Received: 26 May 2022 | Revised: 7 July 2022 | Accepted: 9 July 2022

DOI: 10.1111/cts.13382

ARTICLE

Association between betamethasone levels and respiratory distress syndrome in preterm births: A prospective cohort study

Noah Zafran[1,2] | **Manal Massalha**[1] | **Abeer Suleiman**[1] | **Refaat Massalha**[3] | **Lila Mahagna**[3] | **Scott A. Weiner**[4] | **Shabtai Romano**[1,2] | **Eliezer Shalev**[1,2] | **Raed Salim**[1,2]

[1]Department of Obstetrics and Gynecology, Emek Medical Center, Afula, Israel

[2]The Ruth and Bruce Rappaport Faculty of Medicine, Technion, Haifa, Israel

[3]Endocrinology Laboratory, Emek Medical Center, Afula, Israel

[4]Department of Neonatology, Emek Medical Center, Afula, Israel

Correspondence
Raed Salim, Department of Obstetrics and Gynecology, Emek Medical Center, Afula 18101, Israel.
Email: salim_ra@clalit.org.il

Funding information
Emek Medical Center and Technion, Research and Development Foundation, LTD, Israel, Grant/Award Number: 2022418

Abstract

The recommended fixed dosage of betamethasone for pregnancies at risk of preterm birth was determined in the 1970s, regardless of gestational age (GA), number of fetuses, and maternal weight. We aimed to examine the association between maternal and neonatal betamethasone serum levels and neonatal respiratory distress syndrome (RDS) and to examine whether levels correlate with maternal weight, GA, or number of fetuses. A prospective study was conducted at a single academic medical center between August 2016 and February 2019. Women received betamethasone and delivered between 28^{+0} and 34^{+6} weeks were included. Maternal serum levels (MSLs), and neonatal serum levels (NSLs) of betamethasone at delivery were analyzed using Corticosteroid enzyme-linked immunosorbent assay kit. RDS was diagnosed according to clinical and radiographic findings. We assumed that the sensitivity of NSLs to detect RDS is 95%; hence, 150 neonates were needed (power 80%, alpha 0.05). Overall, 124 women were included; including 96 (77.4%) singletons, 26 (21.0%) twins, and 2 (1.6%) triplets, corresponding to 154 neonates. RDS was diagnosed in 35 neonates (22.7%). After adjusting for GA, time elapsed from the last dose, and number of doses, NSLs were associated with RDS (relative risk: 0.97, 95% confidence interval: 0.94–0.99, $p = 0.011$). A level of 6.00 ng/ml predicted RDS with a sensitivity of 80.0% and specificity of 64.7%. Adjusted MSLs were not associated with RDS. Both maternal and neonatal serum levels were not associated with the number of fetuses and maternal weight. In conclusion, NSLs are associated with RDS whereas MSLs are not.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

The currently recommended betamethasone dosage for fetal lungs' maturity was determined in the 1970s, regardless of gestational age, maternal body mass index,

and number of fetuses. This fixed dosage that is administered to all pregnant women at risk of preterm birth is associated with nonpersistent and an unequal effect on neonatal morbidity and mortality.

WHAT QUESTION DID THIS STUDY ADDRESS?

Is there an association between maternal or fetal betamethasone levels and neonatal outcome, and whether levels differ according to maternal weight, gestational age, or number of fetuses?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Adjusted neonatal betamethasone serum levels were associated with respiratory distress syndrome, whereas adjusted maternal serum levels were not. Both were not associated with maternal weight or number of fetuses, and only weakly associated with gestational age at delivery.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

The results derived from this study suggest that simply adjusting betamethasone dosage according to maternal serum levels, gestational age, number of fetuses, or maternal weight at delivery is unlikely to lead to better determining the optimum betamethasone dosage.

INTRODUCTION

Preterm birth has an occurrence rate of nearly 12% and is considered the main cause of neonatal mortality and morbidity in the Western world.[1] This neonatal morbidity is manifested, among others, in neonatal respiratory distress syndrome (RDS), intracranial hemorrhage, necrotizing enterocolitis, etc.[2] Of the surviving neonates, ~10%–15% remains with significant sequel.[1] Antenatal corticosteroid (ACS) administered to women at risk of preterm birth for accelerating fetal lung maturation is considered the most effective intervention to reduce mortality and morbidity, associated with prematurity, particularly RDS.[2,3]

Nonetheless, there is an unexplained gap in clinical outcomes in ACS therapy administered at any given gestational age (GA). Namely, after ACS administration, a proportion of preterm neonates develop RDS whereas others, with similar obstetric background, born at the same GA or even younger, do not. Moreover, compared to singleton pregnancies, in twins, who are at higher risk to develop RDS, current evidence is insufficient to assess effectiveness of ACS therapy.[3]

Since determined in the early 1970s, the current accepted fixed dosage of betamethasone is administered to all pregnant women at risk of preterm birth, regardless of maternal weight, or body mass index (BMI), GA, or whether they are singleton or multiple pregnancies.[2] Given that a fixed dosage is administered to all pregnant women at risk of preterm birth, combined with the nonpersistent and unequal effect on neonatal morbidity and mortality, may point to a possible association between betamethasone levels and neonatal outcome.[4]

In human subjects, it is uncertain if an association exists between maternal or fetal betamethasone levels and neonatal outcome, and whether levels differ according to maternal weight, GA, or number of fetuses. In the present study, we aimed to determine maternal and neonatal betamethasone levels at birth, among women who received betamethasone between 28^{+0} and 34^{+0} weeks of gestation, and to examine whether an association exists with neonatal RDS.

METHODS

A prospective cohort study was conducted at a single university institution between August 2016 and February 2019. Pregnant women who received betamethasone between 28 and 34 weeks of gestation, due to threatened spontaneous or indicated preterm delivery and delivered between 28^{+0} and 34^{+6}, were included. A complete course of betamethasone (consisting of 50% betamethasone phosphate and 50% betamethasone acetate) comprised of two doses; 12 mg each, 24 h apart. Women who received the first dose only because of failure to delay delivery were also included. Maternal and cord blood were drawn simultaneously at delivery to determine betamethasone levels tested at designated laboratories.

Women who received corticosteroids for other reasons or had fetal malformations diagnosed in the antepartum period or immediately postpartum were

excluded. Eligible women who were recruited before 34 weeks of gestation, but delivered at or beyond 35 weeks of gestation, or delivered at another institution, were also excluded from the analysis. The policy at our institution during the study period was to give a complete course of betamethasone at signs of impending spontaneous preterm delivery or potential indication for medically indicated preterm delivery (<34 weeks). A single rescue course was administered up to 34 weeks, if delivery did not occur within 7 days, but risk of preterm delivery persisted.

Determining betamethasone concentrations

In the present study, both maternal and neonatal (cord) levels were analyzed using the same Corticosteroid enzyme-linked immunosorbent assay (ELISA) kit (Randox Laboratories Ltd.). Preliminary results showed that the kit is valid for determining betamethasone concertation.[5] The assay exhibits very low cross-reactivity for cortisol (<0.5%), which is vital in minimizing interference from endogenous cortisol. In the current study, 3 ml of maternal blood was drawn at delivery of the neonates, and was sent together with blood drawn from the umbilical cord immediately after birth. The paired samples were centrifuged and serum was kept at −70°C until processing. Betamethasone concentration in samples was processed in an SQII (AESKU.SYSTEMS GmbH & Co. KG) and levels were then determined using the Corticosteroid ELISA kit according to the manufacturer's instructions.

Primary outcome

The primary outcome was association between neonatal serum levels (NSLs) of betamethasone and RDS. The diagnosis of RDS was determined within the first 24 h after delivery according to the Vermont–Oxford network criteria; partial pressure of oxygen (PaO2) <50 mmHg in room air, central cyanosis in room air, a requirement for supplemental oxygen to maintain PaO2 >50 mmHg, or a requirement for supplemental oxygen to maintain a pulse oximeter saturation over 85% and a chest radiograph consistent with RDS diagnosis.[6] Neonatologists were aware of the number of courses and time elapsed from betamethasone treatment.

Power analysis

The prevalence of RDS between 28^{+0} and 34^{+0} weeks is nearly 20%.[7] It is unknown whether NSLs of betamethasone predict the occurrence of RDS. In order to detect a change in the percentage value of the sensitivity of NSL to predict RDS from 70% (null hypothesis) to nearly 95% (alternative hypothesis), based on a target significance level of 0.05, and power of 80%, 150 neonates were required.[8]

Statistics

Continuous variables were assessed for approximate normality via Skewness and Kurtosis. As maternal serum levels (MSLs) and the time elapsed from delivery from the last dose were not normally distributed (skewness and kurtosis were greater than the absolute value of 1.5), both measures were log transformed to achieve approximate normality and all analyses were performed on the log. NSLs were approximately normally distributed and all analyses were performed on the raw data. Relative risk (RR) and 95% confidence interval (CI) were computed in order to find associations between the maternal demographic and obstetric characteristics and RDS using Poisson regression.

Poisson regression analysis of RDS using time to delivery from the last dose, measured in (log) hours, was performed to assess serum levels over time adjusting for clinically relevant background variables, such as GA and number of doses. Models were then produced in order to estimate the risk of developing RDS (outcome) according to GA, time elapsed from last dose to delivery (log scale), number of doses of betamethasone, and MSL or NSL of betamethasone. This was repeated for the subgroup receiving only one betamethasone course and for another subgroup who delivered within 1 week from the last betamethasone dose. Area under the curve of serum level for the adjusted RDS outcome was calculated using the receiver operating characteristic procedure with nonparametric distribution. Linear regression analysis was performed to test for serum level differences between singleton and multiple fetuses, adjusting for time (on the log scale), number of doses, GA, and maternal weight at delivery. Significance was considered to be $p < 0.05$. All analyses were carried out using SPSS version 28 (IBM Corp, 2012).

Study approval

The study was approved by the local ethics committee of Emek Medical Center in compliance with the Helsinki Declaration (#0122-11-EMC). Signed informed consent was obtained from each of the participating women.

RESULTS

During the study period, 248 women were eligible and signed an informed consent. Of all eligible, 124 (50%) were excluded; five due to major congenital malformations diagnosed after delivery, one withdrew consent, one woman delivered at another institution, and 117 recruited women who delivered at or beyond 35 weeks of gestation. Overall, 124 eligible women were included in the final analysis; 96 (77.4%) delivered singletons, 26 twins (21.0%), and two triplets (1.6%), corresponding to a total of 154 neonates. Final blood sampling was obtained in February 2019.

In four pairs of twins, one was with RDS and the second without, therefore these women were analyzed with mothers who had an RDS neonate whereas neonatal data were analyzed separately. In all other multiple gestations, all the newborns had similar concurring RDS outcomes. One MSL result was excluded from the analysis because betamethasone concentration (164 ng/ml) was more than three SDs above the mean (outlier). It should be noted that using the statistical analysis with or without this sample did not change the findings significantly.

RDS was diagnosed in 35 (22.7%) neonates. There were no significant differences in maternal demographic and obstetric characteristics among women with and without RDS to the neonate (Table 1). Betamethasone doses and the time elapsed to delivery did not differ significantly between women who delivered neonates with and without RDS. MSLs of betamethasone were comparable among women with and without RDS ($p = 0.20$; Table 2). Women who had neonates with RDS delivered at an earlier GA compared to women who did not (31.0 ± 2.1 vs. 32.6 ± 1.7 weeks; $p = 0.001$). After correcting for time elapsed from the last dose and number of doses, GA was still significantly associated with RDS (RR: 0.92, 95% CI: 0.88–0.95, $p = 0.001$).

Table 3 presents the neonatal characteristics of the 154 neonates included. Neonates who developed RDS had a statistically significant lower birth weight (1550 ± 465 g) and lower mean Apgar score at 1 and 5 min (6.9 ± 2.1, 8.6 ± 1.3, respectively) compared to neonates without RDS (1822 ± 440 g, $p = 0.002$; 8.2 ± 1.5, $p = 0.001$; and 9.4 ± 1.0, $p = 0.001$; respectively). NSLs of betamethasone were comparable among neonates with and without RDS ($p = 0.84$; Table 3). Nevertheless, after correcting for time elapsed from the last dose, GA, and number of doses, NSL was significantly associated with RDS (RR: 0.97, 95% CI: 0.94–0.99, $p = 0.011$). Receiver operator curve analysis revealed that NSLs of 6.00 ng/ml predicted RDS with a sensitivity of 80.0% and specificity of 64.7% (Figure 1). For the subgroup of women who received only one course of betamethasone (either one or two doses; 108 neonates), NSLs predicted RDS as well (RR: 0.97, 95% CI: 0.94–0.995, $p = 0.020$) after adjustment for GA, time elapsed from the last dose, and number of doses. For every 1 ng/ml increase in NSLs, the risk of RDS decreased 3%. Receiver operator curve analysis revealed that NSLs of 5.50 ng/ml predicted RDS with sensitivity of 80.0% and specificity of 71.0% (Figure 1). Additionally, for the subgroup of women who delivered within 1 week from the last dose (125 neonates), NSLs were predictive of RDS as well, after adjustment for the same covariates (RR: 0.97, 95% CI: 0.95–0.966, $p = 0.023$). Receiver operator curve analysis revealed that NSLs of 5.25 ng/ml predicted RDS with sensitivity of 82.8% and specificity of 68.7% (Figure 1).

MSLs did not predict RDS (RR: 1.04, 95% CI: 0.98–1.10, $p = 0.20$). After correcting for GA, time elapsed from the last dose, and number of doses, MSLs were still not associated with RDS (RR: 0.86, 95% CI: 0.69–1.08, $p = 0.202$). For the subgroup of women (87 women) who received only one course of betamethasone (either one or two doses), MSLs were not associated with RDS after adjustment for the same covariates (RR: 0.81, 95% CI: 0.60–1.10, $p = 0.178$). Additionally, for the subgroup of women (103 women) who delivered within 1 week from the last dose, MSLs were still not predictive of RDS (RR: 0.85, 95% CI: 0.63–1.14, $p = 0.271$) after adjustment for the same covariates.

A linear regression was performed to examine the relationship between MSL and NSL with GA, maternal weight at delivery, and number of fetuses (Table 4). After adjusting for time (on the log scale), number of doses, GA, and maternal weight at delivery, there was no significant difference in MSLs ($t = 1.57$, $p = 0.12$) or NSLs ($t = 0.24$, $p = 0.81$) between singleton and multiple fetuses. Additionally, after adjusting for time (on the log scale), number of doses, number of fetuses, and GA, there was no association between MSL and maternal delivery weight ($t = 1.37$, $p = 0.17$) or between NSL and maternal delivery weight ($t = -0.70$, $p = 0.49$). However, after adjusting for time (on the log scale), number of doses, maternal delivery weight, and number of fetuses, there remained an association between MSL and GA ($t = -2.88$, $p = 0.005$; partial $R^2 = 3.4\%$) and between NSL and GA ($t = -2.16$, $p = 0.032$, partial $R^2 = 1.3\%$). For every 1 week increase in GA, the log of MSL decreased by −0.036 ng/ml and NSL decreased by −0.23 ng/ml.

DISCUSSION

In this prospective study, the incidence of RDS among neonates delivered between 28^{+0} and 34^{+6} weeks was 22.7%, comparable to that reported in the literature.[7] GA was a significant predictor for RDS. Other demographic

TABLE 1 Maternal demographic and antepartum obstetric characteristics

	Total ($N = 124$)	RDS ($N = 31$)	No RDS ($N = 93$)	p value	Relative risk (95% CI)
Maternal age, years	30.8 ± 6.2 (26.0–35.8)	30.8 ± 5.9 (26.0–37.0)	30.8 ± 6.3 (26.5–35.5)	0.98	1.00 (0.94–1.06)
Gravidity	2.9 ± 1.9 (1–4)	2.8 ± 2.0 (1–4)	2.9 ± 1.9 (1–4)	0.75	0.98 (0.81–1.18)
Parity	1.3 ± 1.4 (0–2)	1.3 ± 1.5 (0.0–2.0)	1.3 ± 1.4 (0.0–2.0)	0.98	1.00 (0.78–1.28)
Body mass index (pregestational), kg/m^2	26.07 ± 5.93 (21.47–29.46)	25.93 ± 5.37 (21.62–28.48)	26.11 ± 6.13 (21.46–29.75)	0.90	1.00 (0.94–1.06)
Body mass index (at delivery), kg/m^2	29.78 ± 6.20 (25.31–34.12)	29.59 ± 5.83 (25.79–33.79)	29.84 ± 6.35 (24.96–34.14)	0.87	0.99 (0.94–1.06)
Maternal weight at delivery, kg	79.1 ± 16.6 (68.2–90.0)	78.9 ± 17.0 (69.5–86.5)	79.1 ± 16.6 (67.0–90.0)	0.96	1.00 (0.98–1.02)
Smoking	12 (9.7)	3 (9.7)	9 (9.7)	>0.99	1.00 (0.36–2.80)
Creatinine level, mg/dl	0.51 ± 0.2 (0.41–0.57)	0.57 ± 0.40 (0.42–0.57)	0.50 ± 0.10 (0.41–0.57)	0.20	1.89 (0.71–5.03)
Hypertension in pregnancy	25 (20.2)	8 (25.8)	17 (18.3)	0.37	1.38 (0.70–2.70)
Diabetes in pregnancy	13 (10.5)	2 (6.5)	11 (11.8)	0.52	0.59 (0.16–2.18)
Thrombophilia	7 (5.6)	2 (6.5)	5 (5.4)	>0.99	1.15 (0.34–3.88)
Number of fetuses				>0.99	
Singleton	96 (77.4)	24 (77.4)	72 (77.4)		1.00 (reference)
Multiple	28 (22.6)	7 (22.6)	21 (22.6)		1.00 (0.80–1.27)
Twins	26 (21.0)	6 (19.4)	20 (21.5)		0.92 (0.38–2.26)
Triplets	2 (1.6)	1 (3.2)	1 (1.1)		2.00 (0.27–14.78)
Prophylactic progesterone use	15 (12.1)	5 (16.1)	10 (10.8)	0.52	1.40 (0.63–3.08)
Tocolysis use	38 (30.6)	10 (32.3)	28 (30.1)	0.82	1.08 (0.56–2.06)
Prophylactic antibiotics use	40 (32.2)	6 (19.4)	34 (36.6)	0.08	0.50 (0.21–1.23)
Maternal fever ≥38°C	4 (3.2)	1 (3.2)	3 (3.2)	>0.99	1.00 (0.11–9.27)
Betamethasone indication				0.10	
Spontaneous preterm labor	75 (60.5)	15 (48.4)	60 (64.5)	–	1.00 (reference)
Growth restriction	25 (20.2)	7 (22.6)	18 (19.4)	0.41	1.40 (0.65–3.04)
Preeclampsia	20 (16.1)	9 (29.0)	11 (11.8)	0.03	2.25 (1.16–4.37)
Bleeding	4 (3.2)	0 (0.0)	4 (4.3)	>0.99	0.49 (0.03–7.77)*

Note: Data are mean ± SD (IQR) or N (%). Relative risk, 95% CI, p computed from Poisson regression.

Abbreviations: CI, confidence interval; RDS, respiratory distress syndrome.

*Adjusted by adding 0.5 to each cell.

TABLE 2 Betamethasone doses administered before delivery and maternal delivery characteristics

	Total ($N = 124$)	RDS ($N = 31$)	No RDS ($N = 93$)	p value	Relative risk (95% CI)
Complete one course of betamethasone	95 (76.6)	19 (61.3)	76 (81.7)	0.02	0.48 (0.24–0.99)
Start second course of betamethasone	37/95 (38.9)	6/19 (31.6)	31/76 (40.8)	0.46	0.56 (0.23–1.38)
Complete second course of betamethasone	30/37 (81.1)	5/6 (83.3)	25/31 (80.6)	>0.99	1.17 (0.14–9.98)
Number of doses				0.12	
1	29 (23.4)	12 (38.7)	17 (18.3)		1.85 (0.97–3.52)
2	58 (46.8)	13 (41.9)	45 (48.4)		1.00 (reference)
3	7 (5.6)	1 (3.2)	6 (6.5)		0.64 (0.10–4.16)
4	30 (24.2)	5 (16.1)	25 (26.9)		0.74 (0.29–1.89)
Time elapsed from last betamethasone dose to delivery, hours[a]	40.9 ± 85.2 (12.1–97.3)	23.0 ± 89.8 (8.4–98.3)	45.5 ± 80.3 (16.6–97.0)	0.08	0.90 (0.81–1.01)[b]
Maternal serum level (ng/ml)[a]	7.40 ± 7.00 (2.90–9.45)	8.86 ± 7.28 (3.20–15.00)	6.94 ± 6.89 (2.50–8.50)	0.20	1.04 (0.98–1.10)[b]
Gestational age at delivery, weeks	32.2 ± 1.9 (30.9–33.7)	31.0 ± 2.1 (28.9–32.8)	32.6 ± 1.7 (31.4–34.0)	0.001	0.74 (0.62–0.89)
Labor onset				0.84	
Medically indicated	65 (52.4)	17 (54.8)	48 (51.6)		1.10 (0.54–2.24)
Spontaneous	59 (47.6)	14 (45.2)	45 (48.4)		1.00 (reference)
Delivery mode				0.13	
Vaginal	46 (37.1)	8 (25.8)	38 (40.9)		1.00 (reference)
Cesarean section	78 (62.9)	23 (74.2)	55 (59.1)		1.70 (0.76–3.79)

Note: Data are mean ± SD (interquartile range [IQR]) for continuous data or N (%) except where noted. Relative risk, 95% CI, and p were computed from Poisson regression.

Abbreviations: CI, confidence interval; RDS, respiratory distress syndrome.

[a]Data is median ± IQR (IQR).

[b]Based on log of the variable.

and obstetric variables were comparable and did not differ between neonates with and without RDS. Adjusted NSLs of betamethasone at delivery were associated with RDS. A threshold of 6.0 ng/ml had a sensitivity of 80% and specificity of 64.7%. NSLs were associated with RDS also among women who received only one course of betamethasone and among women who delivered within 1 week of betamethasone administration. MSLs of betamethasone at delivery were not associated with RDS. NSLs and MSLs were not associated with maternal weight at delivery or the number of fetuses but were weakly associated with GA because GA explained <5% of the variance in serum levels (3.4% of MSL and 1.3% of NSL).

ACS administration to mothers at risk of preterm birth is one of the main advances in perinatal medicine. The widespread acceptance of this therapy is due to strong evidence demonstrating improved neonatal outcomes following exposure, mainly from maturation of fetal pulmonary function.[3] ACS became a standard of care for pregnancies at risk of preterm birth and is frequently used as a marker of antenatal quality of care.[9,10] Nevertheless, as we approach nearly 50 years since this practice began,[11] the optimum corticosteroid dose in terms of health cost and benefit has not been established, mainly due to lack of dose response studies in the clinical setting.[9,12] Several authors have suggested adjusting betamethasone dosing to maternal weight or BMI, GA, and number of fetuses, which, at least theoretically, may lead to a variation in response and probably reduce unnecessarily excessive maternal or fetal steroid exposure.[13,14] Nevertheless, the results of the current study showed that the current accepted fixed dosage of betamethasone administered to pregnant women at risk of preterm birth produced MSLs and NSLs that were not associated with BMI or number of fetuses and only weakly associated with GA at delivery. Similar results were presented by Gyamfi et al. who reported on a small

TABLE 3 Immediate neonatal outcomes

	Total ($N = 154$)	RDS ($N = 35$)	No RDS ($N = 119$)	p value	Relative risk (95% CI)
Neonatal serum level (ng/ml)	5.33 ± 3.46 (2.65–7.00)	5.45 ± 3.59 (3.20–7.50)	5.29 ± 3.43 (2.50–7.00)	0.84	1.01 (0.92–1.11)
Neonatal gender				0.86	
Male	90 (58.4)	20 (57.1)	70 (58.8)		1.00 (reference)
Female	64 (41.6)	15(42.9)	49 (41.2)		1.06 (0.59–1.90)
Multifetal	58 (37.7)	11 (31.4)	47 (39.5)	0.39	0.94 (0824–1.08)
Birth weight (g)	1760 ± 459 (1409–2072)	1550 ± 465 (1240–1824)	1822 ± 440 (1480–2125)	0.007	0.90 (0.83–0.97) per 100 g
Apgar score at 1 min	7.9 ± 1.7 (7–9)	6.9 ± 2.1 (5–8)	8.2 ± 1.5 (8, 9)	0.001	0.78 (0.67–0.90)
Apgar score <7 at 1 min	29 (18.8)	13 (37.1)	16 (13.4)	0.002	2.01 (1.05–3.86)
Apgar score 5 min	9.2 ± 1.1 (9, 10)	8.6 ± 1.3 (8, 9)	9.4 ± 1.0 (9, 10)	0.002	0.72 (0.59–0.88)
Apgar <7 at 5 min	5 (3.2)	2 (5.7)	3 (2.5)	0.32	2.21 (0.38–12.70)
Cord artery pH[a]	7.3 ± 0.10 (7.2–7.3)	7.2 ± 0.1 (7.22–7.32)	7.3 ± 0.1 (7.23–7.33)	0.33	1.68 (0.59–4.75)
Cord artery pH < 7.1	11 (7.1)	4 (11.4)	7 (5.9)	0.27	1.94 (0.60–6.26)

Note: Data are mean ± SD (interquartile range [IQR]) or N (%). Relative risk, 95% CI, and p value were computed from Poisson regression.

Abbreviations: CI, confidence interval; RDS, respiratory distress syndrome.

[a]Median ± IQR (range).

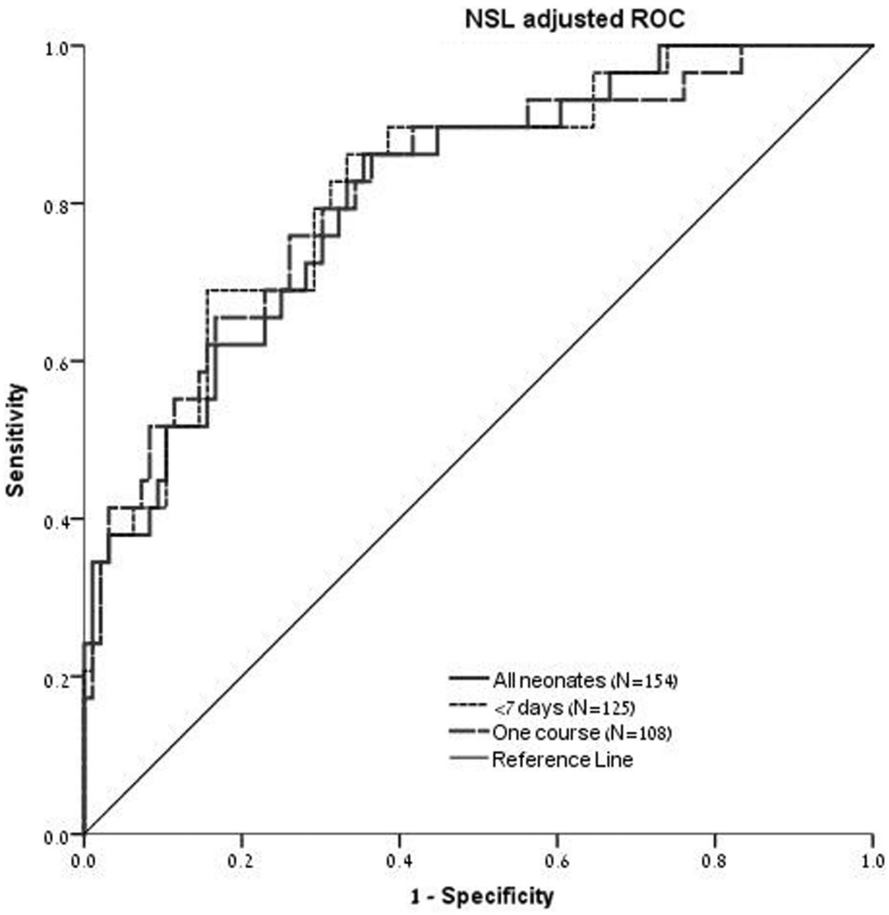

FIGURE 1 Receiver operating characteristic (ROC) curve analysis revealing the cutoff value of neonatal serum levels (NSLs) of betamethasone of all neonates ($N = 154$), neonates who were delivered within 1 week of last dose administered ($N = 125$), and neonates whose mothers received only one course of betamethasone ($N = 108$) after adjustment for gestational age, time elapsed from the last dose, and number of doses.

TABLE 4 Univariate and multivariate linear regression analysis for the relationship between MSL and NSL with gestational age, maternal weight at delivery, and number of fetuses

	MSL (log transformed)							NSL						
	Univariate (unadjusted)				Multivariate[a]			Univariate (unadjusted)				Multivariate[a]		
	b	SE	*p*	R^2 (%)	*b*	SE	*p*	Partial R^2 (%)	*b*	SE	*p*	R^2 (%)	*b*	SE	*p*	Partial R^2 (%)
Multiple gestations	0.016	0.092	0.86	0.00	0.085	0.054	0.12	0.04	0.376	0.572	0.51	0.30	0.095	0.395	0.81	0.10
Weight at delivery, 1 kg	−0.003	0.002	0.26	1.10	−0.002	0.001	0.170	0.04	−0.018	0.017	0.30	0.70	−0.009	0.013	0.49	0.40
Gestational age, weeks	−0.060	0.019	0.003	7.04	−0.036	0.012	0.005	3.40	−0.349	0.148	0.02	3.50	−0.230	0.106	0.032	1.30

Abbreviations: *b*, regression coefficient; MSL, maternal serum level, ng/ml; NSL, neonatal serum levels, ng/ml; *p*, *p* value; R^2, is for coefficient of determination; SE, standard error.

[a]After adjusting for time elapsed from last dose (on the log scale), number of doses, gestational age, and maternal weight at delivery.

number of participants that maternal and umbilical cord blood serum betamethasone concentrations were not different in twin gestations or obese women.[15] These observations suggest that adjusting betamethasone dosing according to maternal weight, GA, or to the number of fetuses will probably not improve the efficacy of betamethasone in decreasing the rate of RDS.

Several animal experiments, measured fetal biochemical response to corticosteroids administration but not clinical response (i.e., RDS).[16–22] It is unknown according to research studies involving human subjects whether an association exists between MSL or NSL of betamethasone and RDS. In the present study, we were able to demonstrate that MSLs were not associated with RDS and for that reason simply adjusting betamethasone dosage according to MSLs is unlikely to lead to better determining the optimum betamethasone dosage in terms of health cost and lower rate of RDS. This may be due to the complex pharmacokinetic and pharmacodynamic relationship of betamethasone in maternal and fetal compartments, mainly owing to the drug nuclear target, the clearance rate, volume of distribution, the facility to cross the human placenta, and binding proteins in maternal and fetal plasma.[9,23] Although adjusted NSLs of betamethasone were associated with RDS, this may be explained by less complex pharmacokinetics and pharmacodynamics, due to a single compartment (fetal only). Still, the sensitivity was only 80%, lower than that hypothesized.

Although the benefits of ACS are clear, nonetheless, ACS are not harmless. Based on evidence from animal and human studies, ACS may have significant long-lasting effects on health, in the form of epigenetic changes found in numerous homeostatic mechanisms.[9]

Furthermore, nearly half of the fetuses whose mothers receive betamethasone, are delivered beyond 35 weeks, and therefore do not benefit from its short-term advantage, and as a result might be pointlessly exposed to long-term consequences.[16,24] Similar findings were observed in the current study in which out of 248 women recruited, 117 (47.2%) delivered at 35 weeks of gestation or beyond.

Several reports, mainly in sheep models, have reported that lower dosing strategies, which may avoid high peak levels, provide betamethasone levels in the target range with a duration adequate to elicit pharmacodynamics benefits.[17–19] In human subjects, similar experiments on short or long-term fetal effects are most likely not practical. Nevertheless, the variation in responses, and the similar MSLs of betamethasone among women who had neonates with and without RDS found in the present study, may pave the way to future trials that may use lower than the standardized betamethasone dosages. Lower dosages may reduce long-term

consequences as well, and may alleviate concerns when a rescue course is advocated.[9]

Strength and limitations

The present study did not examine lower GA (i.e., 24^{+0} to 28^{+0} weeks). The majority of ACS trials included women with singleton pregnancies and moderate prematurity (28^{+0} and 34^{+0} weeks), and, therefore, the evidence of benefits to lower GA is weaker.[9] The very low GA rather than ACS is probably the main factor that might affect the outcome at these GAs.

Moreover, the sample size in terms of certain outcomes examined, as in the case of subgroup analysis, may be small to make definitive conclusions. Additionally, among women who had a rescue dose, blood levels may be affected by a higher basal dose because of prior exposure. Nevertheless, according to a previous publication, the concentration diminished gradually to a level close to the baseline at 5–7 days after the first course of betamethasone.[5] Besides, most women who had a rescue dose did not receive it back-to-back. Finally, we made a subanalysis restricted to women who received one course only. The results were comparable.

In conclusion, NSLs and MSLs were similar among neonates with and without RDS. After adjusting for GA, time elapsed from the last dose, and number of doses, NSLs were associated with RDS whereas MSLs were not. Both MSLs and NSLs were not associated with the number of fetuses and maternal weight at delivery and only weakly associated with GA. The results derived from this and prior studies suggest that simply adjusting betamethasone dosage according to MSL, GA, number of fetuses, or maternal weight is unlikely to lead alone to better determining the optimum betamethasone dosage.

AUTHOR CONTRIBUTIONS

N.Z. and R.S. wrote the manuscript. R.S. and E.S. designed the research. N.Z., M.M., A.S., R.M., L.M., S.A.W., and S.R. performed research. N.Z., E.S., and R.S. analyzed data. R.M. and L.M. contributed to new reagents/analytical tools. All authors approved the final draft.

ACKNOWLEDGEMENT

The authors thank Paula S. Herer, biostatistician, MSc, MPH, for statistical guidance and assistance.

FUNDING INFORMATION

The study was funded by the Emek Medical Center and Technion, Research and Development Foundation, LTD, Israel (#2022418).

CONFLICT OF INTEREST

The authors declared no competing interests for this work.

REFERENCES

1. Meis PJ, Klebanoff M, Thom E, et al. National Institute of Child Health and Human Development maternal-fetal medicine units network. Prevention of recurrent preterm delivery by 17 alpha-hydroxyprogesterone caproate. *N Engl J Med*. 2003;348:2379-2385. doi:10.1056/NEJMoa035140
2. NIH. Consensus development panel on the effect of corticosteroids for fetal maturation on perinatal outcomes. *JAMA*. 1995;273:413-418. doi:10.1001/jama.1995.03520290065031
3. McGoldrick E, Stewart F, Parker R, Dalziel SR. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. *Cochrane Database Syst Rev*. 2020;12:CD004454.
4. Kemp MW, Saito M, Usuda H, et al. The efficacy of antenatal steroid therapy is dependent on the duration of low-concentration fetal exposure: evidence from a sheep model of pregnancy. *Am J Obstet Gynecol*. 2018;219:301.e1-301.e16. doi:10.1016/j.ajog.2018.05.007
5. Salim R, Suleiman A, Colodner R, Nachum Z, Goldstein LH, Shalev E. Measurement of betamethasone concentration in maternal serum treated for fetal lung maturity; is it feasible? *Reprod Biol Endocrinol*. 2016;14:7. doi:10.1186/s12958-016-0142-4
6. Vermont Oxford Network. Manual of operations: part 2. Data definitions & infant data booklets 2022. 2022. https://vtoxford.zendesk.com/hc/en-us/articles/4405064008467-2022-Manual-of-Operations-Part-2-Release-26-2-PDF. Accessed 25. 1 May 2022.
7. Salim R, Zafran N, Nachum Z, Garmi G, Shalev E. Predicting lung maturity in preterm rupture of membranes via lamellar bodies count from a vaginal pool: a cohort study. *Reprod Biol Endocrinol*. 2009;7:112. doi:10.1186/1477-7827-7-112
8. Bujang MA, Adnan TH. Requirements for minimum sample size for sensitivity and specificity analysis. *J Clin Diagn Res*. 2016;10:YE01-YE06. doi:10.7860/JCDR/2016/18129.8744
9. Kemp MW, Newnham JP, Challis JG, Jobe AH, Stock SJ. The clinical use of corticosteroids in pregnancy. *Hum Reprod Update*. 2016;22:240-259. doi:10.1093/humupd/dmv047
10. Henderson ZT, Suchdev DB, Abe K, Johnston EO, Callaghan WM. Perinatal quality collaboratives: improving care for mothers and infants. *J Womens Health (Larchmt)*. 2014;23:368-372. doi:10.1089/jwh.2014.4744
11. Liggins GC, Howie RN. A controlled trial of antepartum glucocorticoid treatment for prevention of the respiratory distress syndrome in premature infants. *Pediatrics*. 1972;50:515-525.
12. Crowther CA, Haslam RR, Hiller JE, Doyle LW, Robinson JS, Australasian collaborative trial of repeat doses of steroids (ACTORDS) study group. Neonatal respiratory distress syndrome after repeat exposure to antenatal corticosteroids: a randomized controlled trial. *Lancet*. 2006;367:1913-1919. doi:10.1016/S0140-6736(06)68846-6
13. Newnham JP, White SW, Meharry S, et al. Reducing preterm birth by a statewide multifaceted program: an implementation study. *Am J Obstet Gynecol*. 2017;216:434-442. doi:10.1016/j.ajog.2016.11.1037
14. Panel ACCPG. *Antenatal Corticosteroids Given to Women Prior to Birth to Improve Fetal, Infant, Child and Adult Health:*

Clinical Practice Guidelines. The University of Auckland, New Zealand: Liggins Institute; 2015.
15. Gyamfi C, Mele L, Wapner RJ, et al. Eunice Kennedy Shriver National Institute of Child Health and Human Development maternal-fetal medicine units network. The effect of plurality and obesity on betamethasone concentrations in women at risk for preterm delivery. *Am J Obstet Gynecol*. 2010;203:219.e1-219.e5. doi:10.1016/j.ajog.2010.04.047
16. McLaughlin KJ, Crowther CA, Walker N, Harding JE. Effects of a single course of corticosteroids given more than 7 days before birth: a systematic review. *Aust N Z J Obstet Gynaecol*. 2003;43:101-106. doi:10.1046/j.0004-8666.2003.00052.x
17. Loehle M, Schwab M, Kadner S, et al. Dose–response effects of betamethasone on maturation of the fetal sheep lung. *Am J Obstet Gynecol*. 2010;202(186):e1-e7. doi:10.1016/j.ajog.2009.09.033
18. Samtani MN, Lohle M, Grant A, Nathanielsz PW, Jusko WJ. Betamethasone pharmacokinetics after two prodrug formulations in sheep: implications for antenatal corticosteroid use. *Drug Metab Dispos*. 2005;33:1124-1130. doi:10.1124/dmd.105.004309
19. Jobe AH, Nitsos I, Pillow JJ, Polglase GR, Kallapur SG, Newnham JP. Betamethasone dose and formulation for induced lung maturation in fetal sheep. *Am J Obstet Gynecol*. 2009;201(611):e1-e7. doi:10.1016/j.ajog.2009.07.014
20. Romejko-Wolniewicz E, Teliga-Czajkowska J, Czajkowski K. Antenatal steroids: can we optimize the dose? *Curr Opin Obstet Gynecol*. 2014;26:77-82. doi:10.1097/GCO.0000000000000047
21. Schmidt AF, Kemp MW, Kannan PS, et al. Antenatal dexamethasone vs. betamethasone dosing for lung maturation in fetal sheep. *Pediatr Res*. 2017;81:496-503. doi:10.1038/pr.2016.249
22. Jobe AH, Kemp M, Schmidt A, Takahashi T, Newnham J, Milad M. Antenatal corticosteroids: a reappraisal of the drug formulation and dose. *Pediatr Res*. 2021;89:318-325. doi:10.1038/s41390-020-01249-w
23. Derendorf H, Möllmann H, Hochhaus G, Meibohm B, Barth J. Clinical PK/PD modelling as a tool in drug development of corticosteroids. *Int J Clin Pharmacol Ther*. 1997;35:481-488.
24. Razaz N, Skoll A, Fahey J, Allen VM, Joseph KS. Trends in optimal, suboptimal, and questionably appropriate receipt of antenatal corticosteroid prophylaxis. *Obstet Gynecol*. 2015;125:288-296. doi:10.1097/AOG.0000000000000629

How to cite this article: Zafran N, Massalha M, Suleiman A, et al. Association between betamethasone levels and respiratory distress syndrome in preterm births: A prospective cohort study. *Clin Transl Sci*. 2022;15:2528-2537. doi:10.1111/cts.13382