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Complete List of Authors:	Bae, Seong Phil; Soon Chun Hyang University Hospital, Pediatrics; Soonchunhyang University School of Medicine, Department of Pediatrics Hahn, Won-Ho; Soonchunhyang University Seoul Hospital, Department of Pediatrics; Soonchunhyang University School of Medicine, Department of Pediatrics Park, Suyeon; Soonchunhyang University Seoul Hospital, Department of Biostatistics; Chung-Ang University, Department of Applied Statistics Jung, Young Hwa; Seoul National University Children's Hospital, Department of Paediatrics; Seoul National University College of Medicine, Department of Pediatrics Park, Jee Yoon; Seoul National University Bundang Hospital, Department of Obstetrics and Gynecology Oh, Kyung Joon; Seoul National University Bundang Hospital, Department of Obstetrics and Gynecology Choi, Chang Won; Seoul National University Bundang Hospital, Department of Pediatrics; Seoul National University College of Medicine, Department of Pediatrics
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Title page

Original article

Effects of antenatal corticosteroids on neonatal outcomes in very-low-birth-weight infants are not different by plurality: a nationwide cohort study

Seong Phil Bae[1,2], M.D., Won-Ho Hahn[1,2], M.D., Ph.D., Suyeon Park[3,4], MS., Young Hwa Jung[5,6], M.D., Ph.D., Jee Yoon Park[7], M.D., Kyung Joon Oh[7], M.D., Ph.D., Chang Won Choi[5,6], M.D., Ph.D.

[1]Department of Pediatrics, Soonchunhyang University Seoul Hospital, Seoul, Republic of Korea

[2]Department of Pediatrics, Soonchunhyang University School of Medicine, Asan, Republic of Korea

[3]Department of Biostatistics, Soonchunhyang University Seoul Hospital, Seoul, Republic of Korea

[4]Department of Applied Statistics, Chung-Ang University, Seoul, Republic of Korea

[5]Department of Pediatrics, Seoul National University Bundang Hospital, Seongnam, Republic of Korea

[6]Department of Pediatrics, Seoul National University College of Medicine, Seoul, Republic of Korea

[7]Department of Obstetrics and Gynecology, Seoul National University Bundang Hospital, Seongnam, Republic of Korea

Correspondence to

Chang Won Choi, M.D., Ph.D.

Department of Pediatrics, Seoul National University Bundang Hospital, 82 Gumi-ro 173 Beon-gil, Bundang-gu, Seongnam-si, Gyeonggi-do, Republic of Korea, E-mail: choicw@snu.ac.kr

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ABSTRACT

Objective

To investigate whether effects of antenatal corticosteroids on neonatal outcomes in preterm infants with very-low-birth-weight were different by plurality.

Design

Nationwide prospective cohort study

Setting

Not applicable

Patients

Twins and singletons with very-low-birth-weight (< 1,500 g) who were born between 23^{+0} and 33^{+6} weeks of gestation and registered in the Korean Neonatal Network from January 2014 to December 2019

Main outcome measures

Morbidity and mortality before discharge from neonatal intensive care unit

Results

Among a total of 9,531 preterm infants with very-low-birth-weight, there were 2,364 (24.8%) twins and 7,167 (75.2%) singletons. While 83.9% of singletons were exposed to at least one dose of antenatal corticosteroids, so were 87.9% of twins.

Interaction analysis demonstrated that there was no significant difference in the effect of antenatal corticosteroids on morbidities or mortality between twins and singletons in either gestational age group (23 to 28 weeks or 29 to 33 weeks).

Antenatal corticosteroids significantly decreased the risk of surfactant use, high-grade intraventricular hemorrhage, periventricular leukomalacia, and mortality in the gestational age group of 23 to 28 weeks. In the gestational age group of 29 to 33 weeks, antenatal corticosteroids significantly decreased the risk of surfactant use and mortality but increased the risk of sepsis.

Conclusion

This study demonstrates that effect of antenatal corticosteroids on neonatal outcomes of preterm infants with very-low-birth-weight does not differ significantly by plurality (twin or singleton pregnancy).

Keywords: antenatal corticosteroids, preterm delivery, mortality, morbidity, twins, very low birth weight infant

Key messages

Why is already known on this topic?

Antenatal corticosteroids (ACS) administered before preterm delivery can decrease neonatal morbidity and mortality in singleton pregnancies. However, data about such effects of ACS in multiple pregnancies are still limited.

What this study adds?

The effect of antenatal corticosteroids administered before preterm delivery on neonatal morbidity and mortality does not differ by plurality (twin or singleton pregnancy).

Introduction

Antenatal corticosteroid (ACS) has been established as the standard therapy for pregnant women at risk of preterm delivery within 7 days between 24^{+0} and 33^{+6} weeks of gestation.[1] Currently, ACS is equally recommended for twin and singleton pregnancies with the same regimen based on previous studies.[2-4] A recent study has shown that a complete course of ACS administered before preterm twin delivery is associated with a significant decrease in neonatal mortality, short-term respiratory morbidity, and severe neurological injury in a magnitude similar to those observed for a singleton pregnancy.[3] However, data that demonstrate comparable effects of ACS therapy in twin pregnancies are limited.

As a result of increasing childbirth age and use of assisted reproductive technology, twin pregnancies are increasing in many countries, including Korea.[5 6] However, twin pregnancies are more likely to have preterm birth, low birth weight, and longer hospital stays than singleton pregnancies.[7 8] In addition, mono-chorionicity, birth weight discordance, and obstetric complications associated with multiple pregnancies may result in adverse perinatal outcomes.[9-11]

Recently, the mortality of extreme preterm multiples has decreased to a level comparable to that of singletons born at the same gestational age along with increased use of ACS therapy.[12] However, many studies have reported conflicting results regarding equivalent effects of ACS on neonatal outcomes of twins and singletons.[3 13-18] To date, there have been no clinical trials designed to investigate the effect of ACS in twins. A meta-analysis on ACS therapy has also highlighted the need for additional studies on multiple pregnancies.[4]

Thus, the purpose of this study was to investigate whether effects of ACS administered before preterm delivery on neonatal outcomes of twins and singletons were comparable.

Materials and Methods

Study design and participants

Information on the study population is presented in Figure 1. Data collected prospectively through the Korean Neonatal Network (KNN), a nationwide very low birth weight (VLBW, <1,500 g) infant registry participated by 61 neonatal intensive care units across South Korea,[19] were used for this study. A total of 11,121 infants with VLBW born preterm (between 23^{+0} and 33^{+6} weeks of gestation) between January 2014 and December 2019 were enrolled. Exclusion criteria were: high-order multiple gestations (≥ triplets), birth outside the hospital, major congenital anomalies, transfer to other hospitals, no or unreliable information on ACS. Finally, 2,364 twins and 7,167 singletons were analyzed. ACS therapy was defined if the pregnant women had received at least one dose of any kind of corticosteroids before preterm delivery. Variables collected included gestational age, birth weight, small for gestational age (SGA), sex, maternal age, maternal diabetes mellitus (DM), maternal hypertension, chorioamnionitis, premature rupture of membrane, cesarean section, *in vitro* fertilization (IVF), surfactant use, sepsis, high-grade intraventricular hemorrhage (IVH), periventricular leukomalacia (PVL), surgically treated patent ductus arteriosus (PDA), bronchopulmonary dysplasia (BPD), advanced retinopathy of prematurity (ROP), and mortality. Definitions for different variables are provided

in Supplemental Materials. The analysis of neonatal morbidity was performed for infants who survived before discharge, while the analysis of mortality was performed for the total population.

Statistical methods

Rates of neonatal outcomes between infants exposed to ACS and infants without such exposure are presented along with risk difference and 95% CIs. To investigate whether associations between ACS and neonatal outcomes were altered by plurality (twins versus singletons), the interaction effect between ACS and twin pregnancies on neonatal outcomes was evaluated by adding interaction terms (ACS × twins) in Poisson regression models that included potential confounders, such as gestational age, birth weight, sex, maternal age, maternal diabetes mellitus, maternal hypertension, premature rupture of membrane, cesarean section, and *in vitro* fertilization. Missing values for chorioamnionitis were substantial (17.6% in twins and 14.5% in singletons). They were excluded from the analyses. In the second model excluding the interaction term to investigate the individual effect of ACS and twins, crude and adjusted relative risk (RR) and 95% CI were calculated by Poisson regression analyses with selection of variables that showed minimal QIC (Quasi-likelihood under the Independence model Criterion) for each outcome. These models were fitted with generalized estimation equations (GEE) approach for the correlation between a pair of twins from a mother. The level of significance was set at $p < 0.05$. All statistical analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA) and R package version 3.3.1 (The R Foundation for Statistical Computing, Vienna, Austria).

Ethic approval

Registration of data in the KNN was approved by the Institutional Review Board (IRB) of each participating center. Informed consent was obtained from parents of each infant prior to participation in the KNN registry. This study was approved by the IRB of Seoul National University Bundang Hospital (approval number: B-1305-202-005).

Results

Exposure to antenatal corticosteroids

Among 2,364 twins, 2,078 (87.9%) infants were exposed to at least one dose of ACS before preterm delivery. Among 7,167 singletons, 6,013 (83.9%) infants were exposed to at least one dose of ACS before preterm delivery (Figure 1).

Comparisons of perinatal baseline characteristics between infants exposed to antenatal corticosteroids and infants without ACS exposure

Twins exposed to ACS were born at a significantly later gestational age compared to those without ACS exposure (Table 1). However, there was no significant difference in gestational age or birth weight between singletons exposed to ACS and those without ACS exposure. Singletons exposed to ACS had significantly higher rates of maternal DM, maternal hypertension, and cesarean section than those without exposure. In both twins and singletons, infants exposed to

Table 1. Comparisons of perinatal baseline characteristics between infants exposed to ACS and infants without ACS exposure

	Twins (n = 2,364)			Singletons (n = 7,167)		
	ACS-exposed (n = 2,078)	ACS-unexposed (n = 286)	*P* value	ACS-exposed (n = 6,013)	ACS-unexposed (n = 1,154)	*P* value
Infant						
Gestational age (weeks), mean (SD)	28.08 (2.19)	27.75 (2.50)	0.034	28.49 (2.52)	28.39 (2.78)	0.256
Birth weight (g), mean (SD)	1,059 (274)	1,034 (301)	0.186	1,060 (281)	1,073 (289)	0.138
SGA, n (%)	147 (7.1)	22 (7.7)	0.704	1080 (18.0)	199 (17.2)	0.560
Male, n (%)	1079 (51.9)	143 (50.0)	0.541	3074 (51.1)	597 (51.7)	0.708
Maternal						
Maternal age (years), mean (SD)	33.41 (3.80)	31.91 (4.66)	<0.001	33.44 (4.43)	32.82 (4.92)	<0.001
Maternal diabetes mellitus, n (%)	238 (11.5)	26 (9.1)	0.221	597 (9.9)	89 (7.7)	0.019
Maternal hypertension, n (%)	196 (9.5)	22 (7.7)	0.329	1749 (29.1)	286 (24.8)	0.003
Chorioamnionitis[a], n (%)	516 (29.9)	63 (28.0)	0.551	2084 (40.2)	351 (37.0)	0.064
Premature rupture of membrane, n (%)	787 (38.1)	84 (29.9)	0.007	2334 (38.9)	312 (27.5)	<0.001
Cesarean section, n (%)	1837 (88.4)	244 (85.3)	0.132	4634 (77.1)	807 (69.9)	<0.001
In vitro fertilization, n (%)	1152 (56.6)	128 (45.1)	<0.001	494 (8.2)	51 (4.4)	<0.001

Data are presented as mean (SD) or n (%).

Abbreviations: ACS, antenatal corticosteroids; SGA, small for gestational age; IVH, intraventricular hemorrhage; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity.

[a] Values were missing for 415 infants in the group of twins and 1039 infants in the group of singletons.

ACS were more likely to have higher rates of premature rupture of membrane and be conceived through IVF from older mothers than those without exposure.

Interaction between ACS and twins pregnancies on neonatal outcomes

We investigated whether effects of ACS on neonatal outcomes differed between twins and singletons. Because neonatal outcomes are substantially dependent on gestational age, comparison of ACS effects on neonatal outcomes by plurality was performed by stratifying the study population into two gestation age groups: an age group of 23 to 28 weeks and an age group of 29 to 33 weeks group.

Among infants in the age group of 23 to 28 weeks, exposure to ACS for twins was significantly associated with a lower rate of mortality, but not with morbidity. However, exposure to ACS for singletons was significantly associated with a lower mortality and a lower morbidity including surfactant use, high-grade IVH, and PVL (Table 2). Among infants in the age group of 29 to 33 weeks, exposure to ACS for twins was significantly associated with lower rates of mortality and surfactant use (Table 3). Exposure to ACS for singletons was significantly associated with a lower rate of mortality, but a higher rate of sepsis. In interaction analyses, there was no significant difference in the association of ACS therapy with any neonatal outcomes between twins and singletons in either gestational age group.

Table 2. Comparisons of neonatal outcomes between infants exposed to ACS and infants without ACS exposure in infants born at 23 to 28 weeks of gestational age

Infants who survived before discharge (n = 4160)	ACS-exposed (n=3606)	ACS-unexposed (n=554)	Risk difference, % (95 CI)	*P* value[a]	*P* value for interaction[b]
Surfactant use, n (%)					
Total	3468/3606 (96.2%)	546/554 (98.6%)	-2.4 (-3.6 to -1.2)	0.005	
Twin	952/991 (96.1%)	108/109 (99.1%)	-3.0 (-5.2 to -0.9)	0.110	0.199
Singleton	2516/2615 (96.2%)	438/445 (98.4%)	-2.2 (-3.6 to -0.8)	0.018	
Sepsis, n (%)					
Total	985/3606 (27.3%)	166/554 (30.0%)	-2.7 (-6.7 to 1.4)	0.195	
By numbers of fetus					
Twin	273/991 (27.5%)	32/109 (29.4%)	-1.8 (-10.8 to 7.2)	0.689	0.706
Singleton	712/2615 (27.2%)	134/445 (30.1%)	-2.9 (-7.5 to 1.7)	0.209	
High-grade IVH, n (%)					
Total	313/3606 (8.7%)	79/554 (14.3%)	-5.6 (-8.6 to -2.5)	<0.001	
By numbers of fetus					
Twin	115/991 (11.6%)	15/109 (13.8%)	-2.2 (-8.9 to 4.6)	0.508	0.224
Singleton	198/2615 (7.6%)	64/445 (14.4%)	-6.8 (-10.2 to -3.4)	<0.001	
Periventricular leukomalacia, n (%)					
Total	327/3606 (9.1%)	74/554 (13.4%)	-4.3 (-7.3 to -1.3)	0.001	
By numbers of fetus					
Twin	86/991 (8.7%)	14/109 (12.8%)	-4.2 (-10.7 to 2.4)	0.151	0.749
Singleton	241/2615 (9.2%)	60/445 (13.5%)	-4.3 (-7.6 to -0.9)	0.005	
Surgically treated PDA, n (%)					
Total	616/3606 (17.1%)	99/554 (17.9%)	-0.8 (-4.2 to 2.6)	0.647	
By numbers of fetus					
Twin	175/991 (17.7%)	23/109 (21.1%)	-3.4 (-11.5 to 4.6)	0.375	0.400
Singleton	441/2615 (16.9%)	76/445 (17.1%)	-0.2 (-4.0 to 3.6)	0.911	
Necrotizing enterocolitis, n (%)					
Total	268/3606 (7.4%)	36/554 (6.5%)	0.9 (-1.3 to 3.2)	0.432	
By numbers of fetus					
Twin	72/991 (7.3%)	8/109 (7.3%)	-0.07 (-5.2 to 5.1)	0.978	0.479
Singleton	196/2615 (7.5%)	28/445 (6.3%)	1.2 (-1.3 to 3.7)	0.368	

Bronchopulmonary dysplasia, n (%)					
Total	1664/3606 (46.1%)	269/554 (48.6%)	-2.4 (-6.9 to 2.1)	0.290	
By numbers of fetus					
Twin	437/991 (44.1%)	51/109 (46.8%)	-2.7 (-12.6 to 7.2)	0.591	0.986
Singleton	1227/2615 (46.9%)	218/445 (49.0%)	-2.1 (-7.1 to 3.0)	0.419	
Advanced ROP, n (%)					
Total	769/3602 (21.3%)	113/554 (20.4%)	1.0 (-2.7 to 4.6)	0.610	
By numbers of fetus					
Twin	232/990 (23.4%)	31/109 (28.4%)	-5.0 (-13.9 to 3.9)	0.245	0.158
Singleton	537/2612 (20.6%)	82/445 (18.4%)	2.1 (-1.8 to 6.1)	0.301	
All infants (n = 5407)	ACS (n=4587)	No ACS (n=820)	Risk difference, %(95 CI)	*P* value	*P* value for interaction*
Mortality, n (%)					
Total	981/4587 (21.4%)	266/820 (32.4%)	-11.1 (-14.5 to -7.6)	<0.001	
By numbers of fetus					
Twin	265/1256 (21.1%)	65/174 (37.4%)	-16.3 (-23.8 to -8.7)	<0.001	0.458
Singleton	716/3331 (21.5%)	201/646 (31.1%)	-9.6 (-13.5 to -5.8)	<0.001	

Data are presented as n (%).

[a] Tests for risk difference within each subgroup.

[b] Tests for interaction between antenatal corticosteroids and twin pregnancies on each outcome. *P*-values for interaction were obtained from multivariable Poisson regression models adjusted for gestational age, birthweight, sex, maternal age, maternal diabetes mellitus, maternal hypertension, premature rupture of membrane, cesarean section, and *in vitro* fertilization using the generalized estimation equations.

Abbreviations: ACS, antenatal corticosteroid; SGA, small for gestational age; IVH, intraventricular hemorrhage; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity.

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Table 3. Comparisons of neonatal outcomes between infants exposed to ACS and infants without ACS exposure in infants born at 29 to 33 weeks of gestational age

Infants who survived before discharge (n = 4,019)	ACS-exposed (n=3428)	ACS-unexposed (n=591)	Risk difference, % (95 CI)	*P*-value[a]	*P*-value for interaction[b]

Surfactant use, n (%)					
Total	2240/3428 (65.3%)	402/591 (68.0%)	-2.7 (-6.8 to 1.4)	0.206	
By numbers of fetus					
Twin	583/801 (72.8)	86/104 (82.7)	-9.9 (-17.8 to -2.0)	0.030	0.243
Singleton	1657/2627 (63.1)	316/487 (64.9)	-1.8 (-6.4 to 2.8)	0.446	
Sepsis, n (%)					
Total	376/3428 (11.0)	41/591 (6.9)	4.0 (1.7 to 6.3)	0.003	
By numbers of fetus					
Twin	90/801 (11.2)	8/104 (7.7)	3.5 (-2.0 to 9.1)	0.274	0.966
Singleton	286/2627 (10.9)	33/487 (6.8)	4.1 (1.6 to 6.6)	0.006	
High-grade IVH, n (%)					
Total	53/3426 (1.5)	14/591 (2.4)	-0.8 (-2.1 to 0.5)	0.150	
By numbers of fetus					
Twin	10/801 (1.2)	2/104 (1.9)	-0.7 (-3.4 to 2.1)	0.572	0.742
Singleton	43/2625 (1.6)	12/487 (2.5)	-0.8 (-2.3 to 0.6)	0.204	
Periventricular leukomalacia, n (%)					
Total	179/3427 (5.2)	30/591 (5.1)	0.2 (-1.8 to 2.1)	0.882	
By numbers of fetus					
Twin	71/801 (8.9)	6/104 (5.8)	3.1 (-1.8 to 8.0)	0.287	0.492
Singleton	108/2626 (4.1)	24/487 (4.9)	-0.8 (-2.9 to 1.3)	0.412	
Surgically treated PDA, n (%)					
Total	95/3428 (2.8)	18/591 (3.0)	-0.3 (-1.8 to 1.2)	0.709	
By numbers of fetus					
Twin	19/801 (2.4)	4/104 (3.8)	-1.5 (-5.3 to 2.4)	0.369	0.955
Singleton	76/2627 (2.9)	14/487 (2.9)	0.02 (-1.6 to 1.6)	0.982	
Necrotizing enterocolitis, n (%)					
Total	85/3427 (2.5)	11/591 (1.9)	0.6 (-0.6 to 1.8)	0.363	
By numbers of fetus					
Twin	21/801 (2.6)	4/104 (3.8)	-1.2 (-5.1 to 2.6)	0.474	0.492
Singleton	64/2626 (2.4)	7/487 (1.4)	1 (-0.2 to 2.2)	0.175	
Bronchopulmonary dysplasia, n (%)					
Total	524/3423 (15.3)	93/591 (15.7)	-0.4 (-3.6 to 2.8)	0.790	
By numbers of fetus					

Twin	114/797 (14.3)	16/104 (15.4)	-1.1 (-8.4 to 6.3)	0.768	0.834
Singleton	410/2626 (15.6)	77/487 (15.8)	-0.2 (-3.7 to 3.3)	0.912	
Advanced ROP, n (%)					
Total	45/3426 (1.3)	10/591 (1.7)	-0.4 (-1.5 to 0.7)	0.465	
By numbers of fetus					
Twin	13/800 (1.6)	3/104 (2.9)	-1.3 (-4.6 to 2.1)	0.359	0.202
Singleton	32/2626 (1.2)	7/487 (1.4)	-0.2 (-1.4 to 0.9)	0.690	
All infants (n = 4,124)	ACS (n=3504)	No ACS (n=620)	Risk difference, %(95 CI)	*P* value	*P* value for interaction*
Mortality, n (%)					
Total	76/3504 (2.2)	29/620 (4.7)	-2.5 (-4.2 to -0.8)	<0.001	
By numbers of fetus					
Twin	21/822 (2.6)	8/112 (7.1)	-4.6 (-9.5 to 0.3)	0.009	0.722
Singleton	55/2682 (2.1)	21/508 (4.1)	-2.1 (-3.9 to-..6 -0.3)	0.005	

Data are presented as n (%).

[a] Tests for risk difference within each subgroup.

[b] Tests for interaction between antenatal corticosteroids and twin pregnancies on each outcome. *P*-values for interaction were obtained from multivariable Poisson regression models adjusted for gestational age, birthweight, sex, maternal age, maternal diabetes mellitus, maternal hypertension, premature rupture of membrane, cesarean section, and *in vitro* fertilization using the generalized estimation equations.

Abbreviations: ACS, antenatal corticosteroid; SGA, small for gestational age; IVH, intraventricular hemorrhage; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity.

Independent effects of ACS and twins on neonatal outcomes

In the second model excluding the interaction term, we calculated adjusted RR and 95% CI for individual effects of ACS and twins on each neonatal outcome. In the age group of 23 to 28 weeks, exposure to ACS was significantly associated with a lower risk of surfactant use (adjusted RR (aRR): 0.972 [95% CI: 0.961 – 0.984]), high-grade intraventricular hemorrhage (aRR: 0.621 [95% CI: 0.487 – 0.794]), periventricular leukomalacia (aRR: 0.728 [95% CI: 0.556 – 0.954]), and mortality (aRR: 0.758 [95% CI: 0.679 – 0.846]) (Figure 2A). Twin were associated with higher risks of high-grade IVH (aRR: 1.466 [95% CI: 1.178 – 1.825]) and advanced ROP (aRR: 1.193 [95% CI: 1.069 – 1.331]) than singletons.

In the age group of 29 to 33 weeks, exposure to ACS was significantly associated with lower risks of surfactant use (aRR: 0.914 [95% CI: 0.861 – 0.970]) and mortality (aRR: 0.409 [95% CI: 0.269 – 0.624]), but a higher risk of sepsis (aRR: 1.416 [95% CI: 1.018 – 1.969]) (Figure 2B). Twins showed a lower risk of BPD (aRR: 0.798 [95% CI: 0.648 – 0.982]), but a higher risk of PVL (aRR: 1.735 [95% CI: 1.256 – 2.396]) than singletons.

Discussion

In this nationwide cohort study, we demonstrated that effects of ACS therapy administered before preterm birth on neonatal outcomes were not significantly different by plurality. Mortality and surfactant use were reduced by ACS exposure in both gestational age groups. ACS exposure was associated with decreased risks of high-grade IVH and PVL in infants born at 23 to 28

weeks of gestational age, but increased the risk of sepsis in infants born at 29 to 33 weeks of gestational age.

ACS therapy for mothers at risk of impending preterm delivery is the most effective evidence-based strategy to reduce the mortality and morbidity of their preterm offspring. However, previous studies that investigated the association between ACS therapy and neonatal outcomes in twin pregnancies not only reported conflicting results, but also lacked quantity.

A recent meta-analysis has reported that ACS therapy is associated with decreased neonatal death, RDS, and intraventricular hemorrhage in singleton pregnancies, but not in multiple pregnancies.[4] Furthermore, a randomized controlled trial on 311 twin infants who were delivered before 34 weeks of gestation age showed that ACS therapy had no association with RDS or composite neonatal morbidity.[20] However, large population-based cohort studies from the mid-2000s reported positive ACS effects in multiple pregnancies.[21-23] A study of 750 twin infants from France has reported that a complete course of ACS therapy administered within 7 days before birth is associated with decreased rates of brain injury and mortality.[21] Another study of 8,274 multiples from Italian Neonatal Network reported that ACS therapy reduced the risk of severe IVH and mortality, although it was less effective in multiple pregnancies than in singleton pregnancies.[22] A study of 2,516 twin infants from Canadian Neonatal Network has reported that twins can benefit from a complete course of ACS therapy as good as singletons in short-term respiratory morbidity, severe brain injury, and mortality.[23] Consistent with results of these large cohort studies, our study using the latest data from KNN revealed that the effectiveness of ACS therapy in twins was comparable to that of singletons. Moreover, its benefits on mortality and

morbidity were shown to be different by gestational groups.

Additionally, we found that twins had a higher rate of morbidity than singletons in this study. Twins were associated with higher risks of high-grade IVH and advanced ROP in infants born at 23 to 28 weeks of GA and PVL in infants born at 29 to 33 weeks of GA than singletons. Although twins are more likely to be delivered preterm than singletons, whether twin pregnancy alone has such adverse effects on neonatal outcomes in preterm infants remains unknown.[24] In a large study of infants born between 23 and 35 weeks of gestation, twins and singletons had comparable average birth weights up to 32 weeks of gestation and similar neonatal outcomes.[25] One study from Australian and New Zealand Neonatal Network has found higher mortality, but not morbidities, in twins than in singletons.[12] Furthermore, two national cohort studies have reported comparable risks for neonatal outcomes except for RDS between twins and singletons.[26 27] However, a population-based European cohort study showed that twins had higher risk for mortality and high-grade IVH in infants born at 24 to 27 weeks of GA.[14] Other studies have revealed disparities in the rate of morbidity such as BPD and ROP between twins and singletons.[8 28]

Possible reasons for conflicting findings in twins include study design and changes in population characteristics over time.[29] We found that twins had lower rates of antenatal complications such as maternal hypertension and chorioamnionitis, and higher rates of obstetric interventions, including IVF, ACS, and cesarean section than singletons in both gestational age groups (Supplemental Table 1). This trend has also been observed across other recent studies.[12 30] The higher rate of IVF in twins reflect the current trend of childbirth in Korea.[5] The reason for higher

ACS exposure in twins is currently unclear. However, more medical attention for twin pregnancies might be one possible cause.[31] One noteworthy result was that the cesarean section rate for twins born between 23 and 28 weeks of GA was 87.2%, which was higher in Korea than in other countries. For similar gestational age, the cesarean section rate for twins was 74.4% in the USA and 61.8% in Australia and New Zealand.[12 15]

Despite the fact that these difference in characteristics between twins and singletons are observed in other studies, an increase in the proportion of the population exposed to ACS along with variation in practice of neonatal care in each country might have complexly contributed to the interaction between ACS and plurality as well as the inherent risk of a twin pregancy.[32] Although we adjusted potential confounders which were different between twins and singletons in the analyses, different population characteristics between twins and singletons and/or between studies should be considered when interpreting our results.

Our study has several limitations. Firstly, the KNN registry had no information about chorionicity in multiple pregnancies. Thus, we were unable to investigate the difference in the effect of ACS therapy on neonatal outcomes according to chorionicity in twins. Secondly, although we used prospective cohort data of preterm infants with VLBW for this study, antenatal information including ACS administration was collected retrospectively because preterm infants were enrolled after they were born. Therefore, it was unknown why ACS was not or incompletely administered in infants who did not receive a complete course of ACS therapy. Notably, the percentage of singletons without exposure to ACS was higher in this study than in other studies.[33 34] Moreover, information on the total amount of ACS administered was not

available in the KNN registry database. Information on total amount of ACS administered will enable a more quantitative analysis of effects of ACS therapy on neonatal outcomes. Lastly, in our cohort, 56.6% of twins were conceived via IVF whereas only 7.1% of singletons were conceived via IVF. Although preterm infants conceived via IVF are known to have comparable morbidity and mortality with their peers conceived via natural pregnancy[35], higher proportion of IVF cases among twins should be taken into account when interpreting our results.

Conclusion

Despite differences in demographic and clinical characteristics according to plurality, ACS therapy administered before birth had comparable positive effects on neonatal outcomes of preterm infants with VLBW regardless of plurality.

Acknowledgements

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Competing interests

None declared.

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Data Availability statement

The Korean Neonatal Network (KNN) Publication Ethics Policy adheres to the following research data management and access guidelines: All information about patients and participating NICUs are confidential. They might be used by individuals for approved research purposes. If an individual or institution that is not affiliated with KNN wants to use the data, it must make an official request to the KNN publication-Ethics Committee and obtain approval from the network.

Author contributions

Seong Phil Bae: Conceptualization, Methodology, Investigation, Writing – Original draft preparation, and Funding acquisition. Won-Ho Han: Investigation, Writing – Review & Editing. Suyeon Park: Formal analysis, Visualization. Young Hwa Jung: Data curation, Writing – Review & Editing. Jee Yoon Park: Writing – Review & Editing. Kyung Joon Oh: Writing – Review &

Editing. Chang Won Choi: Conceptualization, Methodology, Writing – Review & Editing, and Supervision.

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Figure Legends

Figure 1. Flow chart showing the selection of study population.

ACS, antenatal corticosteroid; VLBW, very low birth weight.

Figure 2. Forest plot showing results of logistic regression for effects of antenatal corticosteroids and twins on neonatal outcomes.

(A) 23 to 28 weeks of gestataional age. (B) 29 to 33 weeks of gestational age.

For each neonatal outcome, solid dots represent the aOR and lateral lines represent the 95% CI. [a]Adjusted relative risk (RR) and 95% CI were obtained from Poisson regression models adjusted for gestational age, birthweight, sex, maternal age, maternal diabetes mellitus, maternal hypertension, premature rupture of membrane, cesarean section, and in vitro fertilization using generalized estimation equations..

Abbreviations: ACS, antenatal corticosteroid; CI, confidence interval; IVH, intraventricular hemorrhage; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity.

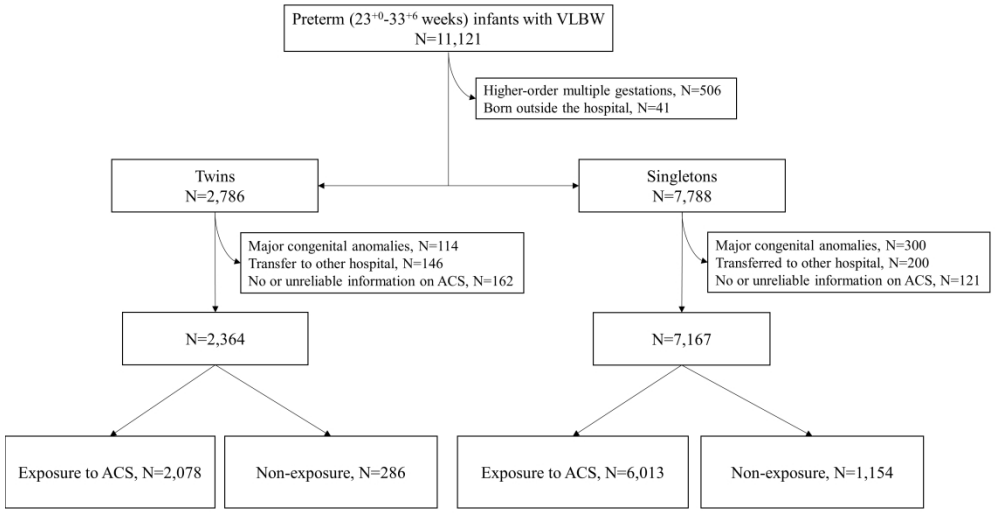

Figure 1. Flow chart showing the selection of study population.
ACS, antenatal corticosteroid; VLBW, very low birth weight.

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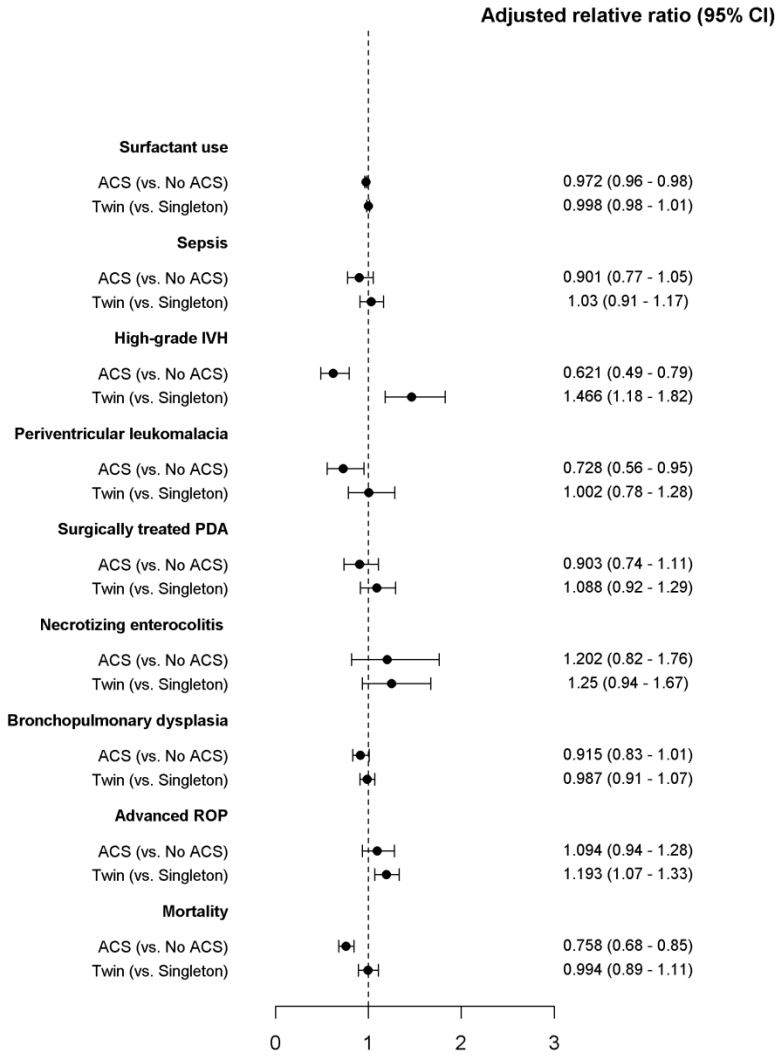

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Abbreviations: ACS, antenatal corticosteroid; CI, confidence interval; IVH, intraventricular hemorrhage; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity.

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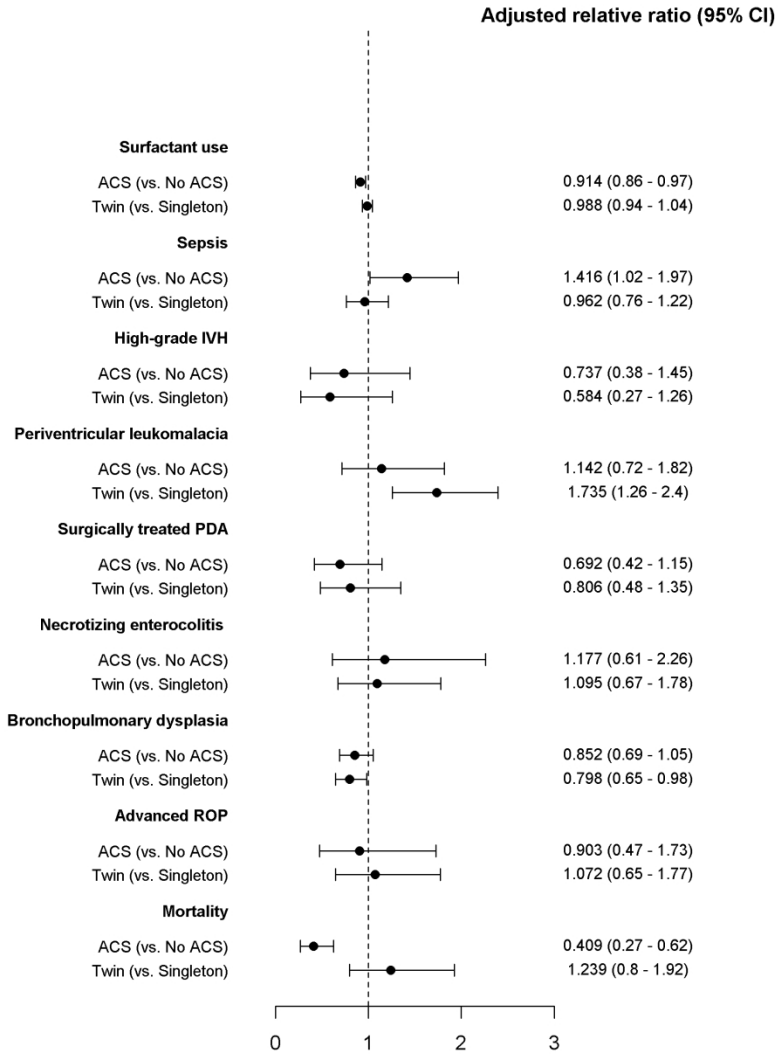

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Supplemental Table 1. Comparison of baseline characteristics between twins and singletons

	23 – 28 weeks of gestational age			29 – 33 weeks of gestational age		
	Twin (n = 1,430)	Singleton (n = 3,977)	*P* value	Twin (n = 934)	Singleton (n = 3,190)	*P* value
Infant						
Gestational age (weeks), mean (SD)	26.63 (1.64)	26.60 (1.58)	0.471	30.19 (0.91)	30.80 (1.34)	0.001<
Birth weight (g), mean (SD)	917 (235)	913 (246)	0.581	1,268 (188)	1,248 (203)	0.005
SGA, n (%)	75 (5.2)	380 (9.6)	0.001<	94 (10.1)	899 (28.2)	0.001<
Male, n (%)	775 (54.2)	2,107 (53.0)	0.434	447 (47.9)	1,564 (49.0)	0.529
Maternal						
Maternal age (years), mean (SD)	33.05 (4.04)	33.33 (4.51)	0.031	33.51 (3.79)	33.36 (4.52)	0.305
Maternal diabetes mellitus, n (%)	138 (9.7)	348 (8.8)	0.268	126 (13.5)	338 (10.6)	0.013
Maternal hypertension, n (%)	74 (5.2)	678 (17.0)	0.001<	144 (15.5)	1357 (42.5)	0.001<
Chorioamnionitis[a], n (%)	446 (37.2)	1701 (49.9)	0.001<	133 (17.7)	734 (27.0)	0.001<
Premature rupture of membrane, n (%)	524 (37.0)	1770 (44.8)	0.001<	347 (37.4)	876 (27.6)	0.001<
Cesarean section, n (%)	1247 (87.2)	2823 (71.0)	0.001<	834 (89.3)	2618 (82.1)	0.001<
In vitro fertilization, n (%)	768 (54.8)	348 (8.8)	0.001<	512 (55.8)	197 (6.2)	0.001<
Antenatal corticosteroid, n (%)	1256 (87.8)	3331 (83.8)	0.001<	822 (88.0)	2,682 (84.1)	0.003

Data are presented as mean (SD) or n (%).

Abbreviations: ACS, antenatal corticosteroid; SGA, small for gestational age; IVH, intraventricular hemorrhage; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity.

[a] Values were missing for 797 infants in the group with 23 to 28 weeks of gestational age and 657 infants in the group with 29 to 33 weeks of gestational age.

Supplemental text 1. The definition of variables

Small for gestational age (SGA) was defined as birth weight below the 10th percentile for gestational age according to the Fenton growth chart.[1] Sepsis was defined as a case of positive blood culture and requiring systemic antibiotics treatment for more than five days. Chorioamnionitis was defined as histologic findings of acute inflammation in the chorion-decidua, amnion, umbilical cord, and chorionic plate by pathologist at each participating facility using the criteria of Salafia et al.[2] modified by Yoon et al.[3] Maternal hypertension included pre-existing hypertension and/or pregnancy-induced hypertension. Maternal diabetes mellitus (DM) included pre-existing and/or pregnancy-induced DM. High-grade intraventricular hemorrhage (IVH) was defined as grade 3 or 4 IVH according to Papile's criteria.[4] Periventricular leukomalacia (PVL) was diagnosed based on brain ultrasound or magnetic resonance imaging obtained at term-equivalent age. Only cystic lesions were counted. Surgically treated patent ductus arteriosus (PDA) was defined as surgical ligation or division of symptomatic PDA. Necrotizing enterocolitis (NEC) was diagnosed and staged according to modified Bell's criteria.[5] Only NEC of stage 2 or higher was counted. Bronchopulmonary dysplasia (BPD) was defined as a need for supplementary oxygen at 36 weeks postmenstrual age (PMA) or discharge according to the National Heart, Lung, and Blood Institute (NHLBI) workshop definition.[6] Advanced retinopathy of prematurity (ROP) was defined as stage 3 or higher according to the International Classification for Retinopathy of Prematurity[7] or having an operation (cryotherapy, laser photocoagulation, or vitrectomy), or intravitreal injection with anti-vascular endothelial growth factor.[8]

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First of all, we would like to thank the reviewers for their constructive criticism of our paper. Based on the advice of reviewers, the paper was revised by enlarging the study population and adopting a new analysis method. We also answered the comments from the reviewers.

Reviewer: 1

Comments to the Author

Thank you for the opportunity to review this manuscript from Bae et al and the Korean Neonatal Network. I follow the ACS literature closely and I appreciate the authors scholarship and efforts. I quite enjoy database scouring for useful insights, thus the authors are to be credited.

The manuscript is generally clear and well-written in translation, and the references are good except in a few instances.

This is a good manuscript but I believe can become a more helpful manuscript with some reasonable modifications. This will strengthen its utility for discerning ADC readers, especially those of us who rely on large data sets to guide potentially better practice selections, and those of us increasingly concerned by ACS misuse and misunderstanding.

1. The KNN database is unusual in one obvious aspect - a strikingly low ACS usage rate overall. The KNN ACS usage is far below that of the Vermont Oxford Network and the NICHD Neonatal Network. Figure 1 documents this - 1316 of 2786 twins got either no or incomplete ACS,....and 3774 of 7788 singletons got either no or incomplete ACS. The authors need to address this more explicitly and reference the VON and NICHD ACS rates 2014 to the present. In the VON collaborative our NICUs participate in the ACS rate in VLBWs is >90 %.
This KNN report is a "natural experiment" 2014-2019 - i.e., "What Does Low ACS Use Look Like".

: Thank you for your comments.

As you mentioned, a recent study of 187,187 VLBW infants born from 2014 to 2018 enrolled in the VON database reported that infant who received ACS therapy was about 86.7%. (Katherine Culbreath et al. Impact of concomitant necrotizing enterocolitis on mortality in very low birth weight infants with intraventricular hemorrhage, PMID: 35715599). In a study using the database of the Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network from 2013 to 2018, Bell et al. reported that 9,571 (88.1%) of 10,867 infants born between 22 weeks and 28 weeks of GA were exposed to ACS. (Bell et al., Mortality, In-Hospital Morbidity, Care Practices, and 2-Year Outcomes for Extremely Preterm Infants in the US, 2013-2018, PMID : 35040888). And, In a study of Australia and New Zealand, exposure rate of ACS in epoch 3 (2007-2012) was 89.4% (19,014/21,606, Improving incidence trends of severe intraventricular haemorrhages in preterm infants < 32weeks gestation: Yeo eta al, a cohort study, PMID : 31201252).

Especially in multiple gestations, the study using data from NICHD reported that the ACS administration rate was 88% (6094/6925) in infants born at 22 to 28 weeks of gestational age (Boghossian et al., Association of Antenatal Corticosteroids with mortality, morbidity, and neurodevelopmental outcomes in extremely preterm multiple gestation infants, PMID : 27088897). This result was comparable with our data (shown in supplemental table 1, 87.8% (1,256/1,430) in twins born at 23 to 28 weeks of GA).

However, in singletons born at 23 to 33 weeks of GA, infants who exposed to ACS were 83.9% (6,013/7,167) in our study. This rate was lower than those of the studies mentioned above.

As you suggested, we described the possible reasons for the difference in exposure to ACS between twins and singletons in the discussion section (page number 18-19), and mentioned that the proportion of

singletons exposed to ACS was lower in this study compare to other studies in the limitation section (page number 19).

2. A tremendous addition to their analysis, the issue in most parts of the "First World" is not too little ACS, it's too much, i.e., way too many fetuses are exposed to supra-physiologic levels of corticosteroids (1/2 of all ACS-exposed fetuses deliver >36 weeks). Mounting evidence has been published that ACS adversely effect neu rodevelopment The (Ninan JAMA Pediatrics April 2022).

This KNN report would have added insight if they would stratify their outcomes analyses by GA - especially by just excluding 31 and 32 week infants where the benefits of ACS are actually quite small. I would like to see their data and forest plots with 31 and 32 week infants excluded.

: We completely agree with your opinion. We are also aware that alternative ACS dosage regimens are being tried and reviewed due to concerns about the neurodevelopmental disorders in preterm infants (Schmitz et al. Neonatal outcomes for women at risk of preterm delivery given half dose versus full dose of antenatal betamethasone: a randomised, multicentre, double-blind, placebo-controlled, non-inferiority trial, PMID: 35988568).

As you mentioned, we re-analyzed the outcomes by stratifying the total study population into two gestational age groups (group of 23 to 28 weeks GA and group of 29 to 33 weeks GA). In addition, we revised our analytical approach in response to reviewer 2's comments, and the findings from this new analysis are shown in Figure 2.

3. Page 6 - "rapider" is not an English word. More rapid is better.

: As we revised the paper this time, that sentence was deleted.

4. page 7 - Was complete ACS course ">24 hours and <7 days"?

: In the KNN manual of operations, a complete course of ACS therapy was defined as four doses of intramuscular dexamethasone at 12-hour intervals or two doses of intramuscular betamethasone at 24-hour intervals administered within 7 days before preterm birth. KNN did not collect the time interval from the administration of the last dose of ACS to delivery. In this revised paper, an ACS therapy was defined as the administration of any dose of ACS before preterm delivery, regardless of the type of drug, completion of the course, or timing of dosing.

5. Page 8 - the definitions in "Supplemental text 1" contain several tautologies - maternal diabetes is defined as pre-existing maternal diabetes, ditto maternal hypertension,.....and hypotension is defined by treatment which i s of course error-prone and not scalable.

: In this study, the definition of maternal diabetes included both pre-existing diabetes and gestational diabetes. In the same way, the definition of maternal hypertension included both pre-existing hypertension and pregnancy-induced hypertension. We excluded hypotension from the outcomes because it is error-prone and not scalable as you commented.

6. Reference 6 is not the NICHD reference listed in the Supplemental text 1.

: We corrected the sentence

according to the National Institute of Child Health and Human Development Workshop definition

-> according to the National Heart, Lung, and Blood Institute (NHLBI) workshop definition

7. The manuscript would be more informative to ADC readers if the authors would make reasoned comments about some striking differences in Table 1 compared to USA and European data sets. Examples - high C/Section rates (even in singletons), PVL rates that are double, triple the VON and NICHD rates, why maternal age effects ACS use (quite perplexing), why maternal hypertension and chorioamnionitis are more common in singletons (I don't believe I have ever seen that in a large data set).

: As you advised, we mentioned a higher rate of cesarean section delivery compared to that of other studies in discussion. (page number 19). And we presented rates of all outcomes, including PVL, for twins and singletons in each gestational age group so that the reader may compare the results of this study with those of other studies (Table 2 and 3).

While the birth rate has dramatically decreased in Korea since 2000s, both childbirth age of mother and use of assisted reproductive technology were increased. In this study, the rate of IVF was significantly higher in twins (54.1%, 1,280/2364) than in singletons (7.6%, 545/7,167), and the rate of twin pregnancies through IVF was higher than US, even in singleton pregnancies (Sunderam et al., Assisted Reproductive technology surveillance-United States, 2018., PMID : 35176012). Why older maternal age was associated with higher ACS coverage in our study is not clear. However, older mothers were more often conceived by IVF and delivered via cesarean section. These older mothers usually might have sought more medical attention because of their concern for adverse pregnancy outcome. These characteristics of old mothers might be associated with higher ACS coverage.

As for higher rates of maternal hypertension and chorioamnionitis in singletons, we reviewed the rates of obstetric complications (hypertension and chorioamnionitis) between twins and singletons in the other trials.

In a population-based cohort study of infants born before 32 weeks of GA, maternal hypertension was less frequent in twin pregnancies than in singleton pregnancies. (Papiernik et al, Difference in outcome between twins and singletons born very preterm: results from a population-based European cohort. PMID – 20118116) In addition, a study from Israeli showed that the rate of maternal hypertension in twins (9.7%) was lower than in singletons (26.4%). (Shinwell et al. Excess risk of mortality in very low birthweight triplets: a national, population based study, PMID – 12496224). A study from Australia and New Zealand also reported a higher rate of pregnancy induced hypertension in singletons than multiples (Yeo eta al., Trends in Morbidity and Mortality of Extremely Preterm Multiple Gestation Newborns, PMID: 26169427)

In matched cohort study comparing twin and singleton pregnancies complicated by PPROM, twins had less clinical and histologic chorioamnionitis than singletons. (Ehsanipoor et al., Twin versus singleton pregnancies complicated by preterm premature rupture of membranes, PMID – 21736498) Similarly, one study comparing twins and singletons with PPROM reported that twin were less likely to have clinical chorioamnionitis or placental abruption. (Kibel et al., The natural history of preterm premature rupture of membranes in twin pregnancies, PMID : 27550343)

I am aware of the work and diligence it takes to examine large data sets and share insights that might be helpful to others and advance the efforts of evidence-
based medicine and quality, thus I admire the authors' intentions and scholarship. But lumping 23 to 32 week infants together without more discriminant analyses by GA is crucial,.....23 and 24 week infants are not comparable to 30-31-
32 week infants, presenting them together (even with logistic regression) is misleading and obscures insigh

ts in the extremely premature infant especially.

: Thank you for your comments. As we mentioned above, we performed the re-analyses and presented the results for each gestational age group (GA 23 to 28 weeks group and GA 29 to 33 weeks group). We believe that our findings can be helpful for understanding on the effect of ACS in twins.

Reviewer: 2

Comments to the Author
This is an interesting study about the effect of a complete course of ACS in preterm twins less than 1500 g birth weight, conducted within the Korean Neonatal Network, comparing the results with singletons. As a s econdary aim, the authors also tested the hypothesis that the effects of ACS are the same in boys and girls. They found no (statistically significant) differences either between twins and singletons or between boys a nd girls.

I found 3 major problems that must be addressed before accepting the results presented by the Authors.

The first is the choice of comparing neonates who received a complete course of ACS vs. no treatment. Once started ACS, the course is complete or partial not for a pre-
defined decision but depending on how well the timing of delivery is estimated, how rapid the course of la bour or the need for obstetrical intervention is, or simply on when the woman presents to the hospital. Ther efore, excluding women with partial treatment can produce bias as this is associated with baseline imbalan ces of groups, ie mixes a possible different effect of ACS with something that is extraneous. And this is wh at actually occurs: twins and singletons differ. The distribution of women receiving complete, partial, and no ACS is different between twins and singletons [page 9, lines 40-45]: the chi-
square yields a $p<0.001$. By excluding partial ACS, although fewer twins received a complete course (44.4 % vs 47.3% in singletons), the ratio of complete/no ACS infants is reversed (3.7 for twins vs. 2.9 for single tons).

As the authors acknowledge, singletons and twins are 2 different populations, with different gestational ag es, pregnancy complications and backgrounds, so simply comparing the results between the 2 groups make s no sense.
To try to mimic as well as possible a trial, they should try to have groups as balanced at baseline as possibl e and should analyze any ACS treatment vs no treatment –
leaving the current comparison (complete vs no treatment) as a sensitivity analysis or comparing the 3 con ditions (no ACS, partial and complete treatments).

: Thank you for your comments. We agree with your opinion and re-analyzed the data

At this time, we included infants born from mothers who received a partial ACS treatment in addition to infants born from mothers who received a complete ACS treatment. So ACS therapy was re-defined as at least one dose of any kind of corticosteroids before preterm delivery.

Additionally, we performed the re-analyses by stratifying the total study population into two gestational age group (23 to 28 weeks group and 29-33 weeks group) in response to reviewer 1's comment. We compared the antenatal baseline characteristics between ACS exposed and ACS-unexposed groups in twins and singletons. Variables that showed a significant (p <0.10) difference between twins and singletons groups, and potential confounders were used as covariates in Poisson regression models.

The second point is that when one finds negative (i.e., non-
significant) results, one should try to see what the power of the study was. Here the sample size is not large

(for instance, only 286 untreated twins), so non-significant results are not a surprise.
Thus, I do not think that the authors can claim that their results show that the effect of ACS are the same be tween groups because some OR are strikingly different though not statistically significant. A non-significant difference between treated and not treated groups is no evidence of no effect.

Linked to this, the "standard" way to assess whether the effect of ACS is the same in the 2 groups if one ha s the individual data as in this study, is not to run different logistic models, extract the coefficients, and sta ndard errors and compare them by a z-
test [page 8, line 48], but rather to include the information of the group [singleton/twin; boy/girl) in a gener al model and test for interaction.
Especially for the comparison boys/girls, we are not interested in whether ACS work, but if their effect is d ifferent!
I would therefore suggest that the authors change and simplify their analysis.

Thank you for your comments. Although we did not calculate the statistical power of this study, as you advised, we got a new results by applying a more advanced statistical method.

To investigate whether associations between ACS and neonatal outcomes were altered by plurality (twins versus singletons), the interaction effect between ACS and twin pregnancies on neonatal outcomes was evaluated by adding the interaction terms (ACS × twins) in Poisson regression models that included potential confounders (page number 8). Similar to the results of the previous version of our paper, there was no statistically significant interaction between ACS and twin pregnancies in this revision (Table 2, 3). Furthermore, in the second model excluding the interaction term, we calculated adjusted RR and 95% CI for the individual effect of ACS and twins on each neonatal outcome (Figure 2A, 2B).

The last point is that the literature search on the effects of ACS in twins is inaccurate. The authors cite only a handful of original papers to support their starting point and results, but the literature is much vaster, and their study does not fall into a void. For instance, the sentence "Currently, ACS is equally recommended f or twin pregnancies […] based on a retrospective study [2,3]" [page 6, lines 13-15] is inaccurate.
Below is a meta-
analysis on this topic (antenatal steroids in twins) published in Obstet Gynecol, that can be of interest to th e Authors if they have to rearrange their study for publication.

Antenatal Corticosteroids and Neonatal Outcomes in Twins: A Systematic Review and Meta-analysis.
Socha P, McGee A, Bhattacharya S, Young C, Wang R.
Obstet Gynecol. 2022 Jul 1;140(1):20-30. doi: 10.1097/AOG.0000000000004835. Epub 2022 Jun 7.
PMID: 35849452

: We appreciate your comments. The studies cited by the ACG Committee on the use of ACS in multiple pregnancies were referenced in the sentence you pointed out. As you mentioned, there have been a huge number of studies on the effects of ACS. But, effect of ACS in twins have been reported with conflicting results among studies.

As you advised, we tried to present various research results by citing several studies, such as original papers and meta-analyses, dealing with the ACS effect in twins at this time. By comparing the various findings of numerous large-scale studies, we attempted to show as much unbiased information as possible.

Title page

Original article

Effects of antenatal corticosteroids on neonatal outcomes in very-low-birth-weight infants are not different by plurality: a nationwide cohort study

~~Effects of antenatal corticosteroids on neonatal outcomes in very-low-birth-weight infants are not different by plurality or sex: a nationwide cohort study~~

Seong Phil Bae[1,2], M.D., Won-Ho Hahn[1,2], M.D., Ph.D., Suyeon Park[3,4], MS., Young Hwa Jung[5,6], M.D., Ph.D., Jee Yoon Park[7], M.D., Kyung Joon Oh[7], M.D., Ph.D., Chang Won Choi[5,6], M.D., Ph.D.

[1]Department of Pediatrics, Soonchunhyang University Seoul Hospital, Seoul, Republic of Korea

[2]Department of Pediatrics, Soonchunhyang University School of Medicine, Asan, Republic of Korea

[3]Department of Biostatistics, Soonchunhyang University Seoul Hospital, Seoul, Republic of Korea

[4]Department of Applied Statistics, Chung-Ang University, Seoul, Republic of Korea

[5]Department of Pediatrics, Seoul National University Bundang Hospital, Seongnam, Republic of Korea

[6]Department of Pediatrics, Seoul National University College of Medicine, Seoul, Republic of Korea

[7]Department of Obstetrics and Gynecology, Seoul National University Bundang Hospital, Seongnam, Republic of Korea

Correspondence to

Chang Won Choi, M.D., Ph.D.

Department of Pediatrics, Seoul National University Bundang Hospital, 82 Gumi-ro 173 Beon-gil, Bundang-gu, Seongnam-si, Gyeonggi-do, Republic of Korea, E-mail: choicw@snu.ac.kr

Word count: ~~2417~~2356

ABSTRACT

Objective

To investigate whether effects of antenatal corticosteroids on neonatal outcomes in preterm infants with very-low-birth-weight were different by plurality.

~~To investigate whether effects of a complete course of antenatal corticosteroid therapy administered within 7 days before preterm birth on neonatal outcomes were different by plurality and sex in preterm infants with very low birth weight.~~

Design

Nationwide prospective cohort study

~~Retrospective cohort study~~

Setting

Not applicable

~~Sixty-one neonatal intensive care units participating in the Korean Neonatal Network~~

Patients

Twins and singletons with very-low-birth-weight (< 1,500 g) who were born between 23^{+0} and 33^{+6} weeks of gestation and registered in the Korean Neonatal Network from January 2014 to December 2019

~~Twins or singletons with very low birth weight (< 1,500 g) born between 23^{+0} and 33^{+6} weeks of gestation from January 2014 to December 2019~~

Main outcome measures

Morbidity and mortality before discharge from neonatal intensive care unit

Results

Among a total of 9,531 preterm infants with very-low-birth-weight, there were 2,364 (24.8%) twins and 7,167 (75.2%) singletons. While 83.9% of singletons were exposed to at least one dose of antenatal corticosteroids, so were 87.9% of twins.

Interaction analysis demonstrated that there was no significant difference in the effect of antenatal corticosteroids on morbidities or mortality between twins and singletons in either gestational age group (23 to 28 weeks or 29 to 33 weeks).

Antenatal corticosteroids significantly decreased the risk of surfactant use, high-grade intraventricular hemorrhage, periventricular leukomalacia, and mortality in the gestational age group of 23 to 28 weeks. In the gestational age group of 29 to 33 weeks, antenatal corticosteroids significantly decreased the risk of surfactant use and mortality but increased the risk of sepsis.

~~A complete course of antenatal corticosteroid therapy was administered to 44.4% (1,050/2,366) of twins and 47.3% (3,393/7,167) of singletons. Antenatal corticosteroid therapy was significantly associated with lower odds of respiratory distress syndrome, early hypotension, high-grade intraventricular hemorrhage, and mortality in both twins and singletons. Decreased~~

~~odds of periventricular leukomalacia and increased odds of intact survival without serious morbidities associated with antenatal corticosteroid therapy were observed only in singletons. However, the effect size of antenatal corticosteroid therapy on each neonatal outcome was not significantly different between twins and singletons. Furthermore, it did not differ significantly by sex either in twins or singletons.~~

Conclusion

This study demonstrates that effect of antenatal corticosteroids on neonatal outcomes of preterm infants with very-low-birth-weight does not differ significantly by plurality (twin or singleton pregnancy).

~~Effects of antenatal corticosteroids on neonatal outcomes of preterm infants with very low birth weight were not significantly different by plurality or sex.~~

Keywords: antenatal corticosteroids, preterm delivery, mortality, morbidity, twins, very low birth weight infant

Key messages

Why is already known on this topic?

Antenatal corticosteroids (ACS) administered before preterm delivery can decrease neonatal morbidity and mortality in singleton pregnancies. However, data about such effects of ACS in

multiple pregnancies are still limited.

- ~~**While effects of antenatal corticosteroids in singleton pregnancies have been well demonstrated, data regarding effects of antenatal corticosteroids on neonatal outcomes according to fetal number and sex are limited**~~

What this study adds?

The effect of antenatal corticosteroids administered before preterm delivery on neonatal morbidity and mortality does not differ by plurality (twin or singleton pregnancy).

- ~~This study provides additional evidence for comparable effects of antenatal corticosteroids on neonatal outcomes of twin pregnancies and singleton pregnancies, especially when the offspring were born with VLBW.~~
- ~~This study demonstrated that effects of antenatal corticosteroids on neonatal outcomes are not significantly different by sex of the offspring, either in twin or single pregnancies.~~

Introduction

Antenatal corticosteroid (ACS) has been established as the standard therapy for pregnant women at risk of preterm delivery within 7 days between 24^{+0} and 33^{+6} weeks of gestation. [1] Currently, ACS is equally recommended for twin and singleton pregnancies ~~twin pregnancies~~ with the same regimen based on a retrospective cohort study. [2 3] A recent study has shown that ~~In that study,~~ a complete course of ACS was associated with a significant decrease in neonatal mortality, short-term respiratory morbidity, and severe neurological injury in a magnitude similar to those observed for singletons.[3] However, data that demonstrate comparable effects of ACS therapy in twin pregnancies are limited ~~data on effects of ACS therapy on neonatal outcomes according to fetal number are still lacking~~ [4 5].

As a result of increasing childbirth age and use of assisted reproductive technology, twin pregnancies are increasing in many countries including Korea. [6 7] However, twin pregnancies are more likely to have preterm birth, low birth weight, and longer hospital stays than singleton pregnancies~~Twin pregnancies are associated with higher risks of preterm birth and low birth weight than singleton pregnancies~~.[8 9] In addition, mono-chorionicity, birth weight discordance, and obstetric complications associated with multiple pregnancies may result in adverse perinatal outcomes~~Because the current regimen of ACS therapy has been optimized for singleton pregnancy, whether the same regimen is equally effective in twin pregnancies remains unclear~~.[10]

Recently, the mortality of extreme preterm multiples has decreased to a level comparable to that of singletons born at the same gestational age along with increased use of ACS therapy~~In addition to fetal number, fetal sex may affect effects of ACS on neonatal outcomes. Estrogen is~~

known to impact fetal lung development and surfactant production.[11 12] However, many studies have reported conflicting results regarding equivalent effects of ACS on neonatal outcomes of twins and singletons.Preterm female infants have been reported to have lower respiratory morbidity and neonatal mortality than their male counterparts.[13] To date, there have been no clinical trials designed to investigate the effect of ACS in twins. A meta-analysis on ACS therapy has also highlighted the need for additional studies on multiple pregnanciesGiven that preterm male infants have experienced rapider declines in respiratory morbidities and mortality over the past decade than their female counterparts coinciding with the widespread use of ACS therapy, it can be speculated that male preterm infants might benefit from ACS therapy more than their female counterparts.[14]

Thus, the purpose of this study was to investigate whether effects of ACS administered before preterm delivery on neonatal outcomes of twins and singletons were comparable.

The primary purpose of this study was to investigate whether effects of a complete course of ACS therapy administered within 7 days before preterm birth on neonatal outcomes were comparable between twins and singletons. The secondary purpose was to examine whether effects of ACS therapy on neonatal outcomes might differ by sex.

Materials and Methods

Study design and participants

Information on the study population is presented in Figure 1. Data collected prospectively

through the Korean Neonatal Network (KNN), a nationwide very low birth weight (VLBW, <1,500 g) infant registry participated by 61 neonatal intensive care units across South Korea [15] were used for this study. A total of 11,121 infants with VLBW born preterm (between 23^{+0} and 33^{+6} weeks of gestation) between January 2014 and December 2019 were enrolled. Exclusion criteria were: high-order multiple gestations (≥triplets), birth outside the hospital, major congenital anomalies, transfer to other hospitals, no or unreliable information on ACS~~, and incomplete ACS therapy~~. Finally, 2,364 twins and 7,167 singletons were analyzed ~~1,336 twins and 4,547 singletons were analyzed~~. ACS therapy was defined if the pregnant women had received at least one dose of any kind of corticosteroids before preterm delivery.

~~A complete course of ACS therapy was defined as four doses of intramuscular dexamethasone at 12-hour intervals or two doses of intramuscular betamethasone at 24-hour intervals administered within 7 days before preterm birth. Otherwise, the administration of any ACS was defined as an incomplete therapy.~~

Variables collected included ~~In this study, the following variables were collected:~~ gestational age, birth weight, small for gestational age (SGA), sex, maternal age, maternal diabetes mellitus (DM), maternal hypertension, chorioamnionitis, premature rupture of membrane, cesarean section, in vitro fertilization (IVF), ~~Apgar score at 5 minutes,~~ surfactant use~~respiratory distress syndrome (RDS)~~, ~~hypotension during the first week of life,~~ sepsis, high-grade intraventricular hemorrhage (IVH), periventricular leukomalacia (PVL), surgically treated patent ductus arteriosus (PDA), bronchopulmonary dysplasia (BPD), ~~treated~~ advanced retinopathy of prematurity (ROP), and mortality~~ before discharge~~. Definitions for different variables are

provided in Supplemental Materials. The analysis of neonatal morbidity was performed for infants who survived before discharge, while the analysis of mortality was performed for the total population.

~~Each variable is defined in the supplemental materials.~~

Statistical methods

Rates of neonatal outcomes between infants exposed to ACS and infants without such exposure are presented along with risk difference and 95% CIs. To investigate whether associations between ACS and neonatal outcomes were altered by plurality (twins versus singletons), the interaction effect between ACS and twin pregnancies on neonatal outcomes was evaluated by adding interaction terms (ACS × twins) in Poisson regression models that included potential confounders, such as gestational age, birth weight, sex, maternal age, maternal diabetes mellitus, maternal hypertension, premature rupture of membrane, cesarean section, and *in vitro* fertilization. Missing values for chorioamnionitis were substantial (17.6% in twins and 14.5% in singletons). They were excluded from the analyses. In the second model excluding the interaction term to investigate the individual effect of ACS and twins, crude and adjusted relative risk (RR) and 95% CI were calculated by Poisson regression analyses with selection of variables that showed minimal QIC (Quasi-likelihood under the Independence model Criterion) for each outcome. These models were fitted with generalized estimation equations (GEE) approach for the correlation between a pair of twins from a mother. The level of significance was set at $p < 0.05$. All statistical analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA) and R package version 3.3.1 (The R Foundation for Statistical Computing, Vienna,

Austria).

Continuous variables were analyzed using Student's *t*-test. Categorical variables were analyzed by *Chi-squared* test or *Fisher's exact* test. Multivariable logistic regression analyses were performed to compare neonatal outcomes between infants who received ACS therapy and infants who did not receive ACS therapy after adjusting for covariates. Baseline characteristics that showed a significant (p <0.10) difference between infants who received ACS therapy and infant who did not in univariate analysis and potential confounders were used as covariates in multivariate logistic regression analyses. The level of significance was set at p <0.05. Generalized estimation equations (GEE) were used to access associations while adjusting for correlation of outcomes in twins. To compare differences in effects of ACS therapy on neonatal outcomes by plurality, Z-score was calculated for each neonatal outcome using the following formula: $(X1 - X2)/\sqrt{(SE1)^2 + (SE2)^2}$, where X1 was β coefficient of ACS therapy for twins, X2 was β coefficient of ACS for singletons, SE1 was standard error of twins, and SE2 was standard error of singletons. For comparisons by sex, the same statistics was used. The significance of the Z-test was then assessed using two-sided p-values at 5% significance level. All statistical analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA) and R package version 3.3.1 (The R Foundation for Statistical Computing, Vienna, Austria).

Ethic approval

The registration of data in the KNN was approved by the Institutional Review Board (IRB) of each participating center. Informed consent was obtained from parents of each infant prior to participation in the KNN registry. This study was approved by the IRB of Seoul National University Bundang Hospital (approval number: B-1305-202-005).

Results

Exposure to antenatal corticosteroids

~~**Antenatal corticosteroid therapy**~~

Among 2,366 twins, 2,078 (87.9%) infants were exposed to at least one dose of ACS before preterm delivery. Among 7,167 singletons, 6,013 (83.9%) infants were exposed to at least one dose of ACS before preterm delivery (Figure 1).~~1,050 (44.4%), 1,030 (43.5%), and 286 (12.1%) infants received a complete course of ACS therapy, an incomplete course of ACS therapy, and no ACS, respectively (Figure 1). Among 7,167 singletons, 3,393 (47.3%) 2,620 (36.6%), and 1,154 (16.1%) infants received a complete course of ACS therapy, an incomplete course of ACS therapy, and no ACS, respectively. Comparisons were done between infants who received a complete course of ACS therapy and those who did not receive ACS.~~

Comparison of baseline characteristics between infants exposed to antenatal corticosteroids and infants without ACS exposure

~~and neonatal outcomes between twins and singletons~~

Twins exposed to ACS were born at a significantly later gestational age compared to those without ACS exposure (Table 1). However, there was no significant difference in gestational age or birth weight between singletons exposed to ACS and those without ACS exposure. Singletons exposed to ACS had significantly higher rates of maternal DM, maternal hypertension, and cesarean section than those without exposure. In both twins and singletons, infants exposed to

Table 1. Comparisons of perinatal baseline characteristics between infants exposed to ACS and infants without ACS exposure

	Twins (n = 2,364)			Singletons (n = 7,167)		
	ACS-exposed (n = 2,078)	ACS-unexposed (n = 286)	*P* value	ACS-exposed (n = 6,013)	ACS-unexposed (n = 1,154)	*P* value
Infant						
Gestational age (weeks), mean (SD)	28.08 (2.19)	27.75 (2.50)	0.034	28.49 (2.52)	28.39 (2.78)	0.256
Birth weight (g), mean (SD)	1,059 (274)	1,034 (301)	0.186	1,060 (281)	1,073 (289)	0.138
SGA, n (%)	147 (7.1)	22 (7.7)	0.704	1080 (18.0)	199 (17.2)	0.560
Male, n (%)	1079 (51.9)	143 (50.0)	0.541	3074 (51.1)	597 (51.7)	0.708
Maternal						
Maternal age (years), mean (SD)	33.41 (3.80)	31.91 (4.66)	<0.001	33.44 (4.43)	32.82 (4.92)	<0.001
Maternal diabetes mellitus, n (%)	238 (11.5)	26 (9.1)	0.221	597 (9.9)	89 (7.7)	0.019
Maternal hypertension, n (%)	196 (9.5)	22 (7.7)	0.329	1749 (29.1)	286 (24.8)	0.003
Chorioamnionitis[a], n (%)	516 (29.9)	63 (28.0)	0.551	2084 (40.2)	351 (37.0)	0.064
Premature rupture of membrane, n (%)	787 (38.1)	84 (29.9)	0.007	2334 (38.9)	312 (27.5)	<0.001
Cesarean section, n (%)	1837 (88.4)	244 (85.3)	0.132	4634 (77.1)	807 (69.9)	<0.001
In vitro fertilization, n (%)	1152 (56.6)	128 (45.1)	<0.001	494 (8.2)	51 (4.4)	<0.001

Data are presented as mean (SD) or n (%).

Abbreviations: ACS, antenatal corticosteroids; SGA, small for gestational age; IVH, intraventricular hemorrhage; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity.

[a] Values were missing for 415 infants in the group of twins and 1039 infants in the group of singletons.

Maternal and neonatal characteristics were different between twins and singletons (Table 1). Gestational age and birth weight of twins were lower than those of singletons. Infants who were born SGA were significantly more common in singletons than in twins. Rates of Cesarean section and *in-vitro* fertilization (IVF) were significantly higher, whereas rates of maternal hypertension and chorioamnionitis were significantly lower in twins than in singletons. Rates of RDS, hypotension during the first weeks of life, high-grade IVH, surgically treated PDA, and treated ROP were significantly higher in twins than in singletons. Mortality was significantly higher while intact survival without serious morbidities was significantly lower in twins than in singletons. However, there were no significant differences in rates of sepsis, PVL, NEC, or BPD between twins and singletons. ACS were more likely to have higher rates of premature rupture of membrane and be conceived through IVF from older mothers than those without exposure.

Table 1. Comparison of baseline characteristics and neonatal outcomes between twins and singletons

	Twins (n = 1,336)	Singletons (n = 4,547)	*P*-value
Baseline characteristics			
Gestational age (weeks), mean (SD)	27.9 (2.3)	28.5 (2.6)	<0.001
Birth weight (g), mean (SD)	1,036 (285)	1,063 (285)	0.003
SGA, n (%)	108 (8.1)	849 (18.7)	<0.001
Male, n (%)	676 (50.6)	2300 (50.6)	0.992
Maternal age (years), mean (SD)	33.26 (3.91)	33.24 (4.49)	0.865
Maternal diabetes mellitus, n (%)	132 (9.9)	411 (9.0)	0.326
Maternal hypertension, n (%)	124 (9.4)	1316 (28.9)	<0.001
Chorioamnionitis[a], n (%)	337 (30.3)	1519 (38.8)	<0.001
Premature rupture of membrane, n (%)	493 (37.2)	1703 (37.7)	0.741
Cesarean section, n (%)	1173 (87.8)	3381 (74.4)	<0.001
In vitro fertilization, n (%)	740 (56.6)	325 (7.1)	<0.001
Neonatal outcomes			

Apgar score at 5 min, mean (SD)	6.6 (1.9)	6.9 (1.9)	<0.001
Respiratory distress syndrome, n (%)	1153 (86.3)	3643 (80.1)	<0.001
Hypotension during the first week of life, n (%)	391 (29.3)	1106 (24.3)	<0.001
Sepsis, n (%)	284 (21.3)	903 (19.9)	0.278
High-grade IVH, n (%)	136 (10.7)	331 (7.5)	<0.001
Periventricular leukomalacia, n (%)	104 (8.2)	300 (6.8)	0.100
Surgically treated PDA, n (%)	152 (11.4)	427 (9.4)	0.032
Necrotizing enterocolitis, n (%)	81 (6.1)	264 (5.9)	0.726
Bronchopulmonary dysplasia[b], n (%)	394 (34.5)	1289 (32.3)	0.152
Treated ROP[c], n (%)	175 (15.2)	433 (10.7)	<0.001
Mortality, n (%)	210 (15.7)	613 (13.5)	0.038
Intact survival without serious morbidities[d], n (%)	643 (48.1)	2418 (53.2)	0.001

Data are presented as mean (SD) or n (%).

Abbreviations: SGA, small for gestational age; IVH, intraventricular hemorrhage; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity.

[a] Values were missing for 223 infants in the group of twins and 633 infants in the group of singletons.

[b] Values were missing for 195 infants in the group of twins and 553 infants in the group of singletons.

[c] Values were missing for 187 infants in the group of twins and 518 infants in the group of singletons.

[d] Serious morbidity: High-grade IVH, periventricular leukomalacia, bronchopulmonary dysplasia, treated ROP

Interaction between ACS and twins pregnancies on neonatal outcomes

Comparisons of baseline characteristics between infants who received antenatal corticosteroid therapy and infants who did not

We investigated whether effects of ACS on neonatal outcomes differed between twins and singletons. Because neonatal outcomes are substantially dependent on gestational age, comparison of ACS effects on neonatal outcomes by plurality was performed by stratifying the study population into two gestation age groups: an age group of 23 to 28 weeks and an age group of 29 to 33 weeks group.

Among infants in the age group of 23 to 28 weeks, exposure to ACS for twins was significantly

associated with a lower rate of mortality, but not with morbidity. However, exposure to ACS for singletons was significantly associated with a lower mortality and a lower morbidity including surfactant use, high-grade IVH, and PVL (Table 2). Among infants in the age group of 29 to 33 weeks, exposure to ACS for twins was significantly associated with lower rates of mortality and surfactant use (Table 3). Exposure to ACS for singletons was significantly associated with a lower rate of mortality, but a higher rate of sepsis. In interaction analyses, there was no significant difference in the association of ACS therapy with any neonatal outcomes between twins and singletons in either gestational age group.

Table 2 Comparisons of perinatal characteristics between infants who received antenatal corticosteroid and infants who did not

	Twins (n = 1,336)			Singletons (n = 4,547)		
	ACS (n = 1,050)	No ACS (n = 286)	*P* value	ACS (n = 3,393)	No ACS (n = 1154)	*P* value
Baseline characteristics						
Gestational age (weeks), mean (SD)	27.98 (2.26)	27.75 (2.50)	0.160	28.58 (2.52)	28.39 (2.78)	0.035
Birth weight (g), mean (SD)	1,037 (281)	1,034 (301)	0.888	1,059 (284)	1,073 (289)	0.143
SGA, n (%)	86 (8.2)	22 (7.7)	0.784	650 (19.2)	199 (17.2)	0.150
Male, n (%)	533 (50.8)	143 (50.0)	0.819	1703 (50.2)	597 (51.7)	0.366
Maternal age (years), mean (SD)	33.6 (3.60)	31.91 (4.66)	<0.001	33.38 (4.33)	32.82 (4.92)	0.001
Maternal diabetes mellitus, n (%)	106 (10.2)	26 (9.1)	0.594	322 (9.5)	89 (7.7)	0.069
Maternal hypertension, n (%)	102 (9.8)	22 (7.7)	0.277	1030 (30.4)	286 (24.8)	<0.001
Chorioamnionitis[a], n (%)	274 (30.9)	63 (28.0)	0.405	1168 (39.4)	351 (37.0)	0.195
Premature rupture of memebrane, n (%)	409 (39.2)	84 (29.9)	0.004	1391 (41.2)	312 (27.5)	<0.001
Cesarean section, n (%)	929 (88.5)	244 (85.3)	0.148	2574 (75.9)	807 (69.9)	<0.001
In vitro fertilization, n (%)	612 (59.8)	128 (45.1)	<0.001	274 (8.1)	51 (4.4)	<0.001
Neonatal outcomes						
Apgar score at 5 min	6.75 (1.82)	6.19 (1.95)	<0.001	7.04 (1.74)	6.34 (2.12)	<0.001
Respiratory distress syndrome, n (%)	889 (84.7)	264 (92.3)	0.001	2683 (79.1)	960 (83.2)	0.002
Hypotension during the 1st week of life, n (%)	284 (27.0)	107 (37.4)	0.001	758 (22.3)	348 (30.2)	<0.001
Sepsis, n (%)	220 (21.0)	64 (22.4)	0.617	683 (20.2)	220 (19.3)	0.526

High-grade IVH, n (%)	94 (9.3)	42 (16.1)	0.002	199 (6.0)	132 (12.3)	<0.001
Periventricular leukomalacia, n (%)	78 (7.7)	26 (10.0)	0.238	204 (6.1)	96 (9.0)	0.001
Surgically treated PDA, n (%)	115 (11.0)	37 (12.9)	0.349	314 (9.3)	113 (9.8)	0.589
Necrotizing enterocolitis, n (%)	65 (6.2)	16 (5.7)	0.728	202 (6.0)	62 (5.4)	0.494
Bronchopulmonary dysplasia[b], n (%)	323 (35.0)	71 (32.6)	0.498	982 (32.2)	307 (32.5)	0.852
Treated ROP[c], n (%)	140 (15.1)	35 (15.9)	0.755	342 (11.1)	91 (9.5)	0.172
Mortality, n (%)	137 (13.0)	73 (25.5)	<0.001	391 (11.5)	222 (19.2)	<0.001
Intact survival without serious morbidities[d], n (%)	522 (49.7)	121 (42.3)	0.026	1,865 (55.0)	553 (47.9)	<0.001

Data are presented as mean (SD) or n (%).

Abbreviations: ACS, antenatal corticosteroid; SGA, small for gestational age; IVH, intraventricular hemorrhage; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity.

[a] Values were missing for 223 infants in the group of twins and 633 infants in the group of singletons.

[b] Values were missing for 195 infants in the group of twins and 553 infants in the group of singletons.

[c] Values were missing for 187 infants in the group of twins and 518 infants in the group of singletons.

[d] Serious morbidity: High-grade IVH, periventricular leukomalacia, bronchopulmonary dysplasia, treated ROP

Table 2. Comparisons of neonatal outcomes between infants exposed to ACS and infants without ACS exposure in infants born at 23 to 28 weeks of gestational age

Infants who survived before discharge (n = 4160)	ACS-exposed (n=3606)	ACS-unexposed (n=554)	Risk difference, % (95 CI)	*P* value[a]	*P* value for interaction[b]
Surfactant use, n (%)					
Total	3468/3606 (96.2%)	546/554 (98.6%)	-2.4 (-3.6 to -1.2)	0.005	
Twin	952/991 (96.1%)	108/109 (99.1%)	-3.0 (-5.2 to -0.9)	0.110	0.199
Singleton	2516/2615 (96.2%)	438/445 (98.4%)	-2.2 (-3.6 to -0.8)	0.018	
Sepsis, n (%)					
Total	985/3606 (27.3%)	166/554 (30.0%)	-2.7 (-6.7 to 1.4)	0.195	
By numbers of fetus					
Twin	273/991 (27.5%)	32/109 (29.4%)	-1.8 (-10.8 to 7.2)	0.689	0.706
Singleton	712/2615 (27.2%)	134/445 (30.1%)	-2.9 (-7.5 to 1.7)	0.209	
High-grade IVH, n (%)					
Total	313/3606 (8.7%)	79/554 (14.3%)	-5.6 (-8.6 to -2.5)	<0.001	
By numbers of fetus					
Twin	115/991 (11.6%)	15/109 (13.8%)	-2.2 (-8.9 to 4.6)	0.508	0.224
Singleton	198/2615 (7.6%)	64/445 (14.4%)	-6.8 (-10.2 to -3.4)	<0.001	
Periventricular leukomalacia, n (%)					
Total	327/3606 (9.1%)	74/554 (13.4%)	-4.3 (-7.3 to -1.3)	0.001	
By numbers of fetus					
Twin	86/991 (8.7%)	14/109 (12.8%)	-4.2 (-10.7 to 2.4)	0.151	0.749
Singleton	241/2615 (9.2%)	60/445 (13.5%)	-4.3 (-7.6 to -0.9)	0.005	
Surgically treated PDA, n (%)					
Total	616/3606 (17.1%)	99/554 (17.9%)	-0.8 (-4.2 to 2.6)	0.647	
By numbers of fetus					
Twin	175/991 (17.7%)	23/109 (21.1%)	-3.4 (-11.5 to 4.6)	0.375	0.400
Singleton	441/2615 (16.9%)	76/445 (17.1%)	-0.2 (-4.0 to 3.6)	0.911	
Necrotizing enterocolitis, n (%)					
Total	268/3606 (7.4%)	36/554 (6.5%)	0.9 (-1.3 to 3.2)	0.432	
By numbers of fetus					
Twin	72/991 (7.3%)	8/109 (7.3%)	-0.07 (-5.2 to 5.1)	0.978	0.479
Singleton	196/2615 (7.5%)	28/445 (6.3%)	1.2 (-1.3 to 3.7)	0.368	

Bronchopulmonary dysplasia, n (%)					
Total	1664/3606 (46.1%)	269/554 (48.6%)	-2.4 (-6.9 to 2.1)	0.290	
By numbers of fetus					
Twin	437/991 (44.1%)	51/109 (46.8%)	-2.7 (-12.6 to 7.2)	0.591	0.986
Singleton	1227/2615 (46.9%)	218/445 (49.0%)	-2.1 (-7.1 to 3.0)	0.419	
Advanced ROP, n (%)					
Total	769/3602 (21.3%)	113/554 (20.4%)	1.0 (-2.7 to 4.6)	0.610	
By numbers of fetus					
Twin	232/990 (23.4%)	31/109 (28.4%)	-5.0 (-13.9 to 3.9)	0.245	0.158
Singleton	537/2612 (20.6%)	82/445 (18.4%)	2.1 (-1.8 to 6.1)	0.301	
All infants (n = 5407)	ACS (n=4587)	No ACS (n=820)	Risk difference, %(95 CI)	*P* value	*P* value for interaction*
Mortality, n (%)					
Total	981/4587 (21.4%)	266/820 (32.4%)	-11.1 (-14.5 to -7.6)	<0.001	
By numbers of fetus					
Twin	265/1256 (21.1%)	65/174 (37.4%)	-16.3 (-23.8 to -8.7)	<0.001	0.458
Singleton	716/3331 (21.5%)	201/646 (31.1%)	-9.6 (-13.5 to -5.8)	<0.001	

Data are presented as n (%).

[a] Tests for risk difference within each subgroup.

[b] Tests for interaction between antenatal corticosteroids and twin pregnancies on each outcome. *P*-values for interaction were obtained from multivariable Poisson regression models adjusted for gestational age, birthweight, sex, maternal age, maternal diabetes mellitus, maternal hypertension, premature rupture of membrane, cesarean section, and *in vitro* fertilization using the generalized estimation equations.

Abbreviations: ACS, antenatal corticosteroid; SGA, small for gestational age; IVH, intraventricular hemorrhage; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity.

Table 3. Comparisons of neonatal outcomes between infants exposed to ACS and infants without ACS exposure in infants born at 29 to 33 weeks of gestational age

Infants who survived before discharge (n = 4,019)	ACS-exposed (n=3428)	ACS-unexposed (n=591)	Risk difference, % (95 CI)	*P*-value[a]	*P*-value for interaction[b]

Surfactant use, n (%)					
Total	2240/3428 (65.3%)	402/591 (68.0%)	-2.7 (-6.8 to 1.4)	0.206	
By numbers of fetus					
Twin	583/801 (72.8)	86/104 (82.7)	-9.9 (-17.8 to -2.0)	0.030	0.243
Singleton	1657/2627 (63.1)	316/487 (64.9)	-1.8 (-6.4 to 2.8)	0.446	
Sepsis, n (%)					
Total	376/3428 (11.0)	41/591 (6.9)	4.0 (1.7 to 6.3)	0.003	
By numbers of fetus					
Twin	90/801 (11.2)	8/104 (7.7)	3.5 (-2.0 to 9.1)	0.274	0.966
Singleton	286/2627 (10.9)	33/487 (6.8)	4.1 (1.6 to 6.6)	0.006	
High-grade IVH, n (%)					
Total	53/3426 (1.5)	14/591 (2.4)	-0.8 (-2.1 to 0.5)	0.150	
By numbers of fetus					
Twin	10/801 (1.2)	2/104 (1.9)	-0.7 (-3.4 to 2.1)	0.572	0.742
Singleton	43/2625 (1.6)	12/487 (2.5)	-0.8 (-2.3 to 0.6)	0.204	
Periventricular leukomalacia, n (%)					
Total	179/3427 (5.2)	30/591 (5.1)	0.2 (-1.8 to 2.1)	0.882	
By numbers of fetus					
Twin	71/801 (8.9)	6/104 (5.8)	3.1 (-1.8 to 8.0)	0.287	0.492
Singleton	108/2626 (4.1)	24/487 (4.9)	-0.8 (-2.9 to 1.3)	0.412	
Surgically treated PDA, n (%)					
Total	95/3428 (2.8)	18/591 (3.0)	-0.3 (-1.8 to 1.2)	0.709	
By numbers of fetus					
Twin	19/801 (2.4)	4/104 (3.8)	-1.5 (-5.3 to 2.4)	0.369	0.955
Singleton	76/2627 (2.9)	14/487 (2.9)	0.02 (-1.6 to 1.6)	0.982	
Necrotizing enterocolitis, n (%)					
Total	85/3427 (2.5)	11/591 (1.9)	0.6 (-0.6 to 1.8)	0.363	
By numbers of fetus					
Twin	21/801 (2.6)	4/104 (3.8)	-1.2 (-5.1 to 2.6)	0.474	0.492
Singleton	64/2626 (2.4)	7/487 (1.4)	1 (-0.2 to 2.2)	0.175	
Bronchopulmonary dysplasia, n (%)					
Total	524/3423 (15.3)	93/591 (15.7)	-0.4 (-3.6 to 2.8)	0.790	
By numbers of fetus					

Twin	114/797 (14.3)	16/104 (15.4)	-1.1 (-8.4 to 6.3)	0.768	0.834
Singleton	410/2626 (15.6)	77/487 (15.8)	-0.2 (-3.7 to 3.3)	0.912	
Advanced ROP, n (%)					
Total	45/3426 (1.3)	10/591 (1.7)	-0.4 (-1.5 to 0.7)	0.465	
By numbers of fetus					
Twin	13/800 (1.6)	3/104 (2.9)	-1.3 (-4.6 to 2.1)	0.359	0.202
Singleton	32/2626 (1.2)	7/487 (1.4)	-0.2 (-1.4 to 0.9)	0.690	
All infants (n = 4,124)	ACS (n=3504)	No ACS (n=620)	Risk difference, %(95 CI)	*P* value	*P* value for interaction*
Mortality, n (%)					
Total	76/3504 (2.2)	29/620 (4.7)	-2.5 (-4.2 to -0.8)	<0.001	
By numbers of fetus					
Twin	21/822 (2.6)	8/112 (7.1)	-4.6 (-9.5 to 0.3)	0.009	0.722
Singleton	55/2682 (2.1)	21/508 (4.1)	-2.1 (-3.9 to-..6 -0.3)	0.005	

Data are presented as n (%).

[a] Tests for risk difference within each subgroup.

[b] Tests for interaction between antenatal corticosteroids and twin pregnancies on each outcome. *P*-values for interaction were obtained from multivariable Poisson regression models adjusted for gestational age, birthweight, sex, maternal age, maternal diabetes mellitus, maternal hypertension, premature rupture of membrane, cesarean section, and *in vitro* fertilization using the generalized estimation equations.

Abbreviations: ACS, antenatal corticosteroid; SGA, small for gestational age; IVH, intraventricular hemorrhage; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity.

~~received ACS therapy were more frequently conceived through IVF with older mothers than infants who did not receive such therapy for both twins and singletons.~~

Independent effects of ACS and twins on neonatal outcomes

~~**Comparisons of effects of antenatal corticosteroid therapy on neonatal outcomes by plurality**~~

In the second model excluding the interaction term, we calculated adjusted RR and 95% CI for individual effects of ACS and twins on each neonatal outcome. In the age group of 23 to 28 weeks, exposure to ACS was significantly associated with a lower risk of surfactant use (adjusted RR (aRR): 0.972 [95% CI: 0.961 – 0.984]), high-grade intraventricular hemorrhage (aRR: 0.621 [95% CI: 0.487 – 0.794]), periventricular leukomalacia (aRR: 0.728 [95% CI: 0.556 – 0.954]), and mortality (aRR: 0.758 [95% CI: 0.679 – 0.846]) (Figure 2A). Twin were associated with higher risks of high-grade IVH (aRR: 1.466 [95% CI: 1.178 – 1.825]) and advanced ROP (aRR: 1.193 [95% CI: 1.069 – 1.331]) than singletons.

In the age group of 29 to 33 weeks, exposure to ACS was significantly associated with lower risks of surfactant use (aRR: 0.914 [95% CI: 0.861 – 0.970]) and mortality (aRR: 0.409 [95% CI: 0.269 – 0.624]), but a higher risk of sepsis (aRR: 1.416 [95% CI: 1.018 – 1.969]) (Figure 2B). Twins showed a lower risk of BPD (aRR: 0.798 [95% CI: 0.648 – 0.982]), but a higher risk of PVL (aRR: 1.735 [95% CI: 1.256 – 2.396]) than singletons.

~~In both twins and singletons, rates of RDS, hypotension during the first week of life, high-grade~~

IVH, and mortality were significantly lower in infants who received ACS therapy than in infants who did not (Table 2). Infants who received ACS therapy had higher rates of Apgar scores of ≥7 at 5 minutes and intact survival without serious morbidities than infants who did not receive such therapy in both twins and singletons.

Multivariable logistic regression was used to adjust for gestational age, birth weight, sex, maternal age, maternal hypertension, maternal DM, premature rupture of membrane, cesarean section, and IVF. Analysis results of the association between ACS therapy and each neonatal outcome are shown as forest plots in Figure 2. Infants who received ACS therapy had significantly lower odds of RDS, hypotension during the first week of life, high-grade IVH, and mortality and significantly higher odds of Apgar scores of ≥7 at 5 minutes than infants who did not receive ACS therapy in both twins and singletons. However, decreased odds of PVL and increased odds of intact survival without serious morbidities associated with ACS therapy were observed only in singletons.

Z-test *p*-values for all neonatal outcomes were above 0.05 (data not shown), indicating no significant difference in the effect size of ACS therapy on neonatal outcomes between twins and singletons.

Comparisons of the effect of antenatal corticosteroid therapy on neonatal outcomes by sex

Baseline characteristics, neonatal outcomes (Supplementary Tables 1 and 2), and effects of ACS therapy on neonatal outcomes (Table 3) were observed differently by sex in both twins and

singletons. Among twins, ACS therapy was significantly associated with lower odds of hypotension during the first week of life, high-grade IVH, and mortality but higher odds of Apgar scores of ≥7 and intact survival without serious morbidities in female infants after adjusting for gestational age, birth weight, maternal age, maternal hypertension, maternal diabetes mellitus, premature rupture of membrane, cesarean section, and IVF. In male infants, decreased odds associated with ACS therapy were only observed for RDS and mortality. Among singletons, effects of ACS therapy on neonatal outcomes were mostly comparable between male and female infants. After adjusting for the same baseline characteristics used for adjustment in twins, ACS therapy was significantly associated with lower odds of hypotension during the first week of life, high-grade IVH, PVL, and mortality and higher odds of Apgar score of ≥7 at 5 minutes and intact survival without serious morbidities in both sexes. However, decreased odds of RDS associated with ACS therapy were observed only in female infants. There were no significant differences in the effect size of ACS therapy on neonatal outcomes by sex among either twins or singletons as indicated by Z-test *p*-values above 0.05 for all neonatal outcomes.

Table 3 Comparisons of perinatal characteristics between infants who received antenatal corticosteroid and infants who did not

	Twins aOR* (95% CI)[a]			Singleton aOR* (95% CI)		
	Female (n = 660)	Male (n = 676)	*Z*-test *P* value	Female (n = 2,247)	Male (n = 2,300)	*Z-test P value*
Apgar scores of ≥7 at 5 minutes	2.363 (1.464 – 3.814)***	1.531 (0.977 – 2.399)	0.195	1.934 (1.554 – 2.406)***	2.499 (2.029 – 3.077)***	0.097
Respiratory distress syndrome	0.617 (0.314 – 1.214)	0.207 (0.066 - 0.651)**	0.107	0.599 (0.442 – 0.811)**	0.789 (0.585 – 1.064)	0.205
Hypotension during the first week of life	0.530 (0.316 – 0.887)*	0.718 (0.419 – 1.230)	0.424	0.619 (0.480 – 0.799)**	0.661 (0.524 – 0.835)**	0.708
Sepsis	0.829 (0.497 – 1.381)	1.220 (0.711 – 2.091)	0.308	1.212 (0.930 – 1.580)	0.962 (0.753 – 1.229)	0.207
High-grade IVH	0.480 (0.240 – 0.960)*	0.587 (0.329 – 1.050)	0.663	0.341 (0.229 – 0.507)***	0.514 (0.370 – 0.715)***	0.118
Periventricular leukomalacia	0.811 (0.360 – 1.826)	0.915 (0.500 – 1.673)	0.816	0.761 (0.520 – 1.112)	0.655 (0.459 – 0.934)*	0.569
Surgically treated PDA	0.863 (0.481 – 1.548)	1.172 (0.590 – 2.330)	0.506	0.926 (0.656 – 1.305)	1.015 (0.727 – 1.417)	0.706
Necrotizing enterocolitis ≥stage 2	3.289 (0.973 – 11.124)	0.761 (0.336 – 1.726)	0.051	1.330 (0.822 – 2.153)	1.051 (0.709 – 1.559)	0.459
Bronchopulmonary dysplasia	0.810 (0.476 – 1.378)	0.984 (0.555 – 1.743)	0.624	0.780 (0.599 – 1.016)	0.983 (0.764 – 1.264)	0.213
Treated ROP	0.582 (0.281 – 1.206)	0.966 (0.407 – 2.293)	0.381	1.122 (0.736 – 1.708)	1.323 (0.872 – 2.006)	0.585
Mortality	0.467 (0.241 – 0.905)*	0.262 (0.144 – 0.479)***	0.206	0.542 (0.386 – 0.762)***	0.512 (0.380 – 0.688)***	0.801
Intact survival without serious morbidities[b]	1.884 (1.137 – 3.124)*	1.222 (0.715 – 2.087)	0.248	1.520 (1.187 – 1.947)**	1.423 (1.125 – 1.800)**	0.704

*Adjusted for gestational age, birthweight, maternal age, maternal hypertension, maternal diabetes mellitus, premature rupture of membrane, cesarean section, and in vitro fertilization. *P < 0.05; **P < 0.01; ***P < 0.001.

[a] Modeled by logistic regression using generalized estimating equations to account for correlation within twins from the same mother.

[b] Serious morbidity: High-grade IVH, periventricular leukomalacia. bronchopulmonary dysplasia, treated ROP

Abbreviations: ACS, antenatal corticosteroid; aOR, adjusted odds ratio; IVH, intraventricular hemorrhage; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity.

~~Dis~~Discussion

In this nationwide cohort study, we demonstrated that effects of ACS therapy administered before preterm birth on neonatal outcomes were not significantly different by plurality. Mortality and surfactant use were reduced by ACS exposure in both gestational age groups. ACS exposure was associated with decreased risks of high-grade IVH and PVL in infants born at 23 to 28 weeks of gestational age, but increased the risk of sepsis in infants born at 29 to 33 weeks of gestational age.

~~This study showed that effect sizes of a complete course of ACS therapy administered within 7 days before birth on neonatal outcomes were not significantly different by plurality or sex, as indicated by the insignificant z test p-values for all neonatal outcomes.~~

ACS therapy for mothers at risk of impending preterm delivery is the most effective evidence-based strategy to reduce the mortality and morbidity of their preterm offspring. However, previous studies that investigated the association between ACS therapy and neonatal outcomes in twin pregnancies not only reported conflicting results, but also lacked quantity.~~Previous researches have investigated the effect of ACS therapy on neonatal outcomes in twin pregnancies and reported conflicting results, particularly for RDS.~~[16 17]

A recent meta-analysis has reported that ACS therapy is associated with decreased neonatal death, RDS, and intraventricular hemorrhage in singleton pregnancies, but not in multiple pregnancies. Furthermore, a randomized controlled trial on 311 twin infants who were delivered before 34 weeks of gestation age showed that ACS therapy had no association with RDS or

composite neonatal morbidity. However, large population-based cohort studies from the mid-2000s reported positive ACS effects in multiple pregnancies. A study of 750 twin infants from France has reported that a complete course of ACS therapy administered within 7 days before birth is associated with decreased rates of brain injury and mortality.~~Since the use of ACS therapy for impending preterm delivery as a standard management is widespread, whether the current regimen of ACS therapy is also equally effective for twin pregnancy remains unclear. Furthermore, Ballabh et al. have reported a higher clearance of betamethasone in twin pregnancies than in singleton pregnancies.[18] In a large population-based cohort study, Palas et al. have reported that a complete course of ACS therapy administered within 7 days before birth is associated with a decreased rate of brain injury and mortality in preterm twins.~~[18] Another study of 8,274 multiples from Italian Neonatal Network reported that ACS therapy reduced the risk of severe IVH and mortality, although it was less effective in multiple pregnancies than in singleton pregnancies. A study of 2,516 twin infants from Canadian Neonatal Network has reported that twins can benefit from a complete course of ACS therapy as good as singletons in short-term respiratory morbidity, severe brain injury, and mortality.~~Melamed et al. have also reported that preterm twins can benefit from ACS therapy as good as preterm singletons.~~[19] Consistent with results of these large cohort studies, our study using the latest data from KNN revealed that the effectiveness of ACS therapy in twins was comparable to that of singletons. Moreover, its benefits on mortality and morbidity were shown to be different by gestational groups.

Additionally, we found that twins had a higher rate of morbidity than singletons in this study. Twins were associated with higher risks of high-grade IVH and advanced ROP in infants born at

23 to 28 weeks of GA and PVL in infants born at 29 to 33 weeks of GA than singletons. Although twins are more likely to be delivered preterm than singletons, whether twin pregnancy alone has such adverse effects on neonatal outcomes in preterm infants remains unknown. In a large study of infants born between 23 and 35 weeks of gestation, twins and singletons had comparable average birth weights up to 32 weeks of gestation and similar neonatal outcomes. One study from Australian and New Zealand Neonatal Network has found higher mortality, but not morbidities, in twins than in singletons. Furthermore, two national cohort studies have reported comparable risks for neonatal outcomes except for RDS between twins and singletons. However, a population-based European cohort study showed that twins had higher risk for mortality and high-grade IVH in infants born at 24 to 27 weeks of GA. Other studies have revealed disparities in the rate of morbidity such as BPD and ROP between twins and singletons.

~~In their study, preterm twins who received a complete course of ACS therapy had decreased rates of short-term respiratory morbidity, severe brain injury, and mortality. Results of our study support that the current regimen of ACS therapy is equally effective in twins.~~

~~In our study, there were significant differences in baseline characteristics between twins and singletons as shown in Tables 1. Twins had a lower gestational age and birth weight than singletons. Furthermore, obstetric characteristics were quite different between twins and singletons. Cesarean section and IVF were significantly more common in twins, whereas maternal hypertension and chorioamnionitis were more common in singletons. These differences in baseline characteristics between twins and singletons suggest that they are heterogeneous populations with different demographic and clinical backgrounds. Although we adjusted these~~

~~baseline characteristics which were different between twins and singletons in logistic regression analyses of associations between ACS therapy and neonatal outcomes, these different population characteristics between twins and singletons should be considered when interpreting our results.~~

Possible reasons for conflicting findings in twins include study design and changes in population characteristics over time~~Favorable effects of ACS therapy on neonatal survival of preterm infants have been well established.~~[20] We found that twins had lower rates of antenatal complications such as maternal hypertension and chorioamnionitis, and higher rates of obstetric interventions, including IVF, ACS, and cesarean section than singletons in both gestational age groups (Supplemental Table 1). This trend has also been observed across other recent studies. The higher rate of IVF in twins reflect the current trend of childbirth in Korea.[6] The reason for higher ACS exposure in twins is currently unclear. However, more medical attention for twin pregnancies might be one possible cause. One noteworthy result was that the cesarean section rate for twins born between 23 and 28 weeks of GA was 87.2%, which was higher in Korea than in other countries. For similar gestational age, the cesarean section rate for twins was 74.4% in the USA and 61.8% in Australia and New Zealand.~~Similarly, ACS therapy was significantly associated with lower mortality in both twins and singleton in our study. ACS therapy was also significantly associated with higher rate of Apgar score of ≥7 at 5 minutes and lower rates of RDS, hypotension during the first week of life, and high-grade IVH, which could contribute to improved survival.~~

Despite the fact that these difference in characteristics between twins and singletons are observed

in other studies, an increase in the proportion of the population exposed to ACS along with variation in practice of neonatal care in each country might have complexly contributed to the interaction between ACS and plurality as well as the inherent risk of a twin pregancy. ~~In addition, we investigated whether effects of ACS therapy on neonatal outcomes were different by sex. Previous studies have found that preterm male infants are at higher risks of morbidity and mortality than their female peers.~~[21 22] Although we adjusted potential confounders which were different between twins and singletons in the analyses, different population characteristics between twins and singletons and/or between studies should be considered when interpreting our results. ~~This trend was also observed in extremely preterm twins.[23] In our study, a complete course of ACS therapy lowered the odds of mortality in both sexes regardless of whether the infants were twins or singletons. There were no significant differences in the effect size of ACS therapy on neonatal outcomes between male and female infants among either twins or singletons. The faster decline in mortality of preterm male infants over the past decade than that of their female peers suggests that the widespread use of ACS therapy might have mitigated the male disadvantage for neonatal outcomes along with advances in neonatal care.~~[14]

Our study has several limitations. Firstly, the KNN registry had no information on chorionicity in multiple pregnancies. Thus, we were unable to investigate the difference in the effect of ACS therapy on neonatal outcomes according to chorionicity in twins. Secondly, although we used prospective cohort data of preterm infants with VLBW for this study ~~although we used a prospective cohort data of preterm infants with VLBW for this study~~, antenatal information including ACS administration was collected retrospectively because preterm infants were

enrolled after they were born. Therefore, it was unknown why ACS was not or incompletely administered in infants who did not receive a complete course of ACS therapy. Notably, the percentage of singletons without exposure to ACS was higher in this study than in other studies Moreover, information on the total amount of ACS administered was not available in the KNN registry database. Information on total amount of ACS administered will enable a more quantitative analysis of effects of ACS therapy on neonatal outcomes. Lastly, in our cohort, 56.6% of twins were conceived via IVF whereas only 7.1% of singletons were conceived via IVF. ~~56.6% of twins were conceived via IVF, whereas only 7.1% of singletons were so in our cohort.~~ Although preterm infants conceived via IVF are known to have comparable morbidity and mortality with their peers conceived via natural pregnancy[23], higher proportion of IVF cases among twins should be taken into account when interpreting our results.

Conclusion

Despite differences in demographic and clinical characteristics according to plurality ~~and sex~~, ACS therapy administered before birth had comparable positive effects on neonatal outcomes of preterm infants with VLBW regardless of plurality.

~~a complete course of ACS therapy administered within 7 days before birth had comparable positive effects on neonatal outcomes of preterm infants with VLBW regardless of plurality or sex.~~

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Competing interests

None declared.

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Data Availability statement

The Korean Neonatal Network (KNN) Publication Ethics Policy adheres to the following research data management and access guidelines: All information about patients and participating NICUs is confidential and may be used by individuals for approved research purposes. If an individual or institution that is not affiliated with KNN wants to use the data, it must make an official request to the KNN publication-Ethics Committee and obtain approval from the network.

Author contributions

Seong Phil Bae: Conceptualization, Methodology, Investigation, Writing – Original draft preparation, and Funding acquisition. Won-Ho Han: Investigation, Writing – Review & Editing. Suyeon Park: Formal analysis, Visualization. Young Hwa Jung: Data curation, Writing – Review & Editing. Jee Yoon Park: Writing – Review & Editing. Kyung Joon Oh: Writing – Review & Editing. Chang Won Choi: Conceptualization, Methodology, Writing – Review & Editing, and Supervision.

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Figure Legends

Figure 1. Flow chart showing the selection of study population.

ACS, antenatal corticosteroid; VLBW, very low birth weight.

Figure 2. Forest plot showing results of logistic regression for effects of antenatal corticosteroids and twins on neonatal outcomes. ~~Forest plot showing results of logistic regression analysis of the~~

~~association between antenatal corticosteroid therapy and neonatal outcomes in twins and singletons.~~

(1) 23 to 28 weeks of gestataional age. (2) 29 to 33 weeks of gestational age.

For each neonatal outcome, solid dots represent the aOR and lateral lines represent the 95% CI. [a]Adjusted relative risk (RR) and 95% CI were obtained from Poisson regression models adjusted for gestational age, birthweight, sex, maternal age, maternal diabetes mellitus, maternal hypertension, premature rupture of membrane, cesarean section, and in vitro fertilization using generalized estimation equations..

Abbreviations: ACS, antenatal corticosteroid; CI, confidence interval; IVH, intraventricular hemorrhage; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity.

~~For each neonatal outcome, the solid dots represent the aOR, and the lateral lines represent the 95% CI. Generalized estimating equations were used to account for correlation between twins from the same mother. Serious morbidity included high-grade IVH, bronchopulmonary dysplasia, and treated ROP. Adjustments were made for gestational age, birth weight, sex, maternal age, maternal hypertension, maternal diabetes mellitus, premature rupture of membrane, cesarean section, and in vitro ferilization.~~

~~CI, confidence interval; IVH, intraventricular hemorrhage; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity.~~

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Effects of antenatal corticosteroids on neonatal outcomes in very-low-birth-weight infants are not different by plurality: a nationwide cohort study

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Title page

Original article

Effects of antenatal corticosteroids on neonatal outcomes in very-low-birth-weight infants are not different by plurality: a nationwide cohort study

Seong Phil Bae[1,2], M.D., Won-Ho Hahn[1,2], M.D., Ph.D., Suyeon Park[3,4], MS., Young Hwa Jung[5,6], M.D., Ph.D., Jee Yoon Park[7], M.D., Kyung Joon Oh[7], M.D., Ph.D., Chang Won Choi[5,6], M.D., Ph.D.

[1]Department of Pediatrics, Soonchunhyang University Seoul Hospital, Seoul, Republic of Korea

[2]Department of Pediatrics, Soonchunhyang University School of Medicine, Asan, Republic of Korea

[3]Department of Biostatistics, Soonchunhyang University Seoul Hospital, Seoul, Republic of Korea

[4]Department of Applied Statistics, Chung-Ang University, Seoul, Republic of Korea

[5]Department of Pediatrics, Seoul National University Bundang Hospital, Seongnam, Republic of Korea

[6]Department of Pediatrics, Seoul National University College of Medicine, Seoul, Republic of Korea

[7]Department of Obstetrics and Gynecology, Seoul National University Bundang Hospital, Seongnam, Republic of Korea

Correspondence to

Chang Won Choi, M.D., Ph.D.

Department of Pediatrics, Seoul National University Bundang Hospital, 82 Gumi-ro 173 Beon-gil, Bundang-gu, Seongnam-si, Gyeonggi-do, Republic of Korea, E-mail: choicw@snu.ac.kr

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ABSTRACT

Objective

To investigate whether effects of antenatal corticosteroids on neonatal outcomes in preterm infants with very-low-birth-weight were different by plurality.

Design

Nationwide prospective cohort study

Setting

Not applicable

Patients

Twins and singletons with very-low-birth-weight (< 1,500 g) who were born between 23^{+0} and 33^{+6} weeks of gestation and registered in the Korean Neonatal Network from January 2014 to December 2019

Main outcome measures

Morbidity and mortality before discharge from neonatal intensive care unit

Results

Among a total of 9,531 preterm infants with very-low-birth-weight, there were 2,364 (24.8%) twins and 7,167 (75.2%) singletons. While 83.9% of singletons were exposed to at least one dose of antenatal corticosteroids, so were 87.9% of twins.

Interaction analysis demonstrated that there was no significant difference in the effect of antenatal corticosteroids on morbidities or mortality between twins and singletons in either gestational age group (23 to 28 weeks or 29 to 33 weeks).

Antenatal corticosteroids significantly decreased the risk of surfactant use (aRR: 0.972 [95% CI: 0.961 – 0.984]), high-grade intraventricular hemorrhage (aRR: 0.621 [95% CI: 0.487 – 0.794]), periventricular leukomalacia (aRR: 0.728 [95% CI: 0.556 – 0.954]), and mortality (aRR: 0.758 [95% CI: 0.679 – 0.846]) in the gestational age group of 23 to 28 weeks. In the gestational age group of 29 to 33 weeks, antenatal corticosteroids significantly decreased the risk of surfactant use (aRR: 0.914 [95% CI: 0.862 – 0.970]) and mortality (aRR: 0.409 [95% CI: 0.269 – 0.624]) but increased the risk of sepsis (aRR: 1.416 [95% CI: 1.018 – 1.969]).

Conclusion

This study demonstrates that effect of antenatal corticosteroids on neonatal outcomes of preterm infants with very-low-birth-weight does not differ significantly by plurality (twin or singleton pregnancy).

Keywords: antenatal corticosteroids, preterm delivery, mortality, morbidity, twins, very low birth weight infant

Key messages

Why is already known on this topic?

Antenatal corticosteroids (ACS) administered before preterm delivery can decrease neonatal morbidity and mortality in singleton pregnancies. However, data about such effects of ACS in multiple pregnancies are still limited.

What this study adds?

The effect of antenatal corticosteroids administered before preterm delivery on neonatal morbidity and mortality does not differ by plurality (twin or singleton pregnancy).

Introduction

Antenatal corticosteroid (ACS) has been established as the standard therapy for pregnant women at risk of preterm delivery within 7 days between 24^{+0} and 33^{+6} weeks of gestation.[1] Currently, ACS is equally recommended for twin and singleton pregnancies with the same regimen based on previous studies.[2-4] A recent study has shown that a complete course of ACS administered before preterm twin delivery is associated with a significant decrease in neonatal mortality, short-term respiratory morbidity, and severe neurological injury in a magnitude similar to those observed for a singleton pregnancy.[3] However, data that demonstrate comparable effects of ACS therapy in twin pregnancies are limited.

As a result of increasing childbirth age and use of assisted reproductive technology, twin pregnancies are increasing in many countries, including Korea.[5 6] However, twin pregnancies are more likely to have preterm birth, low birth weight, and longer hospital stays than singleton pregnancies.[7 8] In addition, mono-chorionicity, birth weight discordance, and obstetric complications associated with multiple pregnancies may result in adverse perinatal outcomes.[9-11]

Recently, the mortality of extreme preterm multiples has decreased to a level comparable to that of singletons born at the same gestational age along with increased use of ACS therapy.[12] However, many studies have reported conflicting results regarding equivalent effects of ACS on neonatal outcomes of twins and singletons.[3 13-18] To date, there have been no clinical trials designed to investigate the effect of ACS in twins. A meta-analysis on ACS therapy has also highlighted the need for additional studies on multiple pregnancies.[4]

Thus, the purpose of this study was to investigate whether effects of ACS administered before preterm delivery on neonatal outcomes of twins and singletons were comparable.

Materials and Methods

Study design and participants

Information on the study population is presented in Figure 1. Data collected prospectively through the Korean Neonatal Network (KNN), a nationwide very low birth weight (VLBW, <1,500 g) infant registry participated by 61 neonatal intensive care units across South Korea,[19] were used for this study. A total of 11,121 infants with VLBW born preterm (between 23^{+0} and 33^{+6} weeks of gestation) between January 2014 and December 2019 were enrolled. Exclusion criteria were: high-order multiple gestations (≥ triplets), birth outside the hospital, major congenital anomalies, transfer to other hospitals, no or unreliable information on ACS. Finally, 2,364 twins and 7,167 singletons were analyzed. ACS therapy was defined if the pregnant women had received at least one dose of any kind of corticosteroids before preterm delivery. Variables collected included gestational age, birth weight, small for gestational age (SGA), sex, maternal age, maternal diabetes mellitus (DM), maternal hypertension, chorioamnionitis, premature rupture of membrane, cesarean section, *in vitro* fertilization (IVF), surfactant use, sepsis, high-grade intraventricular hemorrhage (IVH), periventricular leukomalacia (PVL), surgically treated patent ductus arteriosus (PDA), bronchopulmonary dysplasia (BPD), advanced retinopathy of prematurity (ROP), and mortality. Definitions for different variables are provided

in Supplemental Materials. The analysis of neonatal morbidity was performed for infants who survived before discharge, while the analysis of mortality was performed for the total population.

Statistical methods

Rates of neonatal outcomes between infants exposed to ACS and infants without such exposure are presented along with risk difference and 95% confidence intervals (CI). To investigate whether associations between ACS and neonatal outcomes were altered by plurality (twins versus singletons), the interaction effect between ACS and twin pregnancies on neonatal outcomes was evaluated by adding interaction terms (ACS × twins) in Poisson regression models. The crude and adjusted relative risks (RR) and 95% CI for the individual effects of ACS and twins were obtained in the second model without the interaction term.

All models were fitted with generalized estimation equations (GEE) approach for the correlation between a pair of twins from a mother. Covariables in regression models were gestational age, birth weight, sex, maternal age, maternal diabetes mellitus, maternal hypertension, premature rupture of membrane, cesarean section, and *in vitro* fertilization. Chorioamnionitis was excluded from the analyses due to substantial missing values (17.6% in twins and 14.5% in singletons).

For each outcomes, a backward-stepwise method was used to remove the variable with the highest *P* value, and variables in final model were selected that showed minimal QIC (Quasi-likelihood under the Independence model Criterion) value.[20-22] To confirm multi-collinearity, variance inflation factors values were checked for all covariates, which were all less than 5,

indicating no significant multi-collinearity (1.002~2.989). The level of significance was set at $p < 0.05$. All statistical analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA) and 'geepack' and 'car' package R version 4.1.2 (The R Foundation for Statistical Computing, Vienna, Austria).

Ethic approval

Registration of data in the KNN was approved by the Institutional Review Board (IRB) of each participating center. Informed consent was obtained from parents of each infant prior to participation in the KNN registry. This study was approved by the IRB of Seoul National University Bundang Hospital (approval number: B-1305-202-005).

Results

Exposure to antenatal corticosteroids

Among 2,364 twins, 2,078 (87.9%) infants were exposed to at least one dose of ACS before preterm delivery. Among 7,167 singletons, 6,013 (83.9%) infants were exposed to at least one dose of ACS before preterm delivery (Figure 1).

Comparisons of perinatal baseline characteristics between infants exposed to antenatal corticosteroids and infants without ACS exposure

Twins exposed to ACS were born at a later gestational age (mean [SD], 28.08 (2.19) vs 27.75 (2.50) weeks) compared to those without ACS exposure (Table 1). However, there was no difference in gestational age (mean [SD], 28.49 (2.52) vs 28.39 (2.78) weeks) or birth weight (mean [SD], 1,060 (281) vs 1,073 (289) g) between singletons exposed to ACS and those without ACS exposure. Singletons exposed to ACS had higher rates of maternal DM (9.9% vs 7.7%; P=0.019), maternal hypertension (29.1% vs 24.8%; P=0.003), and cesarean section (77.1% vs 69.9%; P<0.001) than those without exposure. In both twins and singletons, infants exposed to ACS were more likely to have higher rates of premature rupture of membrane and be conceived through IVF from older mothers than those without exposure.

Table 1. Comparisons of perinatal baseline characteristics between infants exposed to ACS and infants without ACS exposure

	Twins (n = 2,364)			Singletons (n = 7,167)		
	ACS-exposed (n = 2,078)	ACS-unexposed (n = 286)	*P* value[a]	ACS-exposed (n = 6,013)	ACS-unexposed (n = 1,154)	*P* value[a]
Infant						
Gestational age (weeks), mean (SD)	28.08 (2.19)	27.75 (2.50)	0.034	28.49 (2.52)	28.39 (2.78)	0.256
Birth weight (g), mean (SD)	1,059 (274)	1,034 (301)	0.186	1,060 (281)	1,073 (289)	0.138
SGA, n (%)	147 (7.1)	22 (7.7)	0.704	1080 (18.0)	199 (17.2)	0.560
Male, n (%)	1079 (51.9)	143 (50.0)	0.541	3074 (51.1)	597 (51.7)	0.708
Maternal						
Maternal age (years), mean (SD)	33.41 (3.80)	31.91 (4.66)	<0.001	33.44 (4.43)	32.82 (4.92)	<0.001
Maternal diabetes mellitus, n (%)	238 (11.5)	26 (9.1)	0.221	597 (9.9)	89 (7.7)	0.019
Maternal hypertension, n (%)	196 (9.5)	22 (7.7)	0.329	1749 (29.1)	286 (24.8)	0.003
Chorioamnionitis[b], n (%)	516 (29.9)	63 (28.0)	0.551	2084 (40.2)	351 (37.0)	0.064
Premature rupture of membrane, n (%)	787 (38.1)	84 (29.9)	0.007	2334 (38.9)	312 (27.5)	<0.001
Cesarean section, n (%)	1837 (88.4)	244 (85.3)	0.132	4634 (77.1)	807 (69.9)	<0.001
In vitro fertilization, n (%)	1152 (56.6)	128 (45.1)	<0.001	494 (8.2)	51 (4.4)	<0.001

Data are presented as mean (SD) or n (%).

Abbreviations: ACS, antenatal corticosteroids; SD, standard deviation; SGA, small for gestational age; IVH, intraventricular hemorrhage; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity.

[a]*P* value obtained from chi-squared test for categorical variables and Student's *t*-test for continuous variables.

[b] Values were missing for 415 infants in the group of twins and 1039 infants in the group of singletons.

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Interaction between ACS and twins pregnancies on neonatal outcomes

We investigated whether effects of ACS on neonatal outcomes differed between twins and singletons. Because neonatal outcomes are substantially dependent on gestational age, comparison of ACS effects on neonatal outcomes by plurality was performed by stratifying the study population into two gestation age groups: an age group of 23 to 28 weeks and an age group of 29 to 33 weeks group.

Interaction analyses within 23 to 28 weeks (Table 2) and 29 to 33 weeks (Table 3) gestational age groups found no significant difference in the association of ACS therapy with any neonatal outcomes between twins and singletons (P>0.1 for all).

Table 2. Comparisons of neonatal outcomes between infants exposed to ACS and infants without ACS exposure in infants born at 23 to 28 weeks of gestational age

Infants who survived before discharge (n = 4160)	ACS-exposed (n=3606)	ACS-unexposed (n=554)	Risk difference, % (95 CI)	*P* value[a]	*P* value for interaction[b]
Surfactant use, n (%)					
Total	3468/3606 (96.2%)	546/554 (98.6%)	-2.4 (-3.6 to -1.2)	0.005	
Twin	952/991 (96.1%)	108/109 (99.1%)	-3.0 (-5.2 to -0.9)	0.110	0.199
Singleton	2516/2615 (96.2%)	438/445 (98.4%)	-2.2 (-3.6 to -0.8)	0.018	
Sepsis, n (%)					
Total	985/3606 (27.3%)	166/554 (30.0%)	-2.7 (-6.7 to 1.4)	0.195	
By numbers of fetus					
Twin	273/991 (27.5%)	32/109 (29.4%)	-1.8 (-10.8 to 7.2)	0.689	0.706
Singleton	712/2615 (27.2%)	134/445 (30.1%)	-2.9 (-7.5 to 1.7)	0.209	
High-grade IVH, n (%)					
Total	313/3606 (8.7%)	79/554 (14.3%)	-5.6 (-8.6 to -2.5)	<0.001	
By numbers of fetus					
Twin	115/991 (11.6%)	15/109 (13.8%)	-2.2 (-8.9 to 4.6)	0.508	0.224
Singleton	198/2615 (7.6%)	64/445 (14.4%)	-6.8 (-10.2 to -3.4)	<0.001	
Periventricular leukomalacia, n (%)					
Total	327/3606 (9.1%)	74/554 (13.4%)	-4.3 (-7.3 to -1.3)	0.001	
By numbers of fetus					
Twin	86/991 (8.7%)	14/109 (12.8%)	-4.2 (-10.7 to 2.4)	0.151	0.749
Singleton	241/2615 (9.2%)	60/445 (13.5%)	-4.3 (-7.6 to -0.9)	0.005	
Surgically treated PDA, n (%)					
Total	616/3606 (17.1%)	99/554 (17.9%)	-0.8 (-4.2 to 2.6)	0.647	
By numbers of fetus					
Twin	175/991 (17.7%)	23/109 (21.1%)	-3.4 (-11.5 to 4.6)	0.375	0.400
Singleton	441/2615 (16.9%)	76/445 (17.1%)	-0.2 (-4.0 to 3.6)	0.911	
Necrotizing enterocolitis, n (%)					
Total	268/3606 (7.4%)	36/554 (6.5%)	0.9 (-1.3 to 3.2)	0.432	
By numbers of fetus					
Twin	72/991 (7.3%)	8/109 (7.3%)	-0.07 (-5.2 to 5.1)	0.978	0.479
Singleton	196/2615 (7.5%)	28/445 (6.3%)	1.2 (-1.3 to 3.7)	0.368	

Bronchopulmonary dysplasia, n (%)					
Total	1664/3606 (46.1%)	269/554 (48.6%)	-2.4 (-6.9 to 2.1)	0.290	
By numbers of fetus					
Twin	437/991 (44.1%)	51/109 (46.8%)	-2.7 (-12.6 to 7.2)	0.591	0.986
Singleton	1227/2615 (46.9%)	218/445 (49.0%)	-2.1 (-7.1 to 3.0)	0.419	
Advanced ROP, n (%)					
Total	769/3602 (21.3%)	113/554 (20.4%)	1.0 (-2.7 to 4.6)	0.610	
By numbers of fetus					
Twin	232/990 (23.4%)	31/109 (28.4%)	-5.0 (-13.9 to 3.9)	0.245	0.158
Singleton	537/2612 (20.6%)	82/445 (18.4%)	2.1 (-1.8 to 6.1)	0.301	
All infants (n = 5407)	ACS (n=4587)	No ACS (n=820)	Risk difference, %(95 CI)	*P* value	*P* value for interaction*
Mortality, n (%)					
Total	981/4587 (21.4%)	266/820 (32.4%)	-11.1 (-14.5 to -7.6)	<0.001	
By numbers of fetus					
Twin	265/1256 (21.1%)	65/174 (37.4%)	-16.3 (-23.8 to -8.7)	<0.001	0.458
Singleton	716/3331 (21.5%)	201/646 (31.1%)	-9.6 (-13.5 to -5.8)	<0.001	

Data are presented as n (%).

[a] Tests for risk difference within each subgroup.

[b] Tests for interaction between antenatal corticosteroids and twin pregnancies on each outcome. *P*-values for interaction were obtained from multivariable Poisson regression models using the generalized estimation equations.

Abbreviations: ACS, antenatal corticosteroid; CI, confidence interval; SGA, small for gestational age; IVH, intraventricular hemorrhage; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity.

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Table 3. Comparisons of neonatal outcomes between infants exposed to ACS and infants without ACS exposure in infants born at 29 to 33 weeks of gestational age

Infants who survived before discharge (n = 4,019)	ACS-exposed (n=3428)	ACS-unexposed (n=591)	Risk difference, % (95 CI)	*P*-value[a]	*P*-value for interaction[b]
Surfactant use, n (%)					
Total	2240/3428 (65.3%)	402/591 (68.0%)	-2.7 (-6.8 to 1.4)	0.206	
By numbers of fetus					

Twin	583/801 (72.8)	86/104 (82.7)	-9.9 (-17.8 to -2.0)	0.030	0.243
Singleton	1657/2627 (63.1)	316/487 (64.9)	-1.8 (-6.4 to 2.8)	0.446	
Sepsis, n (%)					
Total	376/3428 (11.0)	41/591 (6.9)	4.0 (1.7 to 6.3)	0.003	
By numbers of fetus					
Twin	90/801 (11.2)	8/104 (7.7)	3.5 (-2.0 to 9.1)	0.274	0.966
Singleton	286/2627 (10.9)	33/487 (6.8)	4.1 (1.6 to 6.6)	0.006	
High-grade IVH, n (%)					
Total	53/3426 (1.5)	14/591 (2.4)	-0.8 (-2.1 to 0.5)	0.150	
By numbers of fetus					
Twin	10/801 (1.2)	2/104 (1.9)	-0.7 (-3.4 to 2.1)	0.572	0.742
Singleton	43/2625 (1.6)	12/487 (2.5)	-0.8 (-2.3 to 0.6)	0.204	
Periventricular leukomalacia, n (%)					
Total	179/3427 (5.2)	30/591 (5.1)	0.2 (-1.8 to 2.1)	0.882	
By numbers of fetus					
Twin	71/801 (8.9)	6/104 (5.8)	3.1 (-1.8 to 8.0)	0.287	0.492
Singleton	108/2626 (4.1)	24/487 (4.9)	-0.8 (-2.9 to 1.3)	0.412	
Surgically treated PDA, n (%)					
Total	95/3428 (2.8)	18/591 (3.0)	-0.3 (-1.8 to 1.2)	0.709	
By numbers of fetus					
Twin	19/801 (2.4)	4/104 (3.8)	-1.5 (-5.3 to 2.4)	0.369	0.955
Singleton	76/2627 (2.9)	14/487 (2.9)	0.02 (-1.6 to 1.6)	0.982	
Necrotizing enterocolitis, n (%)					
Total	85/3427 (2.5)	11/591 (1.9)	0.6 (-0.6 to 1.8)	0.363	
By numbers of fetus					
Twin	21/801 (2.6)	4/104 (3.8)	-1.2 (-5.1 to 2.6)	0.474	0.492
Singleton	64/2626 (2.4)	7/487 (1.4)	1 (-0.2 to 2.2)	0.175	
Bronchopulmonary dysplasia, n (%)					
Total	524/3423 (15.3)	93/591 (15.7)	-0.4 (-3.6 to 2.8)	0.790	
By numbers of fetus					
Twin	114/797 (14.3)	16/104 (15.4)	-1.1 (-8.4 to 6.3)	0.768	0.834
Singleton	410/2626 (15.6)	77/487 (15.8)	-0.2 (-3.7 to 3.3)	0.912	
Advanced ROP, n (%)					

Total	45/3426 (1.3)	10/591 (1.7)	-0.4 (-1.5 to 0.7)	0.465	
By numbers of fetus					
Twin	13/800 (1.6)	3/104 (2.9)	-1.3 (-4.6 to 2.1)	0.359	0.202
Singleton	32/2626 (1.2)	7/487 (1.4)	-0.2 (-1.4 to 0.9)	0.690	
All infants (n = 4,124)	ACS (n=3504)	No ACS (n=620)	Risk difference, %(95 CI)	*P* value	*P* value for interaction*
Mortality, n (%)					
Total	76/3504 (2.2)	29/620 (4.7)	-2.5 (-4.2 to -0.8)	<0.001	
By numbers of fetus					
Twin	21/822 (2.6)	8/112 (7.1)	-4.6 (-9.5 to 0.3)	0.009	0.722
Singleton	55/2682 (2.1)	21/508 (4.1)	-2.1 (-3.9 to-..6 -0.3)	0.005	

Data are presented as n (%).

[a] Tests for risk difference within each subgroup.

[b] Tests for interaction between antenatal corticosteroids and twin pregnancies on each outcome. *P*-values for interaction were obtained from multivariable Poisson regression models using the generalized estimation equations.

Abbreviations: ACS, antenatal corticosteroid; CI, confidence interval; SGA, small for gestational age; IVH, intraventricular hemorrhage; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity.

Independent effects of ACS and twins on neonatal outcomes

In the second model excluding the interaction term, we calculated adjusted RR and 95% CI for individual effects of ACS and twins on each neonatal outcome. In the age group of 23 to 28 weeks, exposure to ACS was significantly associated with a lower risk of surfactant use (adjusted RR (aRR): 0.972 [95% CI: 0.961 – 0.984]), high-grade intraventricular hemorrhage (aRR: 0.621 [95% CI: 0.487 – 0.794]), periventricular leukomalacia (aRR: 0.728 [95% CI: 0.556 – 0.954]), and mortality (aRR: 0.758 [95% CI: 0.679 – 0.846]) (Figure 2A). Twin were associated with higher risks of high-grade IVH (aRR: 1.466 [95% CI: 1.178 – 1.825]) and advanced ROP (aRR: 1.193 [95% CI: 1.069 – 1.331]) than singletons.

In the age group of 29 to 33 weeks, exposure to ACS was significantly associated with lower risks of surfactant use (aRR: 0.914 [95% CI: 0.862 – 0.970]) and mortality (aRR: 0.409 [95% CI: 0.269 – 0.624]), but a higher risk of sepsis (aRR: 1.416 [95% CI: 1.018 – 1.969]) (Figure 2B). Twins showed a lower risk of BPD (aRR: 0.798 [95% CI: 0.648 – 0.982]), but a higher risk of PVL (aRR: 1.735 [95% CI: 1.256 – 2.396]) than singletons.

Discussion

In this nationwide cohort study, we demonstrated that effects of ACS therapy administered before preterm birth on neonatal outcomes were not significantly different by plurality. Mortality and surfactant use were reduced by ACS exposure in both gestational age groups. ACS exposure was associated with decreased risks of high-grade IVH and PVL in infants born at 23 to 28

weeks of gestational age, but increased the risk of sepsis in infants born at 29 to 33 weeks of gestational age.

ACS therapy for mothers at risk of impending preterm delivery is the most effective evidence-based strategy to reduce the mortality and morbidity of their preterm offspring. However, previous studies that investigated the association between ACS therapy and neonatal outcomes in twin pregnancies not only reported conflicting results, but also lacked quantity.

A recent meta-analysis has reported that ACS therapy is associated with decreased neonatal death, RDS, and intraventricular hemorrhage in singleton pregnancies, but not in multiple pregnancies.[4] Furthermore, a randomized controlled trial on 311 twin infants who were delivered before 34 weeks of gestation age showed that ACS therapy had no association with RDS or composite neonatal morbidity.[23] However, large population-based cohort studies from the mid-2000s reported positive ACS effects in multiple pregnancies.[24-26] A study of 750 twin infants from France has reported that a complete course of ACS therapy administered within 7 days before birth is associated with decreased rates of brain injury and mortality.[24] Another study of 8,274 multiples from Italian Neonatal Network reported that ACS therapy reduced the risk of severe IVH and mortality, although it was less effective in multiple pregnancies than in singleton pregnancies.[25] A study of 2,516 twin infants from Canadian Neonatal Network has reported that twins can benefit from a complete course of ACS therapy as good as singletons in short-term respiratory morbidity, severe brain injury, and mortality.[26] Consistent with results of these large cohort studies, our study using the latest data from KNN revealed that the effectiveness of ACS therapy in twins was comparable to that of singletons. Moreover, its benefits on mortality and

morbidity were shown to be different by gestational groups.

Additionally, we found that twins had a higher rate of morbidity than singletons in this study. Twins were associated with higher risks of high-grade IVH and advanced ROP in infants born at 23 to 28 weeks of GA and PVL in infants born at 29 to 33 weeks of GA than singletons. Although twins are more likely to be delivered preterm than singletons, whether twin pregnancy alone has such adverse effects on neonatal outcomes in preterm infants remains unknown.[27] In a large study of infants born between 23 and 35 weeks of gestation, twins and singletons had comparable average birth weights up to 32 weeks of gestation and similar neonatal outcomes.[28] One study from Australian and New Zealand Neonatal Network has found higher mortality, but not morbidities, in twins than in singletons.[12] Furthermore, two national cohort studies have reported comparable risks for neonatal outcomes except for RDS between twins and singletons.[29 30] However, a population-based European cohort study showed that twins had higher risk for mortality and high-grade IVH in infants born at 24 to 27 weeks of GA.[14] Other studies have revealed disparities in the rate of morbidity such as BPD and ROP between twins and singletons.[8 31]

Possible reasons for conflicting findings in twins include study design and changes in population characteristics over time.[32] We found that twins had lower rates of antenatal complications such as maternal hypertension and chorioamnionitis, and higher rates of obstetric interventions, including IVF, ACS, and cesarean section than singletons in both gestational age groups (Supplemental Table 1). This trend has also been observed across other recent studies.[12 33] The higher rate of IVF in twins reflect the current trend of childbirth in Korea.[5] The reason for higher

ACS exposure in twins is currently unclear. However, more medical attention for twin pregnancies might be one possible cause.[34] One noteworthy result was that the cesarean section rate for twins born between 23 and 28 weeks of GA was 87.2%, which was higher in Korea than in other countries. For similar gestational age, the cesarean section rate for twins was 74.4% in the USA and 61.8% in Australia and New Zealand.[12 15]

Despite the fact that these difference in characteristics between twins and singletons are observed in other studies, an increase in the proportion of the population exposed to ACS along with variation in practice of neonatal care in each country might have complexly contributed to the interaction between ACS and plurality as well as the inherent risk of a twin pregancy.[35] Although we adjusted potential confounders which were different between twins and singletons in the analyses, different population characteristics between twins and singletons and/or between studies should be considered when interpreting our results.

Our study has several limitations. Firstly, the KNN registry had no information about chorionicity in multiple pregnancies. Thus, we were unable to investigate the difference in the effect of ACS therapy on neonatal outcomes according to chorionicity in twins. Secondly, although we used prospective cohort data of preterm infants with VLBW for this study, antenatal information including ACS administration was collected retrospectively because preterm infants were enrolled after they were born. Therefore, it was unknown why ACS was not or incompletely administered in infants who did not receive a complete course of ACS therapy. Notably, the percentage of singletons without exposure to ACS was higher in this study than in other studies.[36 37] Moreover, information on the total amount of ACS administered was not

available in the KNN registry database. Information on total amount of ACS administered will enable a more quantitative analysis of effects of ACS therapy on neonatal outcomes. Lastly, in our cohort, 56.6% of twins were conceived via IVF whereas only 7.1% of singletons were conceived via IVF. Although preterm infants conceived via IVF are known to have comparable morbidity and mortality with their peers conceived via natural pregnancy[38], higher proportion of IVF cases among twins should be taken into account when interpreting our results.

Conclusion

Despite differences in demographic and clinical characteristics according to plurality, ACS therapy administered before birth had comparable positive effects on neonatal outcomes of preterm infants with VLBW regardless of plurality.

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Competing interests

None declared.

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Data Availability statement

The Korean Neonatal Network (KNN) Publication Ethics Policy adheres to the following research data management and access guidelines: All information about patients and participating NICUs are confidential. They might be used by individuals for approved research purposes. If an individual or institution that is not affiliated with KNN wants to use the data, it must make an official request to the KNN publication-Ethics Committee and obtain approval from the network.

Author contributions

Seong Phil Bae: Conceptualization, Methodology, Investigation, Writing – Original draft preparation, and Funding acquisition. Won-Ho Han: Investigation, Writing – Review & Editing. Suyeon Park: Formal analysis, Visualization. Young Hwa Jung: Data curation, Writing – Review & Editing. Jee Yoon Park: Writing – Review & Editing. Kyung Joon Oh: Writing – Review &

Editing. Chang Won Choi: Conceptualization, Methodology, Writing – Review & Editing, and Supervision.

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Figure Legends

Figure 1. Flow chart showing the selection of study population.

ACS, antenatal corticosteroid; VLBW, very low birth weight.

Figure 2. Forest plot showing results of logistic regression for effects of antenatal corticosteroids and twins on neonatal outcomes.

(A) 23 to 28 weeks of gestataional age. (B) 29 to 33 weeks of gestational age.

For each neonatal outcome, solid dots represent the aOR and lateral lines represent the 95% CI. Adjusted relative risk (RR) and 95% CI were obtained from multivariable Poisson regression models using generalized estimation equations..

Abbreviations: ACS, antenatal corticosteroid; CI, confidence interval; IVH, intraventricular hemorrhage; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity.

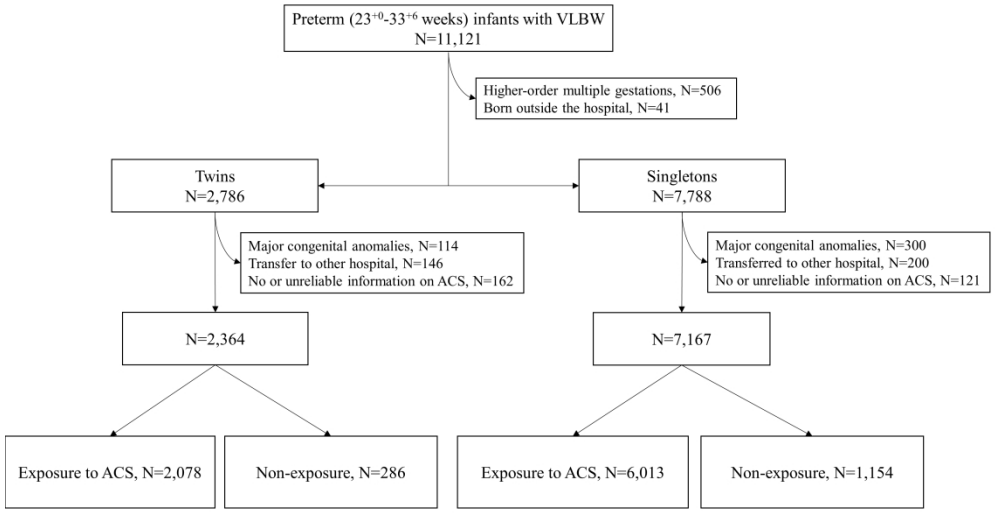

Figure 1. Flow chart showing the selection of study population.
ACS, antenatal corticosteroid; VLBW, very low birth weight.

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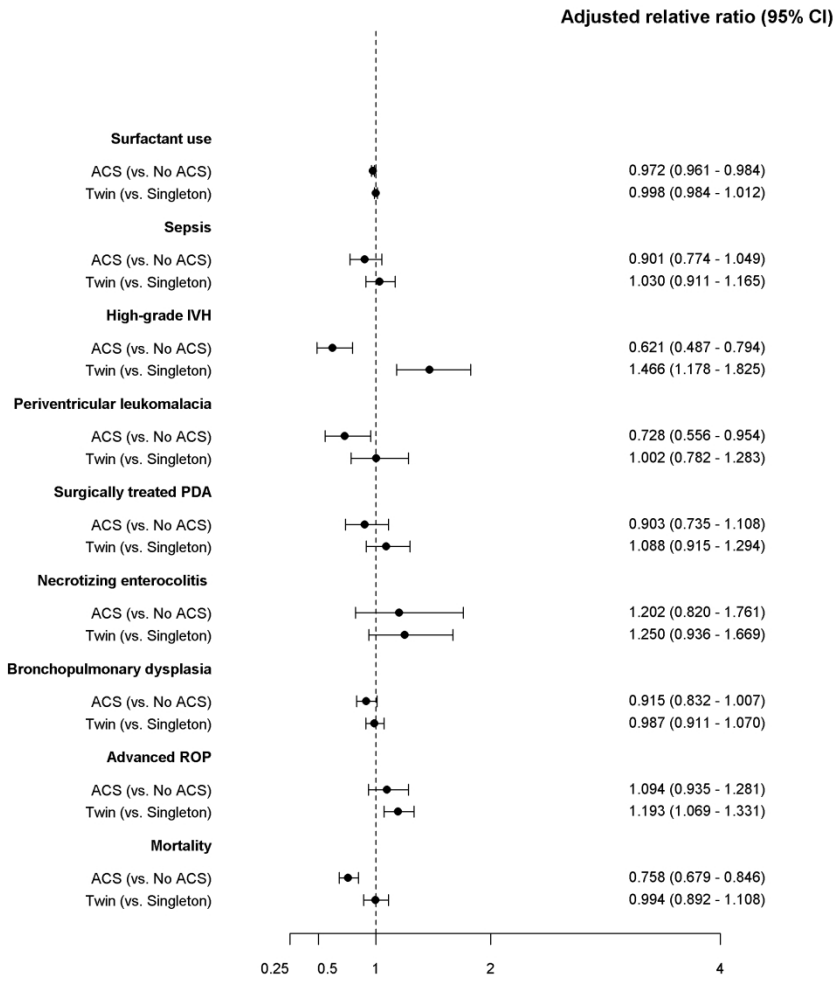
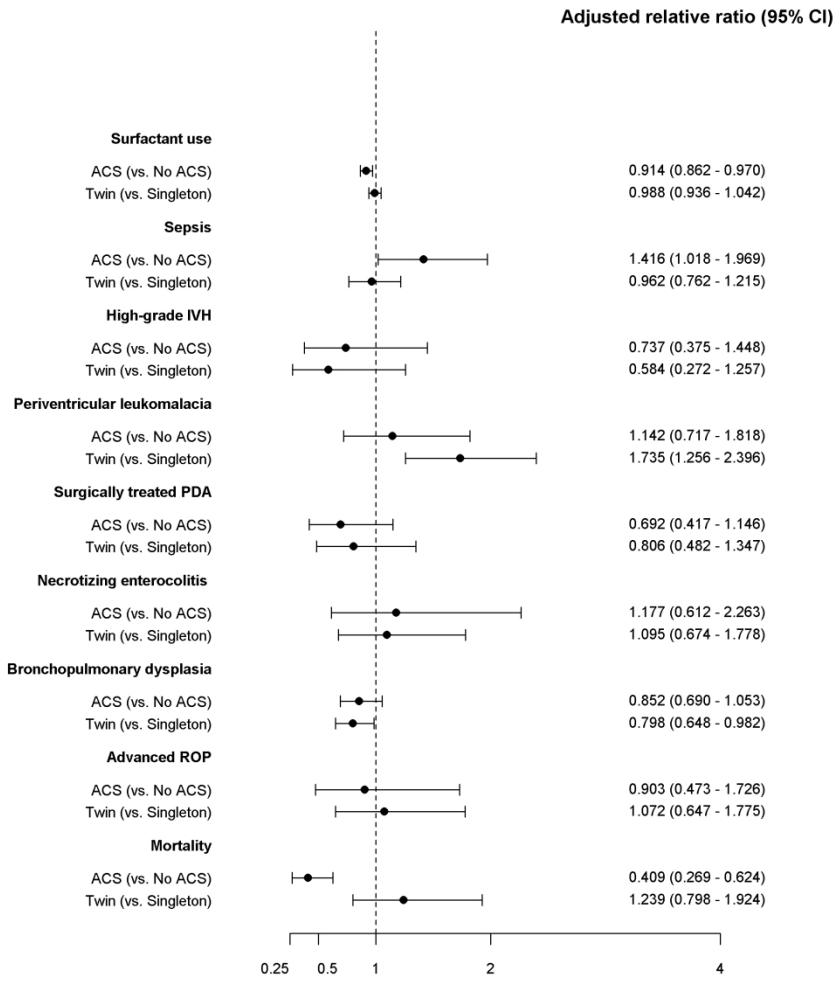


Figure 2. Forest plot showing results of logistic regression for effects of antenatal corticosteroids and twins on neonatal outcomes.
(A) 23 to 28 weeks of gestataional age. (B) 29 to 33 weeks of gestational age.
For each neonatal outcome, solid dots represent the aOR and lateral lines represent the 95% CI. Adjusted relative risk (RR) and 95% CI were obtained from multivariable Poisson regression models using generalized estimation equations..
Abbreviations: ACS, antenatal corticosteroid; CI, confidence interval; IVH, intraventricular hemorrhage; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity.

228x254mm (300 x 300 DPI)



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Supplemental text 1. The definition of variables

Small for gestational age (SGA) was defined as birth weight below the 10th percentile for gestational age according to the Fenton growth chart.[1] Sepsis was defined as a case of positive blood culture and requiring systemic antibiotics treatment for more than five days. Chorioamnionitis was defined as histologic findings of acute inflammation in the chorion-decidua, amnion, umbilical cord, and chorionic plate by pathologist at each participating facility using the criteria of Salafia et al.[2] modified by Yoon et al.[3] Maternal hypertension included pre-existing hypertension and/or pregnancy-induced hypertension. Maternal diabetes mellitus (DM) included pre-existing and/or pregnancy-induced DM. High-grade intraventricular hemorrhage (IVH) was defined as grade 3 or 4 IVH according to Papile's criteria.[4] Periventricular leukomalacia (PVL) was diagnosed based on brain ultrasound or magnetic resonance imaging obtained at term-equivalent age. Only cystic lesions were counted. Surgically treated patent ductus arteriosus (PDA) was defined as surgical ligation or division of symptomatic PDA. Necrotizing enterocolitis (NEC) was diagnosed and staged according to modified Bell's criteria.[5] Only NEC of stage 2 or higher was counted. Bronchopulmonary dysplasia (BPD) was defined as a need for supplementary oxygen at 36 weeks postmenstrual age (PMA) or discharge according to the National Heart, Lung, and Blood Institute (NHLBI) workshop definition.[6] Advanced retinopathy of prematurity (ROP) was defined as stage 3 or higher according to the International Classification for Retinopathy of Prematurity[7] or having an operation (cryotherapy, laser photocoagulation, or vitrectomy), or intravitreal injection with anti-vascular endothelial growth factor.[8]

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Supplemental Table 1. Comparison of baseline characteristics between twins and singletons

	23 – 28 weeks of gestational age			29 – 33 weeks of gestational age		
	Twin (n = 1,430)	Singleton (n = 3,977)	*P* value	Twin (n = 934)	Singleton (n = 3,190)	*P* value
Infant						
Gestational age (weeks), mean (SD)	26.63 (1.64)	26.60 (1.58)	0.471	30.19 (0.91)	30.80 (1.34)	0.001<
Birth weight (g), mean (SD)	917 (235)	913 (246)	0.581	1,268 (188)	1,248 (203)	0.005
SGA, n (%)	75 (5.2)	380 (9.6)	0.001<	94 (10.1)	899 (28.2)	0.001<
Male, n (%)	775 (54.2)	2,107 (53.0)	0.434	447 (47.9)	1,564 (49.0)	0.529
Maternal						
Maternal age (years), mean (SD)	33.05 (4.04)	33.33 (4.51)	0.031	33.51 (3.79)	33.36 (4.52)	0.305
Maternal diabetes mellitus, n (%)	138 (9.7)	348 (8.8)	0.268	126 (13.5)	338 (10.6)	0.013
Maternal hypertension, n (%)	74 (5.2)	678 (17.0)	0.001<	144 (15.5)	1357 (42.5)	0.001<
Chorioamnionitis[a], n (%)	446 (37.2)	1701 (49.9)	0.001<	133 (17.7)	734 (27.0)	0.001<
Premature rupture of membrane, n (%)	524 (37.0)	1770 (44.8)	0.001<	347 (37.4)	876 (27.6)	0.001<
Cesarean section, n (%)	1247 (87.2)	2823 (71.0)	0.001<	834 (89.3)	2618 (82.1)	0.001<
In vitro fertilization, n (%)	768 (54.8)	348 (8.8)	0.001<	512 (55.8)	197 (6.2)	0.001<
Antenatal corticosteroid, n (%)	1256 (87.8)	3331 (83.8)	0.001<	822 (88.0)	2,682 (84.1)	0.003

Data are presented as mean (SD) or n (%).

Abbreviations: ACS, antenatal corticosteroid; SGA, small for gestational age; IVH, intraventricular hemorrhage; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity.

[a] Values were missing for 797 infants in the group with 23 to 28 weeks of gestational age and 657 infants in the group with 29 to 33 weeks of gestational age.

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Title page

Original article

Effects of antenatal corticosteroids on neonatal outcomes in twin and singleton pregnancies: a Korean national cohort study

Seong Phil Bae[1,2], M.D., Won-Ho Hahn[1,2], M.D., Ph.D., Suyeon Park[3,4], MS., Young Hwa Jung[5,6], M.D., Ph.D., Jee Yoon Park[7], M.D., Kyung Joon Oh[7], M.D., Ph.D., Chang Won Choi[5,6], M.D., Ph.D.

[1]Department of Pediatrics, Soonchunhyang University Seoul Hospital, Seoul, Republic of Korea

[2]Department of Pediatrics, Soonchunhyang University School of Medicine, Cheonan, Republic of Korea

[3]Department of Biostatistics, Soonchunhyang University Seoul Hospital, Seoul, Republic of Korea

[4]Department of Applied Statistics, Chung-Ang University, Seoul, Republic of Korea

[5]Department of Pediatrics, Seoul National University Bundang Hospital, Seongnam, Republic of Korea

[6]Department of Pediatrics, Seoul National University College of Medicine, Seoul, Republic of Korea

[7]Department of Obstetrics and Gynecology, Seoul National University Bundang Hospital, Seongnam, Republic of Korea

Correspondence to

Chang Won Choi, M.D., Ph.D.

Department of Pediatrics, Seoul National University Bundang Hospital, 82 Gumi-ro 173 Beon-gil, Bundang-gu, Seongnam-si, Gyeonggi-do, Republic of Korea, E-mail: choicw@snu.ac.kr

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ABSTRACT

Objective

To investigate whether effects of antenatal corticosteroids on neonatal outcomes in preterm infants with very-low-birth-weight were different by plurality.

Design

Nationwide prospective cohort study

Setting

Not applicable

Patients

Twins and singletons with very-low-birth-weight (< 1,500 g) who were born between 23^{+0} and 33^{+6} weeks of gestation and registered in the Korean Neonatal Network from January 2014 to December 2019

Main outcome measures

Morbidity and mortality before discharge from neonatal intensive care unit

Results

Among a total of 9,531 preterm infants with very-low-birth-weight, there were 2,364 (24.8%) twins and 7,167 (75.2%) singletons. While 83.9% of singletons were exposed to at least one dose of antenatal corticosteroids, so were 87.9% of twins.

Interaction analysis demonstrated that there was no significant difference in the effect of antenatal corticosteroids on morbidities or mortality between twins and singletons in either gestational age group (23 to 28 weeks or 29 to 33 weeks).

Antenatal corticosteroids significantly decreased the risk of surfactant use (aRR: 0.972 [95% CI: 0.961 – 0.984]), high-grade intraventricular hemorrhage (aRR: 0.621 [95% CI: 0.487 – 0.794]), periventricular leukomalacia (aRR: 0.728 [95% CI: 0.556 – 0.954]), and mortality (aRR: 0.758 [95% CI: 0.679 – 0.846]) in the gestational age group of 23 to 28 weeks. In the gestational age group of 29 to 33 weeks, antenatal corticosteroids significantly decreased the risk of surfactant use (aRR: 0.914 [95% CI: 0.862 – 0.970]) and mortality (aRR: 0.409 [95% CI: 0.269 – 0.624]) but increased the risk of sepsis (aRR: 1.416 [95% CI: 1.018 – 1.969]).

Conclusion

This study demonstrates that effect of antenatal corticosteroids on neonatal outcomes of preterm infants with very-low-birth-weight does not differ significantly by plurality (twin or singleton pregnancy).

Keywords: antenatal corticosteroids, preterm delivery, mortality, morbidity, twins, very low birth weight infant

Key messages

Why is already known on this topic?

Antenatal corticosteroids (ACS) administered before preterm delivery can decrease neonatal morbidity and mortality in singleton pregnancies. However, data about such effects of ACS in multiple pregnancies are still limited.

What this study adds?

The effect of antenatal corticosteroids administered before preterm delivery on neonatal morbidity and mortality does not differ by plurality (twin or singleton pregnancy).

Infants exposed to ACS were more likely to survive and require less surfactant therapy in both 23 to 28 weeks and 29 to 33 weeks gestational age groups.

How this study might affect research, practice or policy?

This study highlights the need for further investigation to fully understand effects of ACS in multiple pregnancies, given the increasing prevalence of multiple pregnancies worldwide.

Introduction

Antenatal corticosteroid (ACS) has been established as the standard therapy for pregnant women at risk of preterm delivery within 7 days between 24^{+0} and 33^{+6} weeks of gestation.[1] Currently, ACS is equally recommended for twin and singleton pregnancies with the same regimen based on previous studies.[2-4] A recent study has shown that a complete course of ACS administered before preterm twin delivery is associated with a significant decrease in neonatal mortality, short-term respiratory morbidity, and severe neurological injury in a magnitude similar to those observed for a singleton pregnancy.[3] However, data that demonstrate comparable effects of ACS therapy in twin pregnancies are limited.

As a result of increasing childbirth age and use of assisted reproductive technology, twin pregnancies are increasing in many countries, including Korea.[5 6] However, twin pregnancies are more likely to have preterm birth, low birth weight, and longer hospital stays than singleton pregnancies.[7 8] In addition, mono-chorionicity, birth weight discordance, and obstetric complications associated with multiple pregnancies may result in adverse perinatal outcomes.[9-11]

Recently, the mortality of extreme preterm multiples has decreased to a level comparable to that of singletons born at the same gestational age along with increased use of ACS therapy.[12] However, many studies have reported conflicting results regarding equivalent effects of ACS on neonatal outcomes of twins and singletons.[3 13-18] To date, there have been no clinical trials designed to investigate the effect of ACS in twins. A meta-analysis on ACS therapy has also highlighted the need for additional studies on multiple pregnancies.[4]

Thus, the purpose of this study was to investigate whether effects of ACS administered before preterm delivery on neonatal outcomes of twins and singletons were comparable.

Materials and Methods

Study design and participants

Information on the study population is presented in Figure 1. Data collected prospectively through the Korean Neonatal Network (KNN), a nationwide very low birth weight (VLBW, <1,500 g) infant registry participated by 61 neonatal intensive care units across South Korea,[19] were used for this study. A total of 11,121 infants with VLBW born preterm (between 23^{+0} and 33^{+6} weeks of gestation) between January 2014 and December 2019 were enrolled. Exclusion criteria were: high-order multiple gestations (≥ triplets), birth outside the hospital, major congenital anomalies, transfer to other hospitals, no or unreliable information on ACS. Finally, 2,364 twins and 7,167 singletons were analyzed. ACS therapy was defined if the pregnant women had received at least one dose of any kind of corticosteroids before preterm delivery. Variables collected included gestational age, birth weight, small for gestational age (SGA), sex, maternal age, maternal diabetes mellitus (DM), maternal hypertension, chorioamnionitis, premature rupture of membrane, cesarean section, *in vitro* fertilization (IVF), surfactant use, sepsis, high-grade intraventricular hemorrhage (IVH), periventricular leukomalacia (PVL), surgically treated patent ductus arteriosus (PDA), bronchopulmonary dysplasia (BPD), advanced retinopathy of prematurity (ROP), and mortality. Definitions for different variables are provided

in Supplemental Materials. The analysis of neonatal morbidity was performed for infants who survived before discharge, while the analysis of mortality was performed for the total population.

Patent and public involvement

Patient and/or the public were not involved in the design, or recruitment, or conduct, or reporting, or dissemination of plans of the study.

Statistical methods

Rates of neonatal outcomes between infants exposed to ACS and infants without such exposure are presented along with risk difference and 95% confidence intervals (CI). To investigate whether associations between ACS and neonatal outcomes were altered by plurality (twins versus singletons), the interaction effect between ACS and twin pregnancies on neonatal outcomes was evaluated by adding interaction terms (ACS × twins) in Poisson regression models. The crude and adjusted relative risks (RR) and 95% CI for the individual effects of ACS and twins were obtained in the second model without the interaction term.

All models were fitted with generalized estimation equations (GEE) approach for the correlation between a pair of twins from a mother. Covariables in regression models were gestational age, birth weight, sex, maternal age, maternal diabetes mellitus, maternal hypertension, premature rupture of membrane, cesarean section, and *in vitro* fertilization. Chorioamnionitis was excluded

from the analyses due to substantial missing values (17.6% in twins and 14.5% in singletons).

For each outcomes, a backward-stepwise method was used to remove the variable with the highest *P* value, and variables in final model were selected that showed minimal QIC (Quasi-likelihood under the Independence model Criterion) value.[20-22] To confirm multi-collinearity, variance inflation factors values were checked for all covariates, which were all less than 5, indicating no significant multi-collinearity (1.002~2.989). The level of significance was set at $p < 0.05$. All statistical analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA) and 'geepack' and 'car' package R version 4.1.2 (The R Foundation for Statistical Computing, Vienna, Austria).

Ethic approval

Registration of data in the KNN was approved by the Institutional Review Board (IRB) of each participating center. Informed consent was obtained from parents of each infant prior to participation in the KNN registry. This study was approved by the IRB of Seoul National University Bundang Hospital (approval number: B-1305-202-005).

Results

Exposure to antenatal corticosteroids

Among 2,364 twins, 2,078 (87.9%) infants were exposed to at least one dose of ACS before

preterm delivery. Among 7,167 singletons, 6,013 (83.9%) infants were exposed to at least one dose of ACS before preterm delivery (Figure 1).

Comparisons of perinatal baseline characteristics between infants exposed to antenatal corticosteroids and infants without ACS exposure

Twins exposed to ACS were born at a later gestational age (mean [SD], 28.08 (2.19) vs 27.75 (2.50) weeks) compared to those without ACS exposure (Table 1). However, there was no difference in gestational age (mean [SD], 28.49 (2.52) vs 28.39 (2.78) weeks) or birth weight (mean [SD], 1,060 (281) vs 1,073 (289) g) between singletons exposed to ACS and those without ACS exposure. Singletons exposed to ACS had higher rates of maternal DM (9.9% vs 7.7%; P=0.019), maternal hypertension (29.1% vs 24.8%; P=0.003), and cesarean section (77.1% vs 69.9%; P<0.001) than those without exposure. In both twins and singletons, infants exposed to ACS were more likely to have higher rates of premature rupture of membrane and be conceived through IVF from older mothers than those without exposure.

Table 1. Comparisons of perinatal baseline characteristics between infants exposed to ACS and infants without ACS exposure

	Twins (n = 2,364)			Singletons (n = 7,167)		
	ACS-exposed (n = 2,078)	ACS-unexposed (n = 286)	*P* value[a]	ACS-exposed (n = 6,013)	ACS-unexposed (n = 1,154)	*P* value[a]
Infant						
Gestational age (weeks), mean (SD)	28.08 (2.19)	27.75 (2.50)	0.034	28.49 (2.52)	28.39 (2.78)	0.256
Birth weight (g), mean (SD)	1,059 (274)	1,034 (301)	0.186	1,060 (281)	1,073 (289)	0.138
SGA, n (%)	147 (7.1)	22 (7.7)	0.704	1080 (18.0)	199 (17.2)	0.560
Male, n (%)	1079 (51.9)	143 (50.0)	0.541	3074 (51.1)	597 (51.7)	0.708
Maternal						
Maternal age (years), mean (SD)	33.41 (3.80)	31.91 (4.66)	<0.001	33.44 (4.43)	32.82 (4.92)	<0.001
Maternal diabetes mellitus, n (%)	238 (11.5)	26 (9.1)	0.221	597 (9.9)	89 (7.7)	0.019
Maternal hypertension, n (%)	196 (9.5)	22 (7.7)	0.329	1749 (29.1)	286 (24.8)	0.003
Chorioamnionitis[b], n (%)	516 (29.9)	63 (28.0)	0.551	2084 (40.2)	351 (37.0)	0.064
Premature rupture of membrane, n (%)	787 (38.1)	84 (29.9)	0.007	2334 (38.9)	312 (27.5)	<0.001
Cesarean section, n (%)	1837 (88.4)	244 (85.3)	0.132	4634 (77.1)	807 (69.9)	<0.001
In vitro fertilization, n (%)	1152 (56.6)	128 (45.1)	<0.001	494 (8.2)	51 (4.4)	<0.001

Data are presented as mean (SD) or n (%).

Abbreviations: ACS, antenatal corticosteroids; SD, standard deviation; SGA, small for gestational age; IVH, intraventricular hemorrhage; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity.

[a]*P* value obtained from chi-squared test for categorical variables and Student's *t*-test for continuous variables.

[b] Values were missing for 415 infants in the group of twins and 1039 infants in the group of singletons.

Interaction between ACS and twins pregnancies on neonatal outcomes

We investigated whether effects of ACS on neonatal outcomes differed between twins and singletons. Because neonatal outcomes are substantially dependent on gestational age, comparison of ACS effects on neonatal outcomes by plurality was performed by stratifying the study population into two gestation age groups: an age group of 23 to 28 weeks and an age group of 29 to 33 weeks group.

Interaction analyses within 23 to 28 weeks (Table 2) and 29 to 33 weeks (Table 3) gestational age groups found no significant difference in the association of ACS therapy with any neonatal outcomes between twins and singletons (P>0.1 for all).

Table 2. Comparisons of neonatal outcomes between infants exposed to ACS and infants without ACS exposure in infants born at 23 to 28 weeks of gestational age

Infants who survived before discharge (n = 4160)	ACS-exposed (n=3606)	ACS-unexposed (n=554)	Risk difference, % (95 CI)	*P* value[a]	*P* value for interaction[b]
Surfactant use, n (%)					
Total	3468/3606 (96.2%)	546/554 (98.6%)	-2.4 (-3.6 to -1.2)	0.005	
Twin	952/991 (96.1%)	108/109 (99.1%)	-3.0 (-5.2 to -0.9)	0.110	0.199
Singleton	2516/2615 (96.2%)	438/445 (98.4%)	-2.2 (-3.6 to -0.8)	0.018	
Sepsis, n (%)					
Total	985/3606 (27.3%)	166/554 (30.0%)	-2.7 (-6.7 to 1.4)	0.195	
By numbers of fetus					
Twin	273/991 (27.5%)	32/109 (29.4%)	-1.8 (-10.8 to 7.2)	0.689	0.706
Singleton	712/2615 (27.2%)	134/445 (30.1%)	-2.9 (-7.5 to 1.7)	0.209	
High-grade IVH, n (%)					
Total	313/3606 (8.7%)	79/554 (14.3%)	-5.6 (-8.6 to -2.5)	<0.001	
By numbers of fetus					
Twin	115/991 (11.6%)	15/109 (13.8%)	-2.2 (-8.9 to 4.6)	0.508	0.224
Singleton	198/2615 (7.6%)	64/445 (14.4%)	-6.8 (-10.2 to -3.4)	<0.001	
Periventricular leukomalacia, n (%)					
Total	327/3606 (9.1%)	74/554 (13.4%)	-4.3 (-7.3 to -1.3)	0.001	
By numbers of fetus					
Twin	86/991 (8.7%)	14/109 (12.8%)	-4.2 (-10.7 to 2.4)	0.151	0.749
Singleton	241/2615 (9.2%)	60/445 (13.5%)	-4.3 (-7.6 to -0.9)	0.005	
Surgically treated PDA, n (%)					
Total	616/3606 (17.1%)	99/554 (17.9%)	-0.8 (-4.2 to 2.6)	0.647	
By numbers of fetus					
Twin	175/991 (17.7%)	23/109 (21.1%)	-3.4 (-11.5 to 4.6)	0.375	0.400
Singleton	441/2615 (16.9%)	76/445 (17.1%)	-0.2 (-4.0 to 3.6)	0.911	
Necrotizing enterocolitis, n (%)					
Total	268/3606 (7.4%)	36/554 (6.5%)	0.9 (-1.3 to 3.2)	0.432	
By numbers of fetus					
Twin	72/991 (7.3%)	8/109 (7.3%)	-0.07 (-5.2 to 5.1)	0.978	0.479
Singleton	196/2615 (7.5%)	28/445 (6.3%)	1.2 (-1.3 to 3.7)	0.368	

Bronchopulmonary dysplasia, n (%)					
Total	1664/3606 (46.1%)	269/554 (48.6%)	-2.4 (-6.9 to 2.1)	0.290	
By numbers of fetus					
Twin	437/991 (44.1%)	51/109 (46.8%)	-2.7 (-12.6 to 7.2)	0.591	0.986
Singleton	1227/2615 (46.9%)	218/445 (49.0%)	-2.1 (-7.1 to 3.0)	0.419	
Advanced ROP, n (%)					
Total	769/3602 (21.3%)	113/554 (20.4%)	1.0 (-2.7 to 4.6)	0.610	
By numbers of fetus					
Twin	232/990 (23.4%)	31/109 (28.4%)	-5.0 (-13.9 to 3.9)	0.245	0.158
Singleton	537/2612 (20.6%)	82/445 (18.4%)	2.1 (-1.8 to 6.1)	0.301	
All infants (n = 5407)	ACS (n=4587)	No ACS (n=820)	Risk difference, %(95 CI)	*P* value	*P* value for interaction*
Mortality, n (%)					
Total	981/4587 (21.4%)	266/820 (32.4%)	-11.1 (-14.5 to -7.6)	<0.001	
By numbers of fetus					
Twin	265/1256 (21.1%)	65/174 (37.4%)	-16.3 (-23.8 to -8.7)	<0.001	0.458
Singleton	716/3331 (21.5%)	201/646 (31.1%)	-9.6 (-13.5 to -5.8)	<0.001	

Data are presented as n (%).

[a] Tests for risk difference within each subgroup.

[b] Tests for interaction between antenatal corticosteroids and twin pregnancies on each outcome. *P*-values for interaction were obtained from multivariable Poisson regression models using the generalized estimation equations.

Abbreviations: ACS, antenatal corticosteroid; CI, confidence interval; SGA, small for gestational age; IVH, intraventricular hemorrhage; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity.

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Table 3. Comparisons of neonatal outcomes between infants exposed to ACS and infants without ACS exposure in infants born at 29 to 33 weeks of gestational age

Infants who survived before discharge (n = 4,019)	ACS-exposed (n=3428)	ACS-unexposed (n=591)	Risk difference, % (95 CI)	*P*-value[a]	*P*-value for interaction[b]
Surfactant use, n (%)					
Total	2240/3428 (65.3%)	402/591 (68.0%)	-2.7 (-6.8 to 1.4)	0.206	
By numbers of fetus					

Twin	583/801 (72.8)	86/104 (82.7)	-9.9 (-17.8 to -2.0)	0.030	0.243
Singleton	1657/2627 (63.1)	316/487 (64.9)	-1.8 (-6.4 to 2.8)	0.446	
Sepsis, n (%)					
Total	376/3428 (11.0)	41/591 (6.9)	4.0 (1.7 to 6.3)	0.003	
By numbers of fetus					
Twin	90/801 (11.2)	8/104 (7.7)	3.5 (-2.0 to 9.1)	0.274	0.966
Singleton	286/2627 (10.9)	33/487 (6.8)	4.1 (1.6 to 6.6)	0.006	
High-grade IVH, n (%)					
Total	53/3426 (1.5)	14/591 (2.4)	-0.8 (-2.1 to 0.5)	0.150	
By numbers of fetus					
Twin	10/801 (1.2)	2/104 (1.9)	-0.7 (-3.4 to 2.1)	0.572	0.742
Singleton	43/2625 (1.6)	12/487 (2.5)	-0.8 (-2.3 to 0.6)	0.204	
Periventricular leukomalacia, n (%)					
Total	179/3427 (5.2)	30/591 (5.1)	0.2 (-1.8 to 2.1)	0.882	
By numbers of fetus					
Twin	71/801 (8.9)	6/104 (5.8)	3.1 (-1.8 to 8.0)	0.287	0.492
Singleton	108/2626 (4.1)	24/487 (4.9)	-0.8 (-2.9 to 1.3)	0.412	
Surgically treated PDA, n (%)					
Total	95/3428 (2.8)	18/591 (3.0)	-0.3 (-1.8 to 1.2)	0.709	
By numbers of fetus					
Twin	19/801 (2.4)	4/104 (3.8)	-1.5 (-5.3 to 2.4)	0.369	0.955
Singleton	76/2627 (2.9)	14/487 (2.9)	0.02 (-1.6 to 1.6)	0.982	
Necrotizing enterocolitis, n (%)					
Total	85/3427 (2.5)	11/591 (1.9)	0.6 (-0.6 to 1.8)	0.363	
By numbers of fetus					
Twin	21/801 (2.6)	4/104 (3.8)	-1.2 (-5.1 to 2.6)	0.474	0.492
Singleton	64/2626 (2.4)	7/487 (1.4)	1 (-0.2 to 2.2)	0.175	
Bronchopulmonary dysplasia, n (%)					
Total	524/3423 (15.3)	93/591 (15.7)	-0.4 (-3.6 to 2.8)	0.790	
By numbers of fetus					
Twin	114/797 (14.3)	16/104 (15.4)	-1.1 (-8.4 to 6.3)	0.768	0.834
Singleton	410/2626 (15.6)	77/487 (15.8)	-0.2 (-3.7 to 3.3)	0.912	
Advanced ROP, n (%)					

Total	45/3426 (1.3)	10/591 (1.7)	-0.4 (-1.5 to 0.7)	0.465	
By numbers of fetus					
Twin	13/800 (1.6)	3/104 (2.9)	-1.3 (-4.6 to 2.1)	0.359	0.202
Singleton	32/2626 (1.2)	7/487 (1.4)	-0.2 (-1.4 to 0.9)	0.690	
All infants (n = 4,124)	ACS (n=3504)	No ACS (n=620)	Risk difference, %(95 CI)	*P* value	*P* value for interaction*
Mortality, n (%)					
Total	76/3504 (2.2)	29/620 (4.7)	-2.5 (-4.2 to -0.8)	<0.001	
By numbers of fetus					
Twin	21/822 (2.6)	8/112 (7.1)	-4.6 (-9.5 to 0.3)	0.009	0.722
Singleton	55/2682 (2.1)	21/508 (4.1)	-2.1 (-3.9 to-..6 -0.3)	0.005	

Data are presented as n (%).

[a] Tests for risk difference within each subgroup.

[b] Tests for interaction between antenatal corticosteroids and twin pregnancies on each outcome. *P*-values for interaction were obtained from multivariable Poisson regression models using the generalized estimation equations.

Abbreviations: ACS, antenatal corticosteroid; CI, confidence interval; SGA, small for gestational age; IVH, intraventricular hemorrhage; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity.

Independent effects of ACS and twins on neonatal outcomes

In the second model excluding the interaction term, we calculated adjusted RR and 95% CI for individual effects of ACS and twins on each neonatal outcome. In the age group of 23 to 28 weeks, exposure to ACS was significantly associated with a lower risk of surfactant use (adjusted RR (aRR): 0.972 [95% CI: 0.961 – 0.984]), high-grade intraventricular hemorrhage (aRR: 0.621 [95% CI: 0.487 – 0.794]), periventricular leukomalacia (aRR: 0.728 [95% CI: 0.556 – 0.954]), and mortality (aRR: 0.758 [95% CI: 0.679 – 0.846]) (Figure 2A). Twin were associated with higher risks of high-grade IVH (aRR: 1.466 [95% CI: 1.178 – 1.825]) and advanced ROP (aRR: 1.193 [95% CI: 1.069 – 1.331]) than singletons.

In the age group of 29 to 33 weeks, exposure to ACS was significantly associated with lower risks of surfactant use (aRR: 0.914 [95% CI: 0.862 – 0.970]) and mortality (aRR: 0.409 [95% CI: 0.269 – 0.624]), but a higher risk of sepsis (aRR: 1.416 [95% CI: 1.018 – 1.969]) (Figure 2B). Twins showed a lower risk of BPD (aRR: 0.798 [95% CI: 0.648 – 0.982]), but a higher risk of PVL (aRR: 1.735 [95% CI: 1.256 – 2.396]) than singletons.

Discussion

In this nationwide cohort study, we demonstrated that effects of ACS therapy administered before preterm birth on neonatal outcomes were not significantly different by plurality. Mortality and surfactant use were reduced by ACS exposure in both gestational age groups. ACS exposure was associated with decreased risks of high-grade IVH and PVL in infants born at 23 to 28

weeks of gestational age, but increased the risk of sepsis in infants born at 29 to 33 weeks of gestational age.

ACS therapy for mothers at risk of impending preterm delivery is the most effective evidence-based strategy to reduce the mortality and morbidity of their preterm offspring. However, previous studies that investigated the association between ACS therapy and neonatal outcomes in twin pregnancies not only reported conflicting results, but also lacked quantity.

A recent meta-analysis has reported that ACS therapy is associated with decreased neonatal death, RDS, and intraventricular hemorrhage in singleton pregnancies, but not in multiple pregnancies.[4] Furthermore, a randomized controlled trial on 311 twin infants who were delivered before 34 weeks of gestation age showed that ACS therapy had no association with RDS or composite neonatal morbidity.[23] However, large population-based cohort studies from the mid-2000s reported positive ACS effects in multiple pregnancies.[24-26] A study of 750 twin infants from France has reported that a complete course of ACS therapy administered within 7 days before birth is associated with decreased rates of brain injury and mortality.[24] Another study of 8,274 multiples from Italian Neonatal Network reported that ACS therapy reduced the risk of severe IVH and mortality, although it was less effective in multiple pregnancies than in singleton pregnancies.[25] A study of 2,516 twin infants from Canadian Neonatal Network has reported that twins can benefit from a complete course of ACS therapy as good as singletons in short-term respiratory morbidity, severe brain injury, and mortality.[26] Consistent with results of these large cohort studies, our study using the latest data from KNN revealed that the effectiveness of ACS therapy in twins was comparable to that of singletons. Moreover, its benefits on mortality and

morbidity were shown to be different by gestational groups.

Additionally, we found that twins had a higher rate of morbidity than singletons in this study. Twins were associated with higher risks of high-grade IVH and advanced ROP in infants born at 23 to 28 weeks of GA and PVL in infants born at 29 to 33 weeks of GA than singletons. Although twins are more likely to be delivered preterm than singletons, whether twin pregnancy alone has such adverse effects on neonatal outcomes in preterm infants remains unknown.[27] In a large study of infants born between 23 and 35 weeks of gestation, twins and singletons had comparable average birth weights up to 32 weeks of gestation and similar neonatal outcomes.[28] One study from Australian and New Zealand Neonatal Network has found higher mortality, but not morbidities, in twins than in singletons.[12] Furthermore, two national cohort studies have reported comparable risks for neonatal outcomes except for RDS between twins and singletons.[29 30] However, a population-based European cohort study showed that twins had higher risk for mortality and high-grade IVH in infants born at 24 to 27 weeks of GA.[14] Other studies have revealed disparities in the rate of morbidity such as BPD and ROP between twins and singletons.[8 31]

Possible reasons for conflicting findings in twins include study design and changes in population characteristics over time.[32] We found that twins had lower rates of antenatal complications such as maternal hypertension and chorioamnionitis, and higher rates of obstetric interventions, including IVF, ACS, and cesarean section than singletons in both gestational age groups (Supplemental Table 1). This trend has also been observed across other recent studies.[12 33] The higher rate of IVF in twins reflect the current trend of childbirth in Korea.[5] The reason for higher

ACS exposure in twins is currently unclear. However, more medical attention for twin pregnancies might be one possible cause.[34] One noteworthy result was that the cesarean section rate for twins born between 23 and 28 weeks of GA was 87.2%, which was higher in Korea than in other countries. For similar gestational age, the cesarean section rate for twins was 74.4% in the USA and 61.8% in Australia and New Zealand.[12 15]

Despite the fact that these difference in characteristics between twins and singletons are observed in other studies, an increase in the proportion of the population exposed to ACS along with variation in practice of neonatal care in each country might have complexly contributed to the interaction between ACS and plurality as well as the inherent risk of a twin pregancy.[35] Although we adjusted potential confounders which were different between twins and singletons in the analyses, different population characteristics between twins and singletons and/or between studies should be considered when interpreting our results.

Our study has several limitations. Firstly, the KNN registry had no information about chorionicity in multiple pregnancies. Thus, we were unable to investigate the difference in the effect of ACS therapy on neonatal outcomes according to chorionicity in twins. Secondly, although we used prospective cohort data of preterm infants with VLBW for this study, antenatal information including ACS administration was collected retrospectively because preterm infants were enrolled after they were born. Therefore, it was unknown why ACS was not or incompletely administered in infants who did not receive a complete course of ACS therapy. Notably, the percentage of singletons without exposure to ACS was higher in this study than in other studies.[36 37] Moreover, information on the total amount of ACS administered was not

available in the KNN registry database. Information on total amount of ACS administered will enable a more quantitative analysis of effects of ACS therapy on neonatal outcomes. Lastly, in our cohort, 56.6% of twins were conceived via IVF whereas only 7.1% of singletons were conceived via IVF. Although preterm infants conceived via IVF are known to have comparable morbidity and mortality with their peers conceived via natural pregnancy[38], higher proportion of IVF cases among twins should be taken into account when interpreting our results.

Conclusion

Despite differences in demographic and clinical characteristics according to plurality, ACS therapy administered before birth had comparable positive effects on neonatal outcomes of preterm infants with VLBW regardless of plurality.

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Competing interests

None declared.

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Data Availability statement

The Korean Neonatal Network (KNN) Publication Ethics Policy adheres to the following research data management and access guidelines: All information about patients and participating NICUs are confidential. They might be used by individuals for approved research purposes. If an individual or institution that is not affiliated with KNN wants to use the data, it must make an official request to the KNN publication-Ethics Committee and obtain approval from the network.

Author contributions

Seong Phil Bae: Conceptualization, Methodology, Investigation, Writing – Original draft preparation, and Funding acquisition. Won-Ho Han: Investigation, Writing – Review & Editing. Suyeon Park: Formal analysis, Visualization. Young Hwa Jung: Data curation, Writing – Review & Editing. Jee Yoon Park: Writing – Review & Editing. Kyung Joon Oh: Writing – Review &

Editing. Chang Won Choi: Conceptualization, Methodology, Writing – Review & Editing, and Supervision.

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Figure Legends

Figure 1. Flow chart showing the selection of study population.

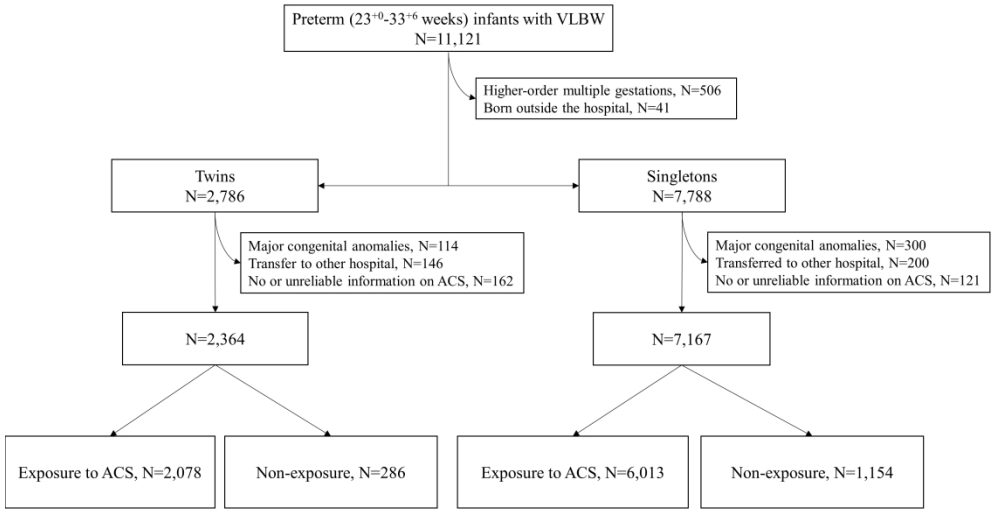
ACS, antenatal corticosteroid; VLBW, very low birth weight.

Figure 2. Forest plot showing results of logistic regression for effects of antenatal corticosteroids and twins on neonatal outcomes.

(A) 23 to 28 weeks of gestataional age. (B) 29 to 33 weeks of gestational age.

For each neonatal outcome, solid dots represent the aOR and lateral lines represent the 95% CI. Adjusted relative risk (RR) and 95% CI were obtained from multivariable Poisson regression models using generalized estimation equations..

Abbreviations: ACS, antenatal corticosteroid; CI, confidence interval; IVH, intraventricular hemorrhage; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity.



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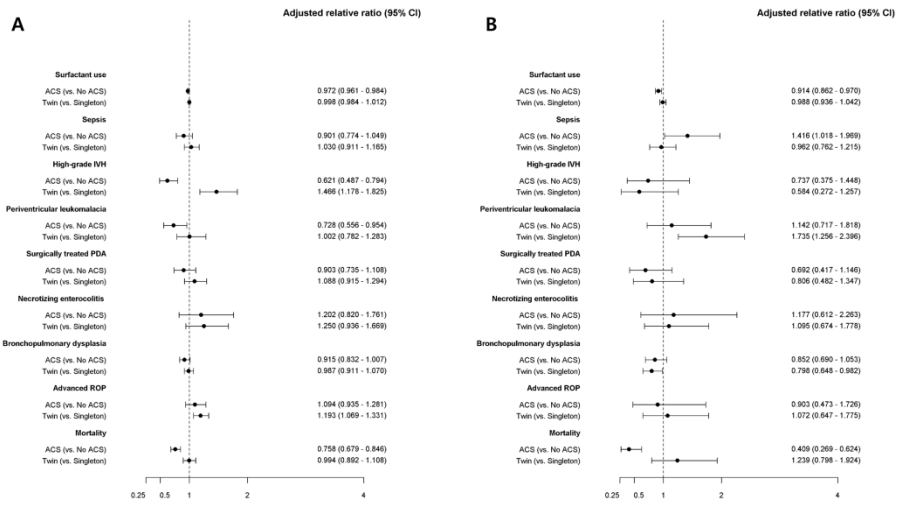

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Supplemental text 1. The definition of variables

Small for gestational age (SGA) was defined as birth weight below the 10th percentile for gestational age according to the Fenton growth chart.[1] Sepsis was defined as a case of positive blood culture and requiring systemic antibiotics treatment for more than five days. Chorioamnionitis was defined as histologic findings of acute inflammation in the chorion-decidua, amnion, umbilical cord, and chorionic plate by pathologist at each participating facility using the criteria of Salafia et al.[2] modified by Yoon et al.[3] Maternal hypertension included pre-existing hypertension and/or pregnancy-induced hypertension. Maternal diabetes mellitus (DM) included pre-existing and/or pregnancy-induced DM. High-grade intraventricular hemorrhage (IVH) was defined as grade 3 or 4 IVH according to Papile's criteria.[4] Periventricular leukomalacia (PVL) was diagnosed based on brain ultrasound or magnetic resonance imaging obtained at term-equivalent age. Only cystic lesions were counted. Surgically treated patent ductus arteriosus (PDA) was defined as surgical ligation or division of symptomatic PDA. Necrotizing enterocolitis (NEC) was diagnosed and staged according to modified Bell's criteria.[5] Only NEC of stage 2 or higher was counted. Bronchopulmonary dysplasia (BPD) was defined as a need for supplementary oxygen at 36 weeks postmenstrual age (PMA) or discharge according to the National Institute of Child Health and Human Development, the National Heart, Lung and Blood Institute, and the Office of Rare Diseases workshop definition.[6] Advanced retinopathy of prematurity (ROP) was defined as stage 3 or higher according to the International Classification for Retinopathy of Prematurity[7] or having an operation (cryotherapy, laser photocoagulation, or vitrectomy), or intravitreal injection with anti-vascular endothelial growth factor.[8]

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Supplemental Table 1. Comparison of baseline characteristics between twins and singletons

	23 – 28 weeks of gestational age			29 – 33 weeks of gestational age		
	Twin (n = 1,430)	Singleton (n = 3,977)	*P* value	Twin (n = 934)	Singleton (n = 3,190)	*P* value
Infant						
Gestational age (weeks), mean (SD)	26.63 (1.64)	26.60 (1.58)	0.471	30.19 (0.91)	30.80 (1.34)	0.001<
Birth weight (g), mean (SD)	917 (235)	913 (246)	0.581	1,268 (188)	1,248 (203)	0.005
SGA, n (%)	75 (5.2)	380 (9.6)	0.001<	94 (10.1)	899 (28.2)	0.001<
Male, n (%)	775 (54.2)	2,107 (53.0)	0.434	447 (47.9)	1,564 (49.0)	0.529
Maternal						
Maternal age (years), mean (SD)	33.05 (4.04)	33.33 (4.51)	0.031	33.51 (3.79)	33.36 (4.52)	0.305
Maternal diabetes mellitus, n (%)	138 (9.7)	348 (8.8)	0.268	126 (13.5)	338 (10.6)	0.013
Maternal hypertension, n (%)	74 (5.2)	678 (17.0)	0.001<	144 (15.5)	1357 (42.5)	0.001<
Chorioamnionitis[a], n (%)	446 (37.2)	1701 (49.9)	0.001<	133 (17.7)	734 (27.0)	0.001<
Premature rupture of membrane, n (%)	524 (37.0)	1770 (44.8)	0.001<	347 (37.4)	876 (27.6)	0.001<
Cesarean section, n (%)	1247 (87.2)	2823 (71.0)	0.001<	834 (89.3)	2618 (82.1)	0.001<
In vitro fertilization, n (%)	768 (54.8)	348 (8.8)	0.001<	512 (55.8)	197 (6.2)	0.001<
Antenatal corticosteroid, n (%)	1256 (87.8)	3331 (83.8)	0.001<	822 (88.0)	2,682 (84.1)	0.003

Data are presented as mean (SD) or n (%).

Abbreviations: ACS, antenatal corticosteroid; SGA, small for gestational age; IVH, intraventricular hemorrhage; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity.

[a] Values were missing for 797 infants in the group with 23 to 28 weeks of gestational age and 657 infants in the group with 29 to 33 weeks of gestational age.