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Complete List of Authors:	Mwita, Stanley; Catholic University of Health and Allied Sciences Kamala, Benjamin; Haydom Lutheran Hospital; Muhimbili University of Health and Allied Sciences, Epidemiology and Biostatistics Konje, Eveline; Catholic University of Health and Allied Sciences Ambrose, Emmanuela; Bugando Medical Centre, Peadiatrics and Child Health Izina, Angelina; Bugando Medical Centre, Department of Radiology Chibwe, Elieza; Catholic University of Health and Allied Sciences, Department of Obstetrics and Gynaecology Kongola, Gilbert; Catholic University of Health and Allied Sciences, Department of Pharmacology Dewey, Deborah ; The University of Calgary , Paediatrics and Community Health Sciences
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The effect of antenatal corticosteroids therapy on perinatal outcomes in preterm singleton and twin pregnancies: A prospective chart review study

Stanley Mwita[1*], Benjamin Kamala[2,3], Eveline Konje[4], Emmanuela Ambrose[5], Angelina Izina[6], Elieza Chibwe[7,] Gilbert Kongola[8], Deborah Dewey[9]

Affiliations

1. School of Pharmacy, Catholic University of Health and Allied Sciences, Mwanza, Tanzania
2. Department of Epidemiology and Biostatistics, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania
3. Department of Research, Haydom Lutheran Hospital, Haydom, Manyara, Tanzania
4. School of Public Health, Catholic University of Health and Allied Sciences, Mwanza, Tanzania
5. Department of Pediatrics, Bugando Medical Centre, Mwanza, Tanzania
6. Department of Radiology, Bugando Medical Centre, Mwanza, Tanzania
7. Department of Obstetrics and Gynaecology, Catholic University of Health and Allied Sciences, Mwanza, Tanzania
8. Department of Pharmacology, Catholic University of Health and Allied Sciences, Mwanza, Tanzania
9. Owerko Centre at the Alberta Children's Hospital Research Institute and Departments of Pediatrics and Community Health Sciences, University of Calgary, Calgary, Canada

***Corresponding author**: Stanley Mwita, P.O.Box 1464, Mwanza-Tanzania

Email: stanleymwita@gmail.com (SM)

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ABSTRACT

Objectives This study aimed to examine the effects of antenatal corticosteroids **(**ACS) treatment in singleton and twin pregnancies delivered before 35 weeks' gestation on adverse perinatal outcomes.

Design A hospital-based prospective chart review study.

Setting This study was conducted in four hospitals located in Mwanza region, Tanzania.

Participants The study population included all singletons and twins delivered between 24 weeks 0 days and 34 weeks 6 days of gestation from July 2019 to February 2020.

Outcome measures The primary outcome was perinatal mortality. Secondary outcomes were, stillbirth, early neonatal mortality, Apgar score <7 at 5 minutes, neonatal sepsis and respiratory distress syndrome (RDS).

Results Of 949 pregnancies, 844 (88.9%) were singletons and 105 (11.1%) were twins (210 twin infants). Three hundred and fourteen singleton (37.2%) and 26 twin (24.8%) pregnancies received at least one dose of ACS. In multivariate analysis, administration of ACS was not associated with reduced risk of any adverse perinatal outcome in twin infants. However, in singletons ACS were significantly associated with a lower risk of perinatal mortality, aRR 0.30 (95%CI 0.21 - 0.42), stillbirth, aRR 0.07 (95%CI 0.03 - 0.16), early neonatal mortality, aRR 0.42 (95%CI 0.25 - 0.69), APGAR scores of < 7 at 5-minute, aRR 0.18 (95%CI 0.12 - 0.29), neonatal sepsis, aRR 0.53 (95%CI 0.34 - 0.82) and RDS, aRR 0.59 (95%CI 0.41- 0. 85).

Conclusion The use of ACS in both singleton and twin pregnancies between 24 weeks 0 days and 34 weeks 6 days of gestation in low resource settings is associated with positive infant outcomes, particularly in singletons, and no adverse effects were noted. Further research that examines the effect of ACS in twin pregnancies is needed.

Strengths and limitations of this study

- This is the first study in Tanzania to evaluate the benefit of ACS in both singleton and twin pregnancies.
- Several confounders were controlled in the multivariable analyses, thus we had unbiased estimate of the true association between ACS exposure and perinatal outcomes.
- Since the study design was observational, causality cannot be inferred.
- Data on the potential confounding factor of chorionicity in twin pregnancies, was not available.
- The number of twins in our sample was small, which may have limited our ability to detect statistically significant results in all of perinatal outcomes in multivariate analysis.

INTRODUCTION

Globally, preterm birth is one of the leading causes of perinatal mortality and morbidity. [1] Women with a twin pregnancy are at higher risk of preterm birth [2] with approximately 60% of twins delivered prematurely. [3] Perinatal mortality includes stillbirth and neonatal death during the first week of life and the overall rate of perinatal mortality is higher in twins than singletons. Findings from a study conducted in Tanzania revealed that the perinatal mortality rate among twins compared to singletons was 91.0 versus 41.1 per 1000 births, (RR = 2.2; 95%CI: 1.7-2.8).[4] Respiratory distress syndrome (RDS), a serious complication of preterm birth, is a significant contributor to perinatal mortality among both singletons and twins. [5]

To reduce perinatal mortality and RDS, interventions need to focus on improving access to high quality antenatal and postnatal care. Antenatal corticosteroids (ACS) are considered a key intervention for moderating the adverse effects of a preterm birth. [6] A single course of ACS is recommended in pregnancies between 24 weeks and 34 weeks of gestation that are at risk of preterm delivery within 7 days. [7] Some research has suggested that the positive effect of ACS exposures in twins is similar to that observed in singletons. [8,9] In contrast, other studies that have investigated the efficacy of ACS in twin pregnancies have not reported similar beneficial effects among twins and singletons. [10,11]

A prospective analysis of data from a registry of preterm multiple-births between 22 to 28 weeks gestational age at 28 National Institute of Child Health and Human Development Neonatal Research Network Centers in United States between January 1998 and December 2013, reported lower in-hospital mortality among infants exposed to ACS compared to infants who were not

exposed (aRR = 0.87; 95% CI, 0.78-0.96), higher-order multiples had a greater reduction in mortality risk than twins with exposure to ACS (aRR = 0.66; 95% CI, 0.53-0.82 vs aRR = 0.90; 95% CI, 0.81-1.01). [8] Further, a retrospective cohort study using data collected on singleton and twin neonates admitted to tertiary neonatal units in Canada who were born between 24 weeks 0 days and 33 weeks 6 days gestational age reported that administration of ACS 1-7 days before birth in twin pregnancies was associated with a clinically significant decrease in neonatal mortality and RDS that was similar in magnitude to that observed among singletons. [9] A retrospective cohort study conducted in China also reported that ACS administration was associated with a reduced risk of RDS in twin preterm infants; however, it did not find a beneficial effect related to neonatal mortality. [11] In contrast, a retrospective medical record review study conducted in Korea did not find a beneficial effect of ACS administration on RDS in twins. [10] This study did not report on neonatal mortality.

A recent Cochrane Review suggested that further research is needed to support the use of ACS in twin pregnancies as there is not enough evidence to support the use of ACS in this group, particularly in low resource countries [12]. To address these issues, we examined the effect of ACS exposure in singleton and twin pregnancies delivered before 35 weeks' gestation on the risk of adverse perinatal outcomes, i.e., perinatal mortality, stillbirth, early neonatal mortality, Apgar score <7 at 5 minutes, neonatal sepsis and RDS, in Tanzania, a low resource country.

MATERIALS AND METHODS

Study design

This was a hospital-based prospective chart review study conducted in four selected hospitals located in Nyamagana and Sengerema districts, which are two of the seven districts of the Mwanza region, northwest Tanzania. The hospital that participated were Bugando Medical Centre (a tertiary consultant zonal referral hospital), Sekou Toure Regional Referral Hospital, Nyamagana District Hospital and Sengerema District Designated Hospital. These hospitals provide obstetric and neonatal care services to a large proportion of the population within the Lake zone in Tanzania.

The study population comprised all singletons and twins delivered between 24 weeks 0 days and 34 weeks 6 days of gestation between July 2019 to February 2020. Data were collected from when pregnant women who were expected to deliver preterm were admitted to hospital to seven days after admission or discharge, whichever came first. Women with one of the following indications for preterm birth were included: antepartum haemorrhage, pre-eclampsia or eclampsia, premature preterm rupture of membrane and preterm labor. Infants born with congenital malformations were excluded. Gestational age was determined based on women's self-reports of their last normal menstrual period, fundal height and/or ultrasound.

The primary predictor variable was ACS (dexamethasone) exposure. The recommended administration guideline for ACS is 4 doses of 6 mg of dexamethasone every 12 hours. [13] We included only women who delivered within 7 days of their first ACS dose. Study participants were classified into two groups, the No-ACS group or the ACS group (women administered at least one does of ACS). The primary outcome was perinatal mortality, which was defined as was defined as stillbirth or early neonatal mortality (i.e., death of a live born neonate between zero and seven days

after birth). Secondary outcomes included stillbirth, early neonatal mortality, Apgar score <7 at 5 minutes, neonatal sepsis and RDS.

The medical records of the women and their infants were reviewed by the principal investigator and two trained research assistants who were enrolled/registered nurses working in the labor wards and neonatal units of each of the hospitals. From each record, we recorded whether or not the woman was exposed or not exposed to ACS and perinatal outcomes. In addition, the following data was obtained from the women's and infant's medical records: parity, marital status, maternal age, maternal education, antenatal care visits (days), gestational age (weeks), mode of deliver, indication for delivery, level of health facility, birthweight (grams), neonate sex, neonatal antibiotic use and fetal heart rate at delivery. Information on the specific neonatal antibiotics used (i.e., Ampicillin, 50 mg/kg every 12 hours; Gentamicin, 4 mg/kg every 24 hours) was also recorded.

Statistical analyses

The data were analyzed using STATA Version 13. Chi-square tests or Fisher exact tests as appropriate, were conducted on the following variables to investigate differences between the No-ACS and ACS groups in singleton and twin pregnancies: parity, marital status, education, antenatal care visits, mode of delivery, indication for preterm delivery and level of health facility. T-tests were used to determine if there were differences in mean maternal age, mean gestational age and birthweight. Differences between the No-ACS and ACS groups in singleton and twin pregnancies were examined using cross-tabulation and chi-square tests for perinatal mortality and the

secondary outcomes (i.e., stillbirth, early neonatal mortality, APGAR score of < 7 at 5 mins, neonatal sepsis and RDS).

Modified Poisson regressions were used to investigate the associations between ACS exposure and perinatal outcomes. To account for the clustering effect and non-independence of twins we used a mixed model's approach. These models were fitted with generalized estimating equations (GEE) to account for associations within a pair of twins from the same mother. Multivariate regression analyses were performed to examine the effects of administration of ACS on perinatal outcomes, controlling for factors with significant associations and those for which there is scientific evidence to consider them as potential confounders, i.e., maternal age, maternal education, antenatal care visit days, gestational age, indication for preterm delivery, level of health facility, birth weight, parity, delivery mode and neonate sex. [9] P-values of less than 0.05 were considered statistically significant. Data are presented as frequencies (percentages), means (standard deviations) and relative risks (RR) with 95% confidence intervals as appropriate.

Patient and public involvement

No patient or member of the public were involved in the design of this study.

RESULTS

Of 949 pregnancies, 844 (88.9%) were singletons and 105 (11.1%) were twins (210 twin infants). Three hundred and fourteen singleton (37.2%) and 26 twin (24.8%) pregnancies received at least one dose of ACS.

In singletons, no significant differences in marital status or mean maternal age were found between women who received ACS and those who did not. However, the groups differ on the following

variables: parity, maternal education, antenatal care visits, gestational age, mode of delivery, indication for delivery and level of health facility where they delivered. Compared to the No-ACS group, a higher proportion of women with singleton pregnancies who received ACS, were nullipara, had a secondary education, made more than 3 visits to antenatal care clinics, had pre-eclampsia or eclampsia, and had a caesarean section delivery. Also, these women had a higher mean gestational age and the majority delivered at the tertiary zonal referral hospital. In the ACS and No-ACS groups, singleton infants had similar birthweights, fetal heart rates at delivery, sex distributions and rates of being prescribed neonatal antibiotics (Table 1).

In twins, no significant differences in parity, mean maternal age, antenatal care visits or indication for delivery were found between women who received ACS and those who did not. However, the groups differed on the following variables: marital status, maternal education, gestational age, mode of delivery and level of health facility where they delivered. Compared to the No-ACS group, a lower proportion of women with twin pregnancies who received ACS were married, and a higher proportion had a secondary education. Also, women who received ACS had a higher mean gestational age, and the majority delivered by caesarean section and at the tertiary zonal referral hospital. There were no differences between the ACS and No-ACS groups in infant sex or fetal heart rate at delivery. Also, the ACS and No-ACS groups had similar rates of being prescribed neonatal antibiotics; however, the birthweights of infants in the ACS group were higher (1926.9±341.2) than those in the No-ACS group (1549.3±443.5) (Table 1).

Table 1: Maternal and infant baseline characteristics of the ACS and No-ACS groups among singletons and twins

	Singletons			Twins		
	ACS (n=314)	**No-ACS (n=530)**	**P-value**	**ACS (n=26)**	**No-ACS (n=79)**	**P-value**
Maternal	**M (SD)/N (%)**	**M (SD)/N (%)**		**M (SD)/N (%)**	**M (SD)/N (%)**	
Nulliparity	119 (37.9)	155 (29.2)	**0.009**	6 (23.1)	25 (31.6)	0.240
Married	272 (86.6)	447 (84.3)	0.366	24 (92.3)	78 (98.7)	**0.016**
Mean Maternal age, years	26.7±5.9	26.5±6.5	0.704	27.3±5.0	27.2±5.5	0.914
Education						
College and above	51 (16.2)	43 (8.1)	**<0.001**	3 (11.5)	4 (5.1)	**0.010**
Secondary education	156 (49.7)	167 (31.5)		12 (46.2)	22 (27.8)	
Primary education	94 (29.9)	276 (52.1)		9 (34.6)	46 (58.2)	
No formal education	13 (4.2)	44 (8.3)		2 (7.7)	7 (8.9)	
More than 3 Antenatal care visits†	152 (47.2)	198 (32.8)	**<0.001**	16 (61.5)	39 (50.6)	0.174
Mean gestational age (weeks)	31.9 ±2.3	31.1±2.5	**<0.001**	32.9±1.5	32.2±2.2	**0.045**
Mode of delivery						
Assisted vaginal	9 (2.9)	10 (1.9)	**<0.001**	3 (11.5)	6 (7.6)	**<0.001**
C- section	134 (42.7)	97 (18.3)		14 (53.9)	15 (19.0)	
Normal vaginal	171 (54.4)	423 (79.8)		9 (34.6)	58 (73.4)	
Indication for delivery						
Antepartum haemorrhage	42 (13.4)	66 (12.4)	**0.017**	3 (11.5)	5 (6.3)	0.272
Pre-eclampsia or Eclampsia	70 (22.3)	84 (15.8)		2 (7.7)	11 (13.9)	
Premature preterm rupture of membrane	45 (14.3)	59 (11.2)		2 (7.7)	7 (8.9)	
Preterm labor	157 (50.0)	321 (60.6)		19 (73.1)	56 (70.9)	

Level of health facility						
Tertiary zonal hospital	228 (72.6)	110 (20.8)	**<0.001**	15 (57.7)	17 (21.5)	**<0.001**
Regional hospital	75 (23.9)	226 (42.6)		9 (34.6)	15 (19.0)	
District hospital	11 (3.5)	194 (36.6)		2 (7.7)	47 (59.5)	
Infants‡						
Birthweight (g)	1980.0±515.5	2021.9± 605.7	0.305	1926.9±341.2	1549.3±443.5	**<0.001**
Sex (male)	170 (54.1)	277 (52.3)	0.598	21 (40.38)	77 (48.73)	0.295
Normal fetal heart rate at delivery	264 (84.1)	414 (78.1)	0.182	41 (78.8)	133 (84.2)	0.133
Received neonatal* antibiotics	201 (65.3)	265 (62.8)	0.494	29 (58.0)	84 (59.6)	0.846

†Denominator included only those who attended antenatal care, singleton (ACS 307 vs No ACS 494) and twins (ACS 26 vs No ACS 77).

‡ Number of twin infants is two times number of twin pregnancies (ACS 52 vs No ACS 158)

*Denominator included live infants only, singleton (ACS 308 vs No ACS 422) and twins (ACS 50 vs No ACS 141).

Perinatal outcomes for the ACS and No-ACS groups among singletons and twins

Among singleton births, those who were exposed to ACS in utero had a lower rate of perinatal mortality (13.4% vs 28.5%), stillbirth (1.9% versus 20.6%), APGAR scores < 7 at 5 minute (7.0% vs 27.2%), neonatal sepsis (10.1% vs 17.3%) and RDS (18.8% vs 25.8%) compared to those not exposed to ACS. However, early neonatal mortality was similar between exposed (11.7%) and unexposed (10.0%) infants (Table 2).

In twins, those who were exposed to ACS in utero had significantly lower rates of APGAR scores < 7 at 5 minute (5.8% vs 21.5%) and RDS (12.0% vs 28.4%) compared to unexposed infants.

However, the two groups did not differ significantly in terms of rates of perinatal mortality (15.4% vs 27.2%), stillbirth (3.9% vs 10.8%), early neonatal mortality (12.0% vs 18.4%) and neonatal sepsis (8.0% vs 27.8%) (Table 2).

Table 2. Perinatal outcomes for the ACS and No-ACS groups among singletons and twins

	Singletons			**Twins**		
	ACS (n=314)	**No-ACS (n=530)**	**P-value**	**ACS (n=52)**	**No-ACS (n=158)**	**P-value**
Outcomes	**N (%)**	**N (%)**		**N (%)**	**N (%)**	
Perinatal Mortality	42 (13.4)	151 (28.5)	**<0.001**	8 (15.4)	43 (27.2)	0.059
Stillbirth	6 (1.9)	109 (20.6)	**<0.001**	2 (3.9)	17 (10.8)	0.104
Early Neonatal Mortality	36 (11.7)	42 (10.0)	0.460	6 (12.0)	26 (18.4)	0.206
Apgar <7 at 5min (0-6)	22 (7.0)	144 (27.2)	**<0.001**	3 (5.8)	34 (21.5)	**0.005**
Neonatal sepsis	31 (10.1)	73 (17.3)	**0.006**	4 (8.0)	18 (27.8)	0.265
Respiratory distress syndrome *	58 (18.8)	109 (25.8)	**0.026**	6 (12)	40 (28.4)	**0.013**

*Denominator included live infants only, singletons (ACS 308 vs No-ACS 422) and twins (ACS 50 vs No-ACS 141).

Univariate and multivariate analyses of the association between ACS exposure and perinatal outcomes among singletons and twins

Unadjusted estimates of perinatal outcomes are presented in Table 3. Using newborns whose mothers who did not receive ACS as a reference, singletons born to women who received ACS

had lower risk of perinatal mortality, RR 0.47 (95%CI 0.34-0.64), stillbirth, RR 0.12 (95%CI 0.06-0.24), APGAR scores of < 7 at 5-minute, RR 0.26 (95%CI 0.18-0.39), neonatal sepsis, RR 0.60 (95%CI 0.42-0.87) and RDS, RR 0.67 (95%CI 0.52-0.88). However, the ACS and No-ACS groups had a similar risk of early neonatal mortality, RR 0.97 (95%CI 0.67-1.39). Twin infants in both groups (ACS and No-ACS) had similar risks of perinatal mortality, RR 0.56 (95%CI 0.27 - 1.18), stillbirth, RR 0.36 (95%CI 0.09- 1.48), early neonatal mortality, RR 0.64 (95%CI 0.25- 1.60), neonatal sepsis RR 0.57 (95%CI 0.16 - 2.00), and RDS, RR 0.43 (95%CI 0.17 -1.02). Twins in the ACS group were less likely to have APGAR scores < 7 at 5-minute, RR 0.27 (95%CI 0.09 -0.82) compared to twins the No-ACS group (Table 3).

Adjusted multivariate analyses revealed that exposure to ACS was significantly associated with a lower likelihood of all perinatal outcomes in singletons: perinatal mortality, aRR 0.30 (95%CI 0.21 - 0.42), stillbirth, aRR 0.07 (95%CI 0.03 - 0.16), early neonatal mortality, aRR 0.42 (95%CI 0.25 - 0.69), APGAR scores of < 7 at 5-minute, aRR 0.18 (95%CI 0.12 - 0.29), diagnoses of neonatal sepsis, aRR 0.53 (95%CI 0.34 - 0.82) and RDS, aRR 0.59 (95%CI 0.41- 0. 85). However, in twin infants, exposure to ACS was not associated with a reduced risk of any perinatal outcomes: perinatal mortality, aRR 0.93 (95%CI 0.45 - 1.92), stillbirth, aRR 0.49 (95%CI 0.09 - 2.72), early neonatal mortality, aRR 1.05 (95%CI 0.44 - 2.50), APGAR scores of < 7 at 5-minute, aRR 0.62 (95%CI 0.20 - 1.89), diagnoses of neonatal sepsis, aRR 0.78 (95%CI 0.28 - 2.20) and RDS, aRR 0.68 (95%CI 0.26 - 1.74) (Table 3).

Table 3. Univariate and multivariate analysis of the associations between ACS exposure and perinatal outcomes among singletons and twins

	Singletons		Twins	
Outcomes	**Crude relative risk (95%CI)**	***Adjusted relative risk (95%CI)**	**Crude relative risk (95%CI)**	***Adjusted relative risk (95%CI)**
Perinatal mortality	0.47 (0.34-0.64)	0.30 (0.21 - 0.42)	0.56 (0.27 - 1.18)	0.93 (0.45 - 1.92)
Stillbirth	0.12 (0.06-0.24)	0.07 (0.03 - 0.16)	0.36 (0.09- 1.48)	0.49 (0.09 - 2.72)
Early Neonatal Mortality	0.97 (0.67-1.39)	0.42 (0.25 - 0.69)	0.64 (0.25- 1.60)	1.05 (0.44 - 2.50)
Apgar < 7 at 5min (0-6)	0.26 (0.18-0.39)	0.18 (0.12 - 0.29)	0.27 (0.09 -0.82)	0.62 (0.20 - 1.89)
Neonatal sepsis	0.60 (0.42-0.87)	0.53 (0.34 - 0.82)	0.57 (0.16 - 2.00)	0.78 (0.28 - 2.20)
Respiratory distress syndrome	0.67 (0.52-0.88)	0.59 (0.41- 0. 85)	0.43 (0.17 -1.02)	0.68 (0.26 - 1.74)

*Model adjusted for maternal age, maternal education, antenatal care visit days, gestational age, indication for preterm delivery, level of health facility, birth weight, parity, delivery mode and neonate sex.

DISCUSSION

ACS are important component in the management of women at risk of preterm delivery as they they stimulate fetal lung maturation and alveolar surfactant production. [14,15] Our study evaluated the effect of ACS administration on adverse perinatal outcomes in singleton and twin pregnancies. We found a significant reduction in the risk adverse perinatal outcomes including perinatal mortality, stillbirth, early neonatal mortality, APGAR scores of < 7 at 5-minute, diagnoses of neonatal sepsis and RDS for singleton infants exposed to at least one dose of ACS; however, exposure to ACS was not associated with a reduction in these adverse perinatal outcomes in twin pregnancies.

Previous studies have reported limited benefits of ACS in reducing rates of mortality and morbidities in twins. [10,16–18] However, these studies are limited by their retrospective design, which leaves them prone to information bias and in the case of some, their small sample size. [10,17] In contrast, several retrospective studies with large samples [9,19] and one with a small sample of twins [20], have reported that the administration of ACS has a protective effect against adverse perinatal outcomes in both singletons and twins born preterm. A study of 1662 twins delivered between 25 weeks 0 days and 34 weeks 6 days of gestation in China from January 2013 to December 2014, reported that administration of at least one dose of ACS was associated with a reduced risk of RDS in twin preterm infants. [11] Also, in a nationwide observational multicentre prospective cohort study of twins born between 22 and 34 completed weeks of gestation in 2011 in France, ACS administered ≤7 days prior to delivery was reported to be significantly associated with a reduced rate of in-hospital mortality. [21]

ACS are used infrequently in low resource countries, although their use is recommended by the World Health Organization (WHO) as essential for reducing infant mortality and morbidities. Currently, the WHO recommends the use of ACS only when gestational age is known, there is no clinical evidence of maternal infection, preterm delivery is imminent, and the delivery is in a facility that can provide adequate care for the mother and the infant. [22] Despite the benefits of ACS in reducing the risk of perinatal adverse perinatal outcomes, particularly in singletons, in the present study less than 40% of singleton pregnancies were administered at least one dose of ACS. One of the main reasons given by health professionals in these setting for not administrating ACS was that women arrived late at the hospital in well-established labor.[23] Another potential impediment to administering ACS in low-resource settings could be difficulty in obtaining an

accurate estimate of gestational age. Before the full positive effects of ACS can be realized in low-resource settings, women and health care professionals need to be educated on the clinical signs associated with preterm delivery, risk factors for preterm delivery and the importance of arriving at the hospital early if preterm delivery is anticipated. In addition, infrastructure in health care facilities that can accurately estimate gestational age and provide adequate care for mothers and their preterm infants is essential. [24]

There is a scarcity of randomized clinical trials that have enrolled women with multiples.[25,26] For example, the landmark study by Liggins and Howie that showed that ACS significantly reduced rates of RDS and neonatal mortality, included only 11 pairs of twins.[27] In medical practice, a similar dose of ACS (i.e., 2 doses of 12 mg of betamethasone every 24 hours or 4 doses of 6 mg of dexamethasone every 12 hours) is given regardless of pregnancy multiplicity. [28] It is possible that this dosing level might not be adequate to produce a therapeutic level for lung maturation in twin pregnancies.[10] Pharmacokinetic data suggest that higher doses of ACS are needed in twin gestations to decrease perinatal morbidities and mortality. Pharmacologically, ACS have been proven to have a higher volume of distribution, shorter half-life and greater clearance in twins compared to singleton pregnancies, which could result in a subtherapeutic dosage for lung maturation. [29–31] Randomized controlled trials need to be conducted to evaluate and develop recommendations regarding the dose levels of ACS that could be effective in improving perinatal outcomes in twin- and higher-level multiple pregnancies. Future studies in low resource settings should also focus on examining associations between ACS therapy and other neonatal morbidities such as bronchopulmonary dysplasia, intraventricular hemorrhage, necrotizing enterocolitis,

retinopathy of prematurity, patent ductus arteriosus, need for mechanical ventilation and neonatal hypoglycemia in both singleton and twin pregnancies.

To the best of our knowledge this is the first study in Tanzania to evaluate the benefit of ACS in both singleton and twin pregnancies in low resource settings. The strengths of our study include the use of prospective study design and controlling for several confounders in the multivariable analyses, thus we had unbiased estimate of the true association between ACS exposure and perinatal outcomes. However, there are some limitations. First, it was a prospective observational study. Thus, the inherent biases associated with observational studies (e.g., selection bias, information bias, confounding) could have influenced our outcomes. Second, data on the potential confounding factor of chorionicity in twin pregnancies, was not available. A third limitation was that the number of twins in our sample was small, which may have limited our ability to detect statistically significant results in all of perinatal outcomes in multivariate analysis. Future studies in low resource settings with larger samples of twins are needed that investigate the associations between ACS exposure and perinatal outcomes. Lastly, the gestational age of some of the infants included in this study was based on the women's self-reported last menstrual period and was not verified by ultrasonographic examination. Thus, some reported gestational ages of study participants may have been incorrect. However, the use of women's self-reported last menstrual period is a common practice in low resource setting and provides valid estimate of gestational age.[32]

CONCLUSION

Our findings add new and important information to the literature on the benefits of ACS in singleton and twin pregnancies in low resource settings. They support the use of ACS in both singleton and twin pregnancies between 24 weeks 0 days and 34 weeks 6 days of gestation in low resource settings as positive effects on infant outcomes were found particularly in singletons and no adverse effects were noted. Further research is necessary to clarify the effects of administration of ACS in twin pregnancies.

Author contributions SM, BK and DD contributed to the conception and design of the study. SM, EK, EA, AI, EC and GK contributed to the acquisition of data, analysis and interpretation of data. All authors were responsible for drafting the manuscript and revising it critically.

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Ethics approval This prospective chart review study was approved by The Catholic University of Health and Allied Sciences and Bugando Medical Centre's Joint Ethics and Research Review Committee (IRB approval No: CREC/368/2019). Secondary data were collected from medical records. No patients were contacted for this study. To ensure confidentiality, all data were anonymized before being accessed by the study team. Medical records were accessed between July 2019 and February 2020. The ethics committee waived the need for participant informed consent.

Data availability statement Data are available on reasonable request.

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The association between of antenatal corticosteroids use and perinatal mortality among preterm singletons and twins in Mwanza, Tanzania: An observational study

Stanley Mwita[1*], Benjamin Kamala[2,3], Eveline Konje[4], Emmanuela Ambrose[5], Angelina Izina[6], Elieza Chibwe[7,] Gilbert Kongola[8], Deborah Dewey[9]

Affiliations

1. School of Pharmacy, Catholic University of Health and Allied Sciences, Mwanza, Tanzania
2. Department of Epidemiology and Biostatistics, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania
3. Department of Research, Haydom Lutheran Hospital, Haydom, Manyara, Tanzania
4. Department of Epidemiology and Biostatistics, Catholic University of Health and Allied Sciences, Mwanza, Tanzania
5. Department of Pediatrics, Bugando Medical Centre, Mwanza, Tanzania
6. Department of Radiology, Bugando Medical Centre, Mwanza, Tanzania
7. Department of Obstetrics and Gynaecology, Catholic University of Health and Allied Sciences, Mwanza, Tanzania
8. Department of Pharmacology, Catholic University of Health and Allied Sciences, Mwanza, Tanzania
9. Owerko Centre at the Alberta Children's Hospital Research Institute and Departments of Pediatrics and Community Health Sciences, University of Calgary, Calgary, Canada

***Corresponding author**: Stanley Mwita, P.O.Box 1464, Mwanza-Tanzania

Email: stanleymwita@gmail.com (SM)

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ABSTRACT

Objectives To examine the association between antenatal corticosteroids **(**ACS) use and perinatal mortality in singletons and twins delivered before 35 weeks' gestation.

Design Secondary analysis of data from an observational prospective chart review study that investigated if exposure to ACS was associated with lower rates of perinatal mortality in preterm infants.

Setting This study was conducted in four hospitals located in Mwanza region, Tanzania.

Participants The study population included all preterm singletons and twins delivered at these hospitals between 24 weeks 0 days and 34 weeks 6 days of gestation from July 2019 to February 2020.

Outcome measures The primary outcome was perinatal mortality; the secondary outcome was respiratory distress syndrome (RDS).

Results The study included 844 singletons and 210 twin infants. Three hundred and fourteen singletons (37.2%) and 52 twins (24.8%) were exposed to at least one dose of ACS. Adjusted multivariate analyses revealed that among singletons exposure to ACS was significantly associated with a lower likelihood of perinatal mortality, aRR 0.30 (95%CI 0.22 - 0.40) and RDS, aRR 0.92 (95%CI 0.87- 0.97). In twin infants, exposure to ACS was associated with a reduced risk of RDS only, aRR 0.87 (95%CI 0.78 – 0.98).

Conclusion The use of ACS between 24 weeks 0 days and 34 weeks 6 days of gestation in both singletons and twins in low resource settings is associated with positive infant outcomes. No adverse effects were noted. Further research that examines the benefits of ACS for twin infants is needed.

Strengths and limitations of this study

- This is the first study in Tanzania to evaluate the benefits of ACS use in both singleton and twin infants.
- Relevant covariates were controlled for in the multivariable analyses which provided an unbiased estimate of the true association between ACS exposure and perinatal outcomes.
- As the study design was observational, causality cannot be inferred.
- Data on the potential confounding factor of chorionicity in twins, was not available.
- The number of twins in our sample was small, which may have limited our ability to detect statistically significant associations between ACS exposure and perinatal mortality.

INTRODUCTION

Globally, preterm birth is one of the leading causes of perinatal mortality and morbidity. [1] Women with a twin pregnancy are at higher risk of preterm birth [2] with approximately 60% of twins are delivered prematurely. [3] Perinatal mortality includes stillbirth and neonatal death during the first week of life and the overall rate of perinatal mortality is higher in twins than singletons.[4] Respiratory distress syndrome (RDS), a serious complication of preterm birth, is a significant contributor to perinatal mortality among both singletons and twins.[5] Antenatal corticosteroids (ACS) are considered a key intervention for moderating the adverse effects of a preterm birth. [6] A single course of ACS is recommended between 24 weeks and 34 weeks of gestation in women that are at risk of preterm delivery within 7 days[7], and may be considered at 22 0/7 weeks to 23 6/7 weeks of gestation if neonatal resuscitation is planned and after appropriate counseling.[8,9]

Some studies have reported that the positive effects of ACS exposure in twins are similar to that observed in singletons; [10,11] however, other have not reported beneficial effects in twins. [12,13] A recent Cochrane Review suggested that further research is needed to support the use of ACS in twin pregnancies, particularly in low resource countries. [14] To address this issue, we examined the association between ACS exposure in singletons and twins delivered before 35 weeks' gestation and the risk of perinatal mortality and RDS in Tanzania, a low resource country.

MATERIALS AND METHODS

Study design

This is a secondary analysis of data from an observational prospective chart review study that investigated if exposure to ACS was associated with lower rates of perinatal mortality in preterm infants.[15] This study was approved by The Catholic University of Health and Allied Sciences and

Bugando Medical Centre's Joint Ethics and Research Review Committee in May 2019. It was conducted in four selected hospitals located in Nyamagana and Sengerema districts, which are two of the seven districts of the Mwanza region, northwest Tanzania. The hospital that participated were Bugando Medical Centre (a tertiary consultant zonal referral hospital), Sekou Toure Regional Referral Hospital, Nyamagana District Hospital and Sengerema District Designated Hospital. These hospitals provide obstetric and neonatal care services to a large proportion of the population within the Lake zone in Tanzania.

The study population included all singletons and twins delivered between July 2019 and February 2020 who met the following inclusion criteria: 1) infants of a mother who displayed indicators of preterm birth including antepartum haemorrhage, pre-eclampsia or eclampsia, premature preterm rupture of membranes or preterm labor, 2) infant delivered in-hospital between 24 weeks 0 days and 34 weeks 6 days of gestation, and 3) infant delivered within 7 days of administration of ACS to the mother. Infants who were reported in the medical record to have a congenital malformation were excluded (Figure 1).

The primary predictor variable was ACS (dexamethasone) exposure administered according to the recommended guideline for ACS, 4 doses of 6 mg of dexamethasone every 12 hours. [16] Study participants were classified into two groups, the No-ACS group and the ACS group (i.e., women administered at least one does of ACS). The primary outcome was perinatal mortality, which was defined as stillbirth or early neonatal mortality (i.e., death of a live born neonate between zero and seven days after birth). The secondary outcome was a diagnosis of RDS.

The medical records of women and their infants were reviewed by the principal investigator and trained research assistants who were enrolled/registered nurses working in the labor wards and neonatal units of the hospitals. To control for selection bias, we collected data on all pregnant women who were admitted to the hospital and were between 24 weeks 0 days and 34 weeks 6 days of gestation. For each participant, we recorded whether the mother was exposed or not exposed to ACS, the perinatal mortality, and the RDS status of the infant. In addition, the following data was obtained from the women's and infant's medical records: parity, marital status, maternal age, maternal education, antenatal care visits (days), gestational age (weeks), mode of delivery, indication for delivery, level of health facility, birthweight (grams) and neonate sex. Gestational age was determined based on women's self-reports of their last normal menstrual period, fundal height and/or ultrasound.

To address the primary research aim of the initial study, based on a power of 95%, an estimated minimum overall sample size of 1010 (both twins and singletons) was determined using Open-Source Epidemiologic Statistics for Public Health (Open Epi).[15,17]

Statistical analyses

The data were analyzed using STATA Version 13. Chi-square tests or Fisher exact tests as appropriate, were conducted to determine if singletons or twins differed by ACS exposure on the following variables: parity, marital status, education, antenatal care visits, mode of delivery, indication for preterm delivery and level of health facility. Mann Whitney U tests were used to determine if there were group differences in median maternal age, gestational age, and birthweight. Differences between singletons and twins in the No-ACS and ACS groups in perinatal mortality and RDS were examined using cross-tabulation and chi-square tests.

For singletons, modified Poisson regressions were used to investigate the associations between ACS exposure and perinatal outcomes. To account for the clustering effect and non-independence of twins we used a mixed model approach. Specifically, the model was fitted with generalized estimating equations (GEE) to account for the associations within a pair of twins from the same mother. Multivariate analyses were performed to examine the effects of ACS on perinatal mortality and RDS controlling for the following factors gestational age, birthweight, level of health facility, and mode of delivery.[15] P-values of less than 0.05 were considered statistically significant. Data are presented as frequencies (percentages), median (interquartile range) and relative risks (RR) with 95% confidence intervals as appropriate.

Patient and public involvement

No patient or member of the public were involved in the design of this study.

RESULTS

The study included 844 singletons and 210 twin infants (Figure 1). Three hundred and fourteen singletons (37.2%) and 52 twins (24.8%) were exposed to at least one dose of ACS. In singletons, no significant differences in marital status or mean maternal age were found between women who received ACS and those who did not. However, the groups differ on the following variables: parity, antenatal care visits, gestational age, mode of delivery, indication for delivery and level of health facility where they delivered (Table 1).

Table 1. Maternal and infant baseline characteristics of the ACS and No-ACS groups among singletons

	ACS (n=314)	No-ACS (n=530)	P-value
Maternal			
Nulliparity, N (%)	119 (37.9)	155 (29.2)	**0.009**
Married, N (%)	272 (86.6)	447 (84.3)	0.366
Maternal age (yrs), M(IQR)	26 (22-31)	26 (21-31)	0.392
More than 3 Antenatal care visits†, N (%)	152 (47.2)	198 (32.8)	**<0.001**
Gestational age (wks), M (IQR)	33 (31-34)	32 (28-34)	**<0.001**
Mode of delivery, N (%)			
Assisted vaginal	9 (2.9)	10 (1.9)	**<0.001**
C- section	134 (42.7)	97 (18.3)	
Normal vaginal	171 (54.4)	423 (79.8)	
Indication for delivery, N (%)			
Antepartum haemorrhage	42 (13.4)	66 (12.4)	**0.017**
Pre-eclampsia or Eclampsia	70 (22.3)	84 (15.8)	
Premature preterm rupture of membrane	45 (14.3)	59 (11.2)	
Preterm labor	157 (50.0)	321 (60.6)	
Level of health facility, N (%)			
Tertiary zonal hospital	228 (72.6)	110 (20.8)	**<0.001**
Regional hospital	75 (23.9)	226 (42.6)	
District hospital	11 (3.5)	194 (36.6)	
Infants			
Birthweight (g), M (IQR)	2000 (1600-2350)	2000 (1600-2500)	0.567
Sex (male), N (%)	170 (54.1)	277 (52.3)	0.598

†Denominator included only those who attended antenatal care (ACS 307 vs No ACS 494)

In twins, no significant differences in parity, mean maternal age, antenatal care visits, gestational age, or indication for delivery were found between women who received ACS and those who did not. However, the groups differed on the following variables: marital status, mode of delivery, level of health facility where they delivered, and infant birthweight (Table 2).

Table 2. Maternal and infant baseline characteristics of the ACS and No-ACS groups among twins

	ACS	No-ACS	P-value
Maternal ‡	**(n=52)**	**(n=158)**	
Nulliparity, N (%)	6 (23.1)	25 (31.6)	0.240
Married, N (%)	24 (92.3)	78 (98.7)	**0.016**
Maternal age (yrs)M (IQR)	26 (24-30)	25 (23-32)	0.760
More than 3 Antenatal care visits†, N (%)	16 (61.5)	39 (50.6)	0.174
Gestational age (wks) M (IQR)	33 (32-34)	34 (30-34)	0.244
Mode of delivery, N (%)			
Assisted vaginal	3 (11.5)	6 (7.6)	**<0.001**
C- section	14 (53.9)	15 (19.0)	
Normal vaginal	9 (34.6)	58 (73.4)	
Indication for delivery, N (%)			
Antepartum haemorrhage	3 (11.5)	5 (6.3)	0.272
Pre-eclampsia or Eclampsia	2 (7.7)	11 (13.9)	
Premature preterm rupture of membrane	2 (7.7)	7 (8.9)	
Preterm labor	19 (73.1)	56 (70.9)	
Level of health facility, N (%),			
Tertiary zonal hospital	15 (57.7)	17 (21.5)	**<0.001**
Regional hospital	9 (34.6)	15 (19.0)	
District hospital	2 (7.7)	47 (59.5)	
Infants			
Birthweight, (g), M (IQR)	1950 (1750-2100)	1500 (1150-1900)	**<0.001**
Sex (male), N (%)	21 (40.38)	77 (48.73)	0.295

‡ Number of women are half the number of twin infants (ACS 26 vs No ACS 79)

†Denominator included only those who attended antenatal care (ACS 26 vs No ACS 77).

Perinatal outcomes for the ACS and No-ACS groups among singletons and twins

Unadjusted estimates of perinatal outcomes are presented in Table 3. Among singleton births, those who were exposed to ACS in utero had a lower rate of perinatal mortality (13.4% vs 28.5%) and RDS (18.8% vs 25.8%) compared to those not exposed to ACS. In twins, those who were

exposed to ACS in utero had a significantly lower rate of RDS (12.0% vs 28.4%) compared to unexposed infants; a trend difference was observed for perinatal mortality (15.4% vs 27.2%).

Table 3. Perinatal outcomes for the ACS and No ACS groups

	Singletons			Twins		
	ACS (n=314)	**No-ACS (n=530)**	**P-value**	**ACS (n=52)**	**No-ACS (n=158)**	**P-value**
Outcomes	**N (%)**	**N (%)**		**N (%)**	**N (%)**	
Perinatal Mortality	42 (13.4)	151 (28.5)	**<0.001**	8 (15.4)	43 (27.2)	0.059
Respiratory distress syndrome *	58 (18.8)	109 (25.8)	**0.026**	6 (12.0)	40 (28.4)	**0.013**

*Denominator included live infants only, singletons (ACS 308 vs No-ACS 422) and twins (ACS 50 vs No-ACS 141).

Multivariate analyses of the association between ACS exposure and perinatal outcomes among singletons and twins

Adjusted multivariate analyses in singletons revealed that exposure to ACS was significantly associated with a lower likelihood of perinatal mortality, aRR 0.30 (95% CI 0.22 - 0.40), P<0.001 and RDS, aRR 0.92 (95% CI 0.87- 0.97), P=0.001. However, in twin infants, exposure to ACS was associated with a reduced risk of RDS only, aRR 0.87 (95% CI 0.78 – 0.98), P=0.026 (Table 4).

Table 4. Multivariate analysis of the associations between ACS exposure and perinatal outcomes among singletons and twins

	Singletons		Twins	
Outcomes	***Adjusted relative risk (95%CI)**	**P Value**	***Adjusted relative risk (95%CI)**	**P Value**
Perinatal mortality	0.30 (0.22 - 0.40)	<0.001	0.78 (0.39 - 1.56)	0.488
Respiratory distress syndrome	0.92 (0.87 - 0.97)	0.001	0.87 (0.78 – 0.98)	0.026

*Model adjusted for gestational age, birthweight, level of health facility, and mode of delivery

DISCUSSION

ACS are an important component in the management of women at risk of preterm delivery as they they stimulate fetal lung maturation and alveolar surfactant production. [18,19] Our study investigated the association between ACS use and perinatal outcomes in singleton and twin infants. We found a significantly lower risk of perinatal mortality and RDS for singleton infants exposed to at least one dose of ACS. In twins, exposure to ACS was associated with a lower risk of RDS. ACS administration was not associated with a reduced risk of perinatal mortality in twins.

Consistent with current findings, a study of 1662 twins delivered between 25 weeks 0 days and 34 weeks 6 days of gestation in China from January 2013 to December 2014, reported that administration of at least one dose of ACS was associated with a reduced risk of RDS in twin preterm infants. [13] Previous research has reported limited benefits of ACS in reducing rates of mortality and morbidities in twins; [12,20–22] however, these studies are limited by their retrospective design[12,20,22], which leaves them prone to information and selection bias. In addition, the sample sizes of some of these studies was small, which may have limited their ability to detect significant

associations. [12,21] In contrast, two retrospective studies with large samples [11,23] and one with a small sample of twins [24], reported that the administration of ACS had a protective effect against adverse perinatal outcomes in both singletons and twins born preterm. Also, in a nationwide observational multicentre prospective cohort study of twins born between 22 and 34 completed weeks of gestation in 2011 in France, ACS administered ≤7 days prior to delivery were reported to be significantly associated with a reduced rate of in-hospital mortality. [25]

ACS are not frequently used in women at risk for preterm delivery in low resource countries, although their use is recommended by the World Health Organization (WHO) as essential for reducing infant mortality and morbidities. Currently, the WHO recommends the use of ACS only when gestational age is known, there is no clinical evidence of maternal infection, preterm delivery is imminent, and the delivery is in a facility that can provide adequate care for the mother and the infant. [26] Despite the benefits of ACS in reducing the risk of adverse perinatal outcomes, particularly in singletons, in the present study less than 40% of the mothers of singletons were administered at least one dose of ACS. The main reason given by health professionals in these low resource settings for not administrating ACS was that women arrived late at the hospital in well-established labor.[15] Another potential impediment to administering ACS in low resource settings could be difficulty in obtaining an accurate estimate of gestational age. Before the positive effects of ACS can be realized in low resource settings, women and health care professionals need to be educated on the clinical signs associated with preterm delivery, risk factors for preterm delivery, and the importance of arriving at the hospital early if preterm delivery is anticipated. In addition, infrastructure in health care facilities that can accurately estimate gestational age and provide adequate care for mothers and their preterm infants is essential. [27]

There is a scarcity of randomized clinical trials that have enrolled women with multiples.[28,29] For example, the landmark study by Liggins and Howie that showed that ACS significantly reduced rates of RDS and neonatal mortality, included only 11 pairs of twins.[30] In medical practice, a similar dose of ACS (i.e., 2 doses of 12 mg of betamethasone every 24 hours or 4 doses of 6 mg of dexamethasone every 12 hours) is given regardless of pregnancy multiplicity. [31] It is possible that this dosing level used for singletons might not be adequate to produce a therapeutic level for lung maturation in twins.[12] Pharmacokinetic data suggest that higher doses of ACS are needed in twin gestations to decrease perinatal morbidities and mortality. Pharmacologically, ACS have been shown to have a higher volume of distribution, shorter half-life, and greater clearance in twins compared to singleton pregnancies, which could result in a subtherapeutic dosage for lung maturation. [32–34] Randomized controlled trials need to be conducted to evaluate and develop recommendations regarding the dose levels of ACS that could be effective in improving perinatal outcomes in twin- and higher-level multiple pregnancies. Future studies in low resource settings should also focus on examining associations between ACS therapy and other neonatal morbidities such as bronchopulmonary dysplasia, intraventricular hemorrhage, necrotizing enterocolitis, retinopathy of prematurity, patent ductus arteriosus, need for mechanical ventilation, and neonatal hypoglycemia in both singleton and twins.

To the best of our knowledge this is the first study in Tanzania to evaluate the benefit of ACS in singletons and twins in low resource settings. A strength of our study was its ability to control for relevant covariates in the multivariable analyses. This resulted in unbiased estimates of the true association between ACS exposure and perinatal outcomes in singletons and twins. However, there

are some limitations. First, it was an observational study. Thus, the inherent biases associated with observational studies (e.g., selection bias, information bias, confounding bias) could have influenced our outcomes. A second limitation was that the small number of twins in our sample. This reduced the power of the study and may have limited our ability to detect statistically significant associations between ACS exposure and perinatal mortality. Future studies in low resource settings with larger samples of twins are needed to clarify the associations between ACS exposure and perinatal outcomes. A third limitation of this study is that data on the potential covariate of chorionicity in twins, was not available in this low resource setting. Fourth, the gestational age of some of the infants included in this study was based on the women's self-reported last menstrual period and was not verified by ultrasonographic examination. Thus, some reported gestational ages of study participants may have been incorrect. However, the use of women's self-reported last menstrual period is a common practice in low resource settings and has been shown to provide a valid estimate of gestational age.[35] Finally, the current study was conducted in four hospitals only, which may limit the generalizability of the findings to other health care settings.

CONCLUSION

Our findings add new and important information to the literature on the benefits of ACS for singleton and twin infants in low resource settings. They also support the use of ACS in both singleton and twin pregnancies at risk for delivery between 24 weeks 0 days and 34 weeks 6 days of gestation in low resource settings as reduced risk of perinatal mortality and RDS were found and no adverse effects were noted. Further research is necessary to clarify the benefits of administration of ACS in twin pregnancies.

Author contributions SM, BK and DD contributed to the conception and design of the study. SM, EK, EA, AI, EC and GK contributed to the acquisition of data, analysis and interpretation of data. All authors were responsible for drafting the manuscript and revising it critically.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study was approved by The Catholic University of Health and Allied Sciences and Bugando Medical Centre's Joint Ethics and Research Review Committee (IRB approval No: CREC/368/2019). Secondary data were collected from medical records. No patients were contacted for this study. To ensure confidentiality, all data were anonymized before being accessed by the study team. Medical records were accessed between July 2019 and February 2020. The ethics committee waived the need for participant informed consent.

Data availability statement Data are available on reasonable request.

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FIGURES

Figure 1. Flow diagram of study population inclusion and exclusion

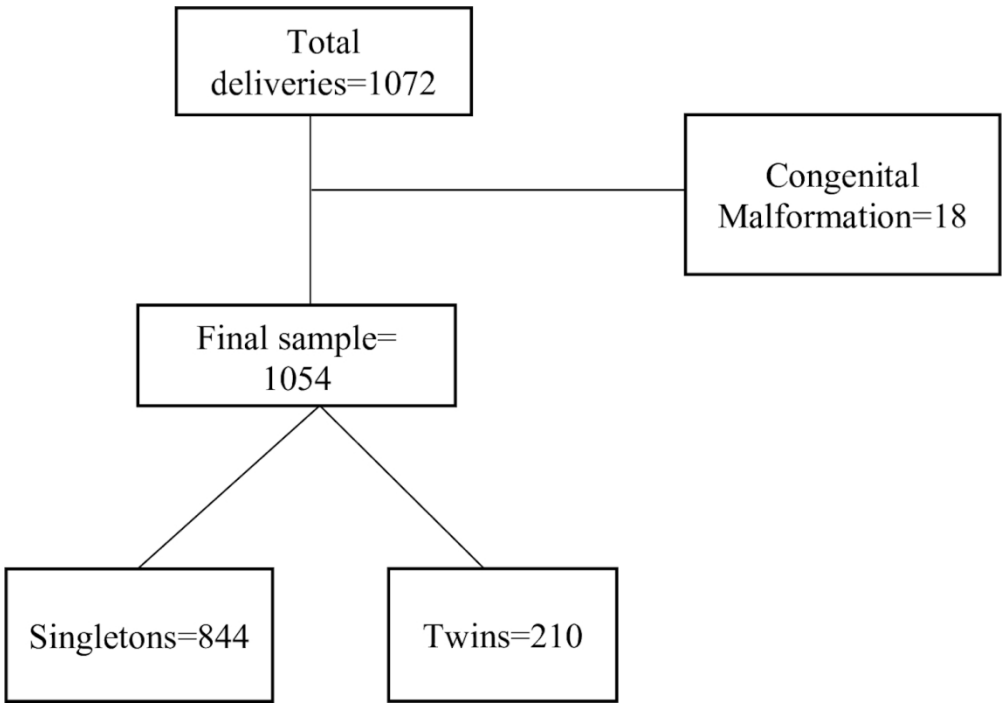

Figure 1. Flow diagram of study population inclusion and exclusion

130x91mm (300 x 300 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	N/A
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A
		(*e*) Describe any sensitivity analyses	N/A
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Figure 1 attached
		(b) Give reasons for non-participation at each stage	Figure 1 attached
		(c) Consider use of a flow diagram	Figure 1 attached
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7-8
		(b) Indicate number of participants with missing data for each variable of interest	N/A
		(c) Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	Report numbers of outcome events or summary measures over time	10
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10-11
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	11
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12- 14
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	15

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.