	-0.85 (-1.33, -0.36)	**-0.86 (-1.43, -0.29),** N=2129*	**0.02**
Head circumference at birth (cm)	30.91 (0.11), N=1132	31.54 (0.10), N=1097	-0.59 (-0.89, -0.30)	**-0.48 (-0.83, -0.13),** N=2225*	**0.04**

Legend:

ACS– antenatal corticosteroids; OR – odds ratio; CI – confidence interval; RDS – respiratory distress syndrome; IVH – intraventricular haemorrhage; N/A– not applicable/not reported as biologically plausible variables could not be adjusted for due to poor model fit/low event rate; cPVL – cystic periventricular leukomalacia; NEC – necrotizing enterocolitis; SE– standard error; MD – mean difference; **bold** – statistically significant association for adjusted models using modified Bonferroni corrected adjusted $p<0.05$

* Total number of infants analyzed due to missing data for adjusted variables

**Severe RDS was defined as needing assisted ventilation via endotracheal tube and supplemental oxygen both within the first 24 hours of life and for 24 hours or more, and either a radiographic scan compatible with respiratory distress syndrome or surfactant given between the first 2–24 hours of life.

†IVH was diagnosed by cranial ultrasound and categorized based on Papile and colleagues' classification.

‡cPVL was defined as periventricular cystic changes in the white matter, excluding subependymal and choroid plexus cysts, diagnosed by cranial ultrasound.

§NEC was defined as either perforation of intestine, pneumatosis intestinalis, or air in the portal vein, diagnosed by radiographic scan or at surgery

[a] Analyses included an interaction term of treatment status*sex; national perinatal mortality rate; gestational age at randomization; preterm premature rupture of membranes at enrollment; multiple-gestation pregnancy; pre-eclampsia; antenatally suspected intrauterine growth restriction; fetal anomalies; maternal smoking; maternal diabetes; hypertension needing treatment; maternal age; maternal substance abuse; maternal antibiotics prior to 2 weeks of enrollment and time from first dose or pre-study corticosteroids to randomization.

[b] Analyses included an interaction term of treatment status*sex; national perinatal mortality rate; multiple-gestation pregnancy; pre-eclampsia; antenatally suspected intrauterine growth restriction; fetal anomalies; maternal smoking and maternal diabetes.

[c] Analyses included an interaction term of treatment status*sex; gestational age at randomization; multiple-gestation pregnancy; preterm premature rupture of membranes at enrollment; antenatally suspected intrauterine growth restriction; pre-eclampsia; maternal diabetes; maternal smoking; maternal antibiotics prior to 2 weeks of enrollment; hypertension needing treatment; fetal anomalies; maternal substance abuse and time from first dose or pre-study corticosteroids to randomization.

[d] Linear regression models were fitted with generalized estimating equations with exchangeable correlation structure applied to account for the interdependency of multiple-gestation pregnancy.

[e] Linear regression models were fitted with generalized estimating equations with exchangeable correlation structure applied to account for the interdependency of multiple-gestation pregnancy. Furthermore, analyses included an interaction term of treatment status*sex; multiple-gestation pregnancy; fetal anomalies; maternal smoking; preeclampsia; maternal substance abuse; maternal age, maternal hypertension requiring treatment and gestational age at randomization.

Table S1: Exposure and biologically plausible covariates to consider in adjusted models

Perinatal outcomes	**Pre-defined variables to consider within final adjusted models***
Composite of perinatal mortality or clinically significant neonatal morbidity	• ACS exposure variable (when applicable) • Infant sex (when applicable) • Interaction term between treatment status and infant sex (when applicable) • Nationals perinatal mortality rate • Gestational age at randomization, weeks • PPROM at enrollment • Multiple-gestation pregnancy • Pre-eclampsia • Antenatally suspected IUGR • Fetal anomalies • Maternal smoking • Maternal diabetes • Hypertension needing treatment • Maternal age • Maternal substance abuse • Maternal antibiotics prior to 2 weeks of enrollment • Time from first dose of pre-study corticosteroids to randomization (days)
Perinatal mortality (i.e., stillbirth or neonatal death)	• ACS exposure variable (when applicable) • Infant sex (when applicable) • Interaction term between treatment status and infant sex (when applicable) • National perinatal mortality rate • Multiple-gestation pregnancy • Pre-eclampsia • Antenatally suspected IUGR • Fetal anomalies • Maternal smoking • Maternal diabetes • Hypertension needing treatment • Maternal age • Maternal substance abuse

Perinatal outcomes	**Pre-defined variables to consider within final adjusted models***
Severe RDS	• ACS exposure variable (when applicable) • Infant sex (when applicable) • Interaction term between treatment status and infant sex (when applicable) • Gestational age at randomization, weeks • Multiple pregnancy • PPROM at enrollment • Antenatal suspected IUGR • Pre-eclampsia • Maternal diabetes • Smoking • Maternal antibiotics prior to 2 weeks of enrollment • Hypertension needing treatment • Fetal anomalies • Maternal substance abuse • Time from first dose of pre-study corticosteroids to randomization (days)
IVH (grade III or IV)† cPVL† NEC (stage II or III)†	• ACS exposure variable (when applicable) • Infant sex (when applicable) • Interaction term between treatment status and infant sex (when applicable)
Birthweight (g) Length at birth (cm) Head circumference at birth (cm)	• ACS exposure variable (when applicable) • Infant sex (when applicable) • Interaction term between treatment status and infant sex (when applicable) • Multiple pregnancy • Fetal anomalies • Smoking • Pre-eclampsia • Maternal substance abuse • Maternal age • Hypertension needing treatment • Gestational age at randomization

Legend:

ACS – antenatal corticosteroids; RDS – respiratory distress syndrome; IVH – intraventricular haemorrhage; cPVL – cystic periventricular leukomalacia; NEC – necrotizing enterocolitis; PPROM – preterm premature rupture of membranes; IUGR – intrauterine growth restriction

* Final models included as many of these pre-defined biologically plausible variables as feasible while following the five events per variable rule of thumb. For each outcome, variables were organized according to relevance based on clinical expertise and review of the literature.
†Additional biologically plausible variables were not considered due to small numbers of outcome events.

Table S2: Perinatal outcomes for female versus male infants exposed to multiple courses of ACS (a post-hoc analysis)

Perinatal mortality or clinically significant neonatal morbidity	Male infants [reference category], n (%)	Female infants, n (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI), N of infants*	Modified Bonferroni adjusted p-value
Total number of infants	N=616	N=546	-	-	
Composite of perinatal mortality or clinically significant neonatal morbidity	84 (13.64%)	64 (11.72%)	0.82 (0.58, 1.16)	0.86 (0.59, 1.25)[a], N=1155*	0.93
Secondary components of the composite					
Perinatal mortality (i.e., stillbirth or neonatal death)	22 (3.57%)	19 (3.47%)	0.97 (0.52, 1.81)	1.00 (0.53, 1.90)[b], N=1155*	0.99
Surviving infants	N=594	N=527	-	-	
• Severe RDS**	51 (8.59%)	36 (6.83%)	0.75 (0.48, 1.16)	0.79 (0.49, 1.26)[c], N=1114*	0.94
• IVH (grade III or IV)†	5 (0.84%)	1 (0.19%)	0.22 (0.03, 1.92)	N/A	N/A
• cPVL‡	7 (1.18%)	2 (0.38%)	0.32 (0.06, 1.66)	N/A	N/A
• NEC§	5 (0.84%)	5 (0.95%)	1.36 (0.37, 5.02)	N/A	N/A
Other secondary outcomes					
Anthropometry	Male infants, mean (robust SE), N of infants	Female infants, mean (robust SE), N of infants	Unadjusted MD (95% CI)[d]	Adjusted MD (95% CI)[e]	Modified Bonferroni adjusted p-value
Birthweight (g)	2191.76 (35.03), N=616	2148.46 (36.96), N=545	-41.74 (-115.22, 31.74)	N/A	N/A
Length at birth (cm)	44.40 (0.24), N=568	43.91 (0.26), N=509	-0.39 (-0.91, 0.13)	N/A	N/A
Head circumference at birth (cm)	31.10 (0.14), N=598	30.69 (0.16), N=534	-0.37 (-0.69, -0.05)	N/A	N/A

Legend:

ACS– antenatal corticosteroids; OR – odds ratio; CI – confidence interval; RDS – respiratory distress syndrome; IVH – intraventricular haemorrhage; N/A– not applicable/not reported, as biologically plausible variables could not be adjusted for due to poor model fit/low event rate; cPVL – cystic periventricular leukomalacia; NEC – necrotizing enterocolitis; SE– standard error; MD – mean difference; **bold** – statistically significant association for adjusted models using modified Bonferroni corrected adjusted $p<0.05$

* Total number of infants analyzed due to missing data for adjusted variables
**Severe RDS was defined as needing assisted ventilation via endotracheal tube and supplemental oxygen both within the first 24 hours of life and for 24 hours or more, and either a radiographic scan compatible with respiratory distress syndrome or surfactant given between the first 2–24 hours of life
†IVH was diagnosed by cranial ultrasound and categorized based on Papile and colleagues' classification
‡cPVL was defined as periventricular cystic changes in the white matter, excluding subependymal and choroid plexus cysts, diagnosed by cranial ultrasound
§NEC was defined as either perforation of intestine, pneumatosis intestinalis, or air in the portal vein, diagnosed by radiographic scan or at surgery

[a] Analyses included national perinatal mortality rate; gestational age at randomization; preterm premature rupture of membranes at enrollment; multiple-gestation pregnancy; pre-eclampsia; antenatally suspected intrauterine growth restriction; fetal anomalies; maternal smoking; maternal diabetes; hypertension needing treatment; maternal age; maternal substance abuse; maternal antibiotics prior to 2 weeks of enrollment and time from first dose or pre-study corticosteroids to randomization.

[b] Analyses included national perinatal mortality rate; multiple-gestation pregnancy; pre-eclampsia; antenatally suspected intrauterine growth restriction; fetal anomalies; maternal smoking and maternal diabetes.

[c] Analyses included gestational age at randomization; multiple-gestation pregnancy; preterm premature rupture of membranes at enrollment; antenatally suspected intrauterine growth restriction; pre-eclampsia; maternal diabetes; maternal smoking; maternal antibiotics prior to 2 weeks of enrollment prior to 2 weeks of enrollment; hypertension needing treatment; fetal anomalies; maternal substance abuse and time from first dose or pre-study corticosteroids to randomization.

[d] Linear regression models were fitted with generalized estimating equations with exchangeable correlation structure applied to account for the interdependency of multiple-gestation pregnancy.

[e] Linear regression models were fitted with generalized estimating equations with exchangeable correlation structure applied to account for the interdependency of multiple-gestation pregnancy. Furthermore, models were adjusted for multiple-gestation pregnancy; fetal anomalies; maternal smoking; preeclampsia; maternal substance abuse; maternal age, maternal hypertension requiring treatment and gestational age at randomization.

Table S3: Perinatal outcomes for female versus male infants exposed to a single course of ACS (a post-hoc analysis)

Perinatal mortality or clinically significant neonatal morbidity	Male infants [reference category], n (%)	Female infants, n (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI), N of infants*	Modified Bonferroni adjusted p-value
Total number of infants	n=598	n=540	-	-	
Composite of perinatal mortality or clinically significant neonatal morbidity	78 (13.04%)	65 (12.04%)	0.90 (0.62, 1.31)	0.90 (0.59, 1.38)[a], N=1137*	1.00
Secondary components of the composite outcome					
Perinatal mortality (i.e., stillbirth or neonatal death)	19 (3.18%)	21 (3.89%)	1.27 (0.67, 2.40)	1.13 (0.58, 2.21)[b], N=1137*	1.00
Surviving infants	n=579	n=519	-	-	
• Severe RDS**	46 (7.94%)	31 (5.97%)	0.70 (0.42, 1.16)	0.72 (0.42, 1.22)[c], N=1097*	0.67
• IVH (grade III or IV)†	7 (1.21%)	2 (0.39)%	0.32 (0.07, 1.54)	N/A	N/A
• cPVL‡	3 (0.52%)	7 (1.35%)	2.63 (0.67, 10.22)	N/A	N/A
• NEC§	6 (1.04%)	6 (1.16%)	1.12 (0.51, 2.47)	N/A	N/A
Other secondary outcomes					
Anthropometry	Male infants, mean (robust SE), N of infants	Female infants, mean (robust SE), N of infants	Unadjusted MD (95% CI)[d]	Adjusted MD (95% CI)[e]	Modified Bonferroni adjusted p-value
Birthweight (g)	2312.18 (34.43), N=598	2248.48 (37.28), N=540	-55.70 (-121.39, 9.99)	N/A	N/A
Length at birth (cm)	45.39 (0.23), N=556	44.64 (0.25), N=499	-0.57 (-1.05, -0.09)	N/A	N/A
Head circumference at birth (cm)	31.64 (0.14), N=575	31.42 (0.14) N=521	-0.25 (-0.57, 0.07)	N/A	N/A

Legend:

ACS– antenatal corticosteroids; OR – odds ratio; CI – confidence interval; RDS – respiratory distress syndrome; IVH – intraventricular haemorrhage; N/A– not applicable/not reported as biologically plausible variables could not be adjusted for due to poor model fit/low event rate; cPVL – cystic periventricular leukomalacia; NEC – necrotizing enterocolitis; SE– standard error; MD – mean difference; **bold** – statistically significant association for adjusted models using modified Bonferroni corrected adjusted $p<0.05$

* Total number of infants analyzed due to missing data for adjusted variables
**Severe RDS was defined as needing assisted ventilation via endotracheal tube and supple mental oxygen both within the first 24 hours of life and for 24 hours or more, and either a radiographic scan compatible with respiratory distress syndrome or surfactant given between the first 2–24 hours of life
†IVH was diagnosed by cranial ultrasound and categorized based on Papile and colleagues' classification
‡cPVL was defined as periventricular cystic changes in the white matter, excluding subependymal and choroid plexus cysts, diagnosed by cranial ultrasound
§NEC was defined as either perforation of intestine, pneumatosis intestinalis, or air in the portal vein, diagnosed by radiographic scan or at surgery

[a] Analyses included national perinatal mortality rate; gestational age at randomization; preterm premature rupture of membranes at enrollment; multiple-gestation pregnancy; pre-eclampsia; antenatally suspected intrauterine growth restriction; fetal anomalies; maternal smoking; maternal diabetes; hypertension needing treatment; maternal age; maternal substance abuse; maternal antibiotics prior to 2 weeks of enrollment and time from first dose or pre-study corticosteroids to randomization.

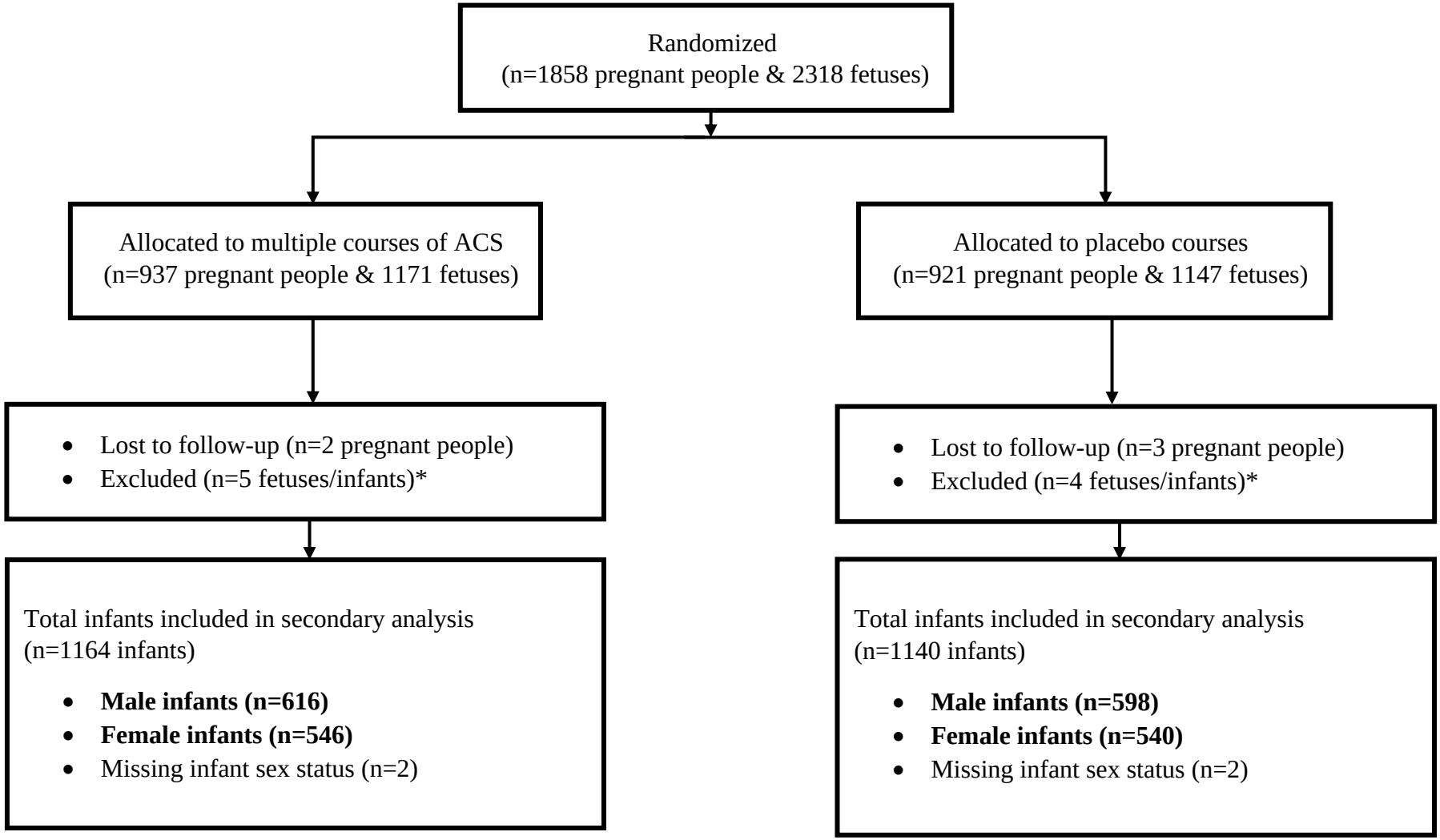
[b] Analyses included national perinatal mortality rate; multiple-gestation pregnancy; pre-eclampsia; antenatally suspected intrauterine growth restriction; fetal anomalies; maternal smoking; maternal diabetes.

[c] Analyses included gestational age at randomization; multiple-gestation pregnancy; preterm premature rupture of membranes at enrollment; antenatally suspected intrauterine growth restriction; pre-eclampsia; maternal diabetes; maternal smoking; maternal antibiotics prior to 2 weeks of enrollment; hypertension needing treatment; fetal anomalies; maternal substance abuse and time from first dose or pre-study corticosteroids to randomization.

[d] Linear regression models were fitted with generalized estimating equations with exchangeable correlation structure applied to account for the interdependency of multiple-gestation pregnancy.

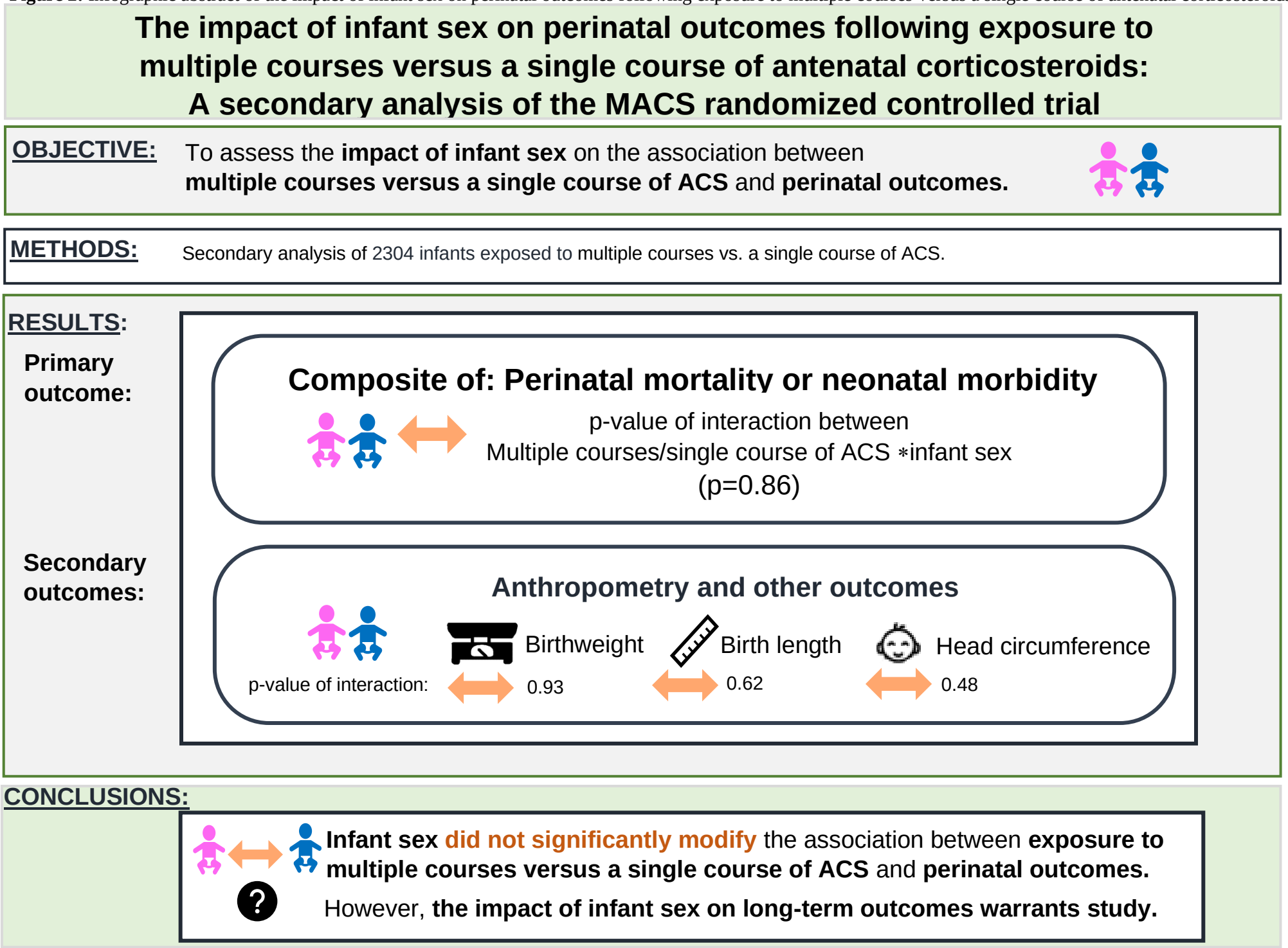
[e] Linear regression models were fitted with generalized estimating equations with exchangeable correlation structure applied to account for the interdependency of multiple-gestation pregnancy. Furthermore, models were adjusted for multiple-gestation pregnancy; fetal anomalies; maternal smoking; preeclampsia; maternal substance abuse; maternal age, maternal hypertension requiring treatment and gestational age at randomization.

Figure 1: Participants included in the secondary analysis of the MACS trial



Legend: *stillbirths in multiple-gestation pregnancy that took place before randomization

Figure 2: Infographic abstract of the impact of infant sex on perinatal outcomes following exposure to multiple courses versus a single course of antenatal corticosteroids



Legend: ACS– antenatal corticosteroids; ⟷ – non-significant outcome