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# Dosage escalation of antenatal steroids in preterm twin pregnancies does not improve long-term outcome

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#### **Abstract**

**Objectives:** To analyze long-term effects of antenatal betamethasone (<16 mg, =24 mg and >24 mg) in preterm twins on infant and childhood morbidity.

**Methods:** Retrospective cohort study among 198 preterm twins. Three follow up time points, including a total of 84 outcomes, were evaluated: first neonatal examination after birth and in the neonatal period up to 10 days after birth using data from the clinic charts; examination from the 21st to the 24th month of life and examination from the 60th to the 64th months, using data from copies of the children's examination booklets sent back by the parents. Dosage-dependent and sex-specific long-term effects of antenatal betamethasone treatment on neonatal, infant and early childhood development and morbidity up to 5.3 years of age were analyzed.

**Results:** Dosage escalation of >24 mg was not associated with improved neonatal, infant or early child hood

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outcome, independent of twin pair structure. In contrast, higher doses >24 mg were significantly linked to increased rates of congenital infections (OR 5.867, 95% CI 1.895–18.167). Male sex as a factor was obvious for lower rates of apnea-bradycardia-syndrome in neonates, higher rates of no free steps after 15 months in infancy and highest rates of motor clumsiness in early childhood.

**Conclusions:** Betamethasone dosage escalation >24 mg in twins born between 23<sup>+5</sup> and 33<sup>+6</sup> weeks of gestation did not improve neonatal, infant or early childhood morbidity. In contrast, higher doses >24 mg total dose resulted in significantly higher rates of congenital infections and are not recommended. For males, 24 mg betamethasone appears to be the preferable dose.

**Keywords:** antenatal betamethasone; antenatal steroid (ANS); dosage; fetal programming; follow up; multiple pregnancy; sex.

#### Introduction

Although in singleton pregnancies threatened with preterm birth, antenatal steroid (ANS) treatment to induce fetal lung maturation and to reduce neonatal morbidity and mortality is common practice [1], in twin pregnancies, the optimal treatment strategy of ANS has not been finally clarified [2]. Currently, the ANS therapy regimen for twin pregnancies corresponds thereto of singletons with Betamethasone (BET)  $2\times12\,\mathrm{mg}$  intramuscularly 24 h apart.

The effects of antenatal lung maturation with gluco-corticoids in multiple pregnancies still remains unclear, as trials in twins are limited and evidence is lacking [1, 3–9]. In a Cochrane meta-analysis from 2017 of randomized controlled studies with multiples, no significant reduction within the occurrence of respiratory distress syndrome (RDS) and intraventricular hemorrhage (IVH) or within the rate of neonatal mortality was found after ANS therapy [2]. Choi et al. concluded that ANS significantly reduce the occurrence of RDS only in singletons, but not in twins [10]. In contrast, other studies confirmed the benefit of ANS in

twins and demonstrated a major reduction in RDS with the currently recommended BET dosage regimen [4, 11-14]. Furthermore, Melamed et al. showed that ANS in twins and in singletons led to a similarly strong reduction in neonatal mortality rate, the necessity for ventilation, the occurrence of RDS and therefore the occurrence of severe neurological damage [15].

We have previously shown that ANS treatment in normally grown singleton and twin pregnancies reduced in an exceedingly dose-dependent and sex-specific manner fetal weight gain, length and head circumference compared with gestational age-matched controls [16, 17]. ANS dose escalation in singletons even increased the negative effects on fetal growth, but did not improve neonatal morbidity and mortality at birth [16]. Very recently we reported in a follow up study in twins, that the negative effects of ANS treatment on fetal growth trajectories persisted even beyond birth into early childhood. Most sensitive to antenatal betamethasone treatment were female-female and mixed pairs [18].

Impaired fetal growth and reduced birth weight as a rough surrogate marker for hormonal or nutritional changes within the intrauterine environment is related to a variety of long-term developmental and health implications [19, 20]. Intrauterine exposure to high levels of glucocorticoids is amongst the proposed mechanisms of perinatal programming [19, 21, 22]. Further studies are needed to understand the mechanism of antenatal glucocorticoid treatment and its impact on long-term development and morbidity, considering the special needs in twin pregnancies.

The objective of the present twin follow-up study was to investigate the effect of ANS dosage and sex on infant's and early child's morbidity up to 5.3 years of age.

### Materials and methods

The original birth cohort has been described previously [17, 18]. Twins born between December 1993 and January 2011 were enrolled in a prospective cohort study in a tertiary referral center at the Department of Obstetrics, Charité University Hospital Berlin, Germany. Women with twin pregnancies and also the diagnosis of preterm labor with symptomatic contractions and cervical ripening, premature rupture of membranes or vaginal bleeding and exposed to antenatal betamethasone (Celestan®, MSD GmbH, Haar, Germany) between 23 weeks plus five days of pregnancy (23<sup>+5</sup> weeks) and 33<sup>+6</sup> weeks were included into the study. Exclusion criteria were: pathological umbilical or uterine doppler findings, chromosomal anomalies, twin-to-twin transfusion syndrome, malformations, high numbered multiple pregnancies, intrauterine death, other fetal or maternal diseases. To rule out possible growth restricting by an already existing placental insufficiency, fetuses

with sonographically estimated fetal weight below the 10th centile at the time of the initial betamethasone treatment were defined as small for gestational age and excluded from the study [17]. Outcome assessment was based on analyzing data after birth until discharge from hospital charts and two standardized examinations provided for all children in Germany free of charge. Results of these examinations, performed by pediatricians and general practitioners, are noted in the infant's examination booklet (Kinderuntersuchungsheft) [23] which all parents receive after the birth of their infant [18]. Parents were contacted by telephone and mail and asked to provide copies of the examinations scheduled for 21-24 and 60-64 months of age.

The protocol for steroid administration changed over time at our institution according to different local and international clinical recommendations in effect during the study period [17, 18]. The betamethasone treatment regimen ranged from 2 × 8 mg (≤16 mg) or  $2 \times 12 \,\mathrm{mg}$  (=24 mg) given once during pregnancy, to repeated doses of betamethasone (8 mg weekly or  $2 \times 12$  mg every second week) when the diagnosis of preterm labor still persisted (collectively grouped as ≥24 mg). Betamethasone dosage effects were analyzed among three different dosages: ≤16, 24 and >24 mg. Gender specificity was tested for male-male, female-female and mixed-pairs of twins. The Charité Hospital Ethics Review Committee approved the trial (EA2\_111\_15).

#### **Statistics**

Sent copies of the children's examination booklets were analyzed. Crossed were interpreted as true, unchecked items as inaccurate and thus transferred to an Excel database. Statistical analyzes were performed using the SPSS 25 software (IBM, SPSS Statistics, version 25, IBM Corporation, Armonk, NY, USA). Data were tested for normality by assessing the histogram and using the Kolmogorov-Smirnov test. Only outcome parameters of the neonatal period and pediatric examinations, which occurred at a frequency of more than 5% of children, were later included in the group comparison (Supplementary Material S1; comparative examination of categorial outcome features are presented in crosstabs). The significance test was performed using the Pearson Chi-square test with a Bonferroni post-hoc test if adequate. If significant, subgroup analysis for dosis, twin pair structure and dosis + twin-pair structure were performed. For all tests, statistical significance was set at p<0.05 and reported if all expected cell frequencies were greater than five.

## Results

### Study population

Briefly, 198 children of twin pregnancies, who had received antenatal betamethasone treatment and were born between 25<sup>+0</sup> and 33<sup>+6</sup> weeks of gestation (median:  $33 \pm 1$  weeks), were included into this follow-up study. Birth weight in female-female pairs 1782  $\pm$  460 g (29.7  $\pm$  42.96 centile) was significantly lower compared to mixed pairs 1907  $\pm$  909 g (52  $\pm$  42.1 centile), but not different from

male-male pairs 1755  $\pm$  775 g (41.8  $\pm$  46.6 centile; median  $\pm$  IQR). The main indication for ANS treatment was premature contractions with cervical shortening (80%), followed by premature rupture of membranes (PROM) (12%). The mean gestational length after ANS treatment was not significantly different within the twin-pair structure. Maternal characteristics were not significantly different between the groups. Neither the number and distribution within the twin-pair structure nor its relation to the dosage-group was significant different. Eighty four categorial outcome parameters in total were analyzed at neonatal, infant and early childhood according to the effect of antenatal betamethasone treatment and twin pair structure, if the group prevalence in the different subsets was >5%, shown in Table 1 in descending order.

## Dosage effect

The prevalence rates of neonatal outcomes within the overall cohort of twins which had received antenatal betamethasone and were born between 23<sup>+5</sup> and 34<sup>+0</sup> weeks of gestation are shown in Table 2A: RDS (37.4%), apnoea-bradycardia syndrome (30.4%), the need for non-invasive ventilation ≥2 continuous hrs (50.8%), ≥24 continuous hrs (39.4%) or endotracheal intubation (24.1%). In infancy, 93.3% presented with no first free steps after 15 months. Dosis escalation did not improve neonatal, infant (21–24 months) or early childhood (60-64 months) outcome (Tables 2A-C). In contrast, higher doses of betamethasone >24 mg vs. ≤24 mg were associated with even higher rates of congenital infections (OR 5.867, 95% CI 1.895-18.167).

#### Twin pair structure

With regard to the twin pair structure, a few differences in categorial outcome parameters could be detected. Female-female pairs had significantly lower rates of apnea-bradycardia-syndrome as neonates compared to male-male or mixed pairs (OR 0.165, 95% CI 0.059-0.457; Table 3A). In infancy (21–24 months), male-male pairs presented with significantly higher rates of no free steps after 15 months compared to female-female and mixed

**Table 1:** Overall outcome parameters with a prevalence of  $\geq 5.0\%$ .

Categorial outcome parameters	Neonatal n (%)	Infant n (%)	Early childhood n (%)
Neonatology admission	118 of 189 (59.6)	_	_
Non-invasive ventilation≥2 continuous hrs	67 of 132 (50.8)	_	-
Non-invasive ventilation≥24 continuous hrs	52 of 132 (39.4)	_	-
Antibiotic treatment	52 of 134 (38.8)	-	-
RDS	52 of 139 (37.4)	_	-
Congenital infection	44 of 135 (32.6)	_	-
Apnoea-bradycardia-syndrome	41 of 135 (30.4)	-	-
Endotracheal intubation	32 of 133 (24.1)	-	-
Coffein application	28 of 131 (21.4)	-	-
Wet lung	22 of 133 (16.5)	_	-
Surfactant	19 of 133 (14.3)	-	-
Respiratory insufficiency	19 of 135 (14.1)	-	-
Sepsis	14 of 135 (10.5)	-	-
Intraventricular hemorrhage (IVH)	7 of 134 (5.2)	_	-
No first free steps after the 15th month of life	_	181 of 194 (93.3)	31 of 187 (16.6)
Overall impression: no age-appropriate development	_	31 of 194 (16.0)	n.a.
Health disorders	_	14 of 194 (7.2)	n.a.
Pronunciation disorders <sup>a</sup>	_	n.a.	34 of 187 (18.2)
Speech disorders <sup>b</sup>	_	n.a.	25 of 187 (13.4)
Motor clumsiness <sup>c</sup>	_	n.a.	21 of 187 (11.3)
Eye abnormalities <sup>d</sup>	-	n.a.	16 of 187 (8.6)
Does not paint or tinker or reluctantly	-	n.a.	12 of 187 (6.4)
Behavioral problems <sup>e</sup>	_	n.a.	11 of 186 (5.9)

 $<sup>^{</sup>a}$ E.g. stammering, stuttering, rumbling;  $^{b}$  significant grammatical and/or sentence errors;  $^{c}$ e.g. when playing ball, running, jumping;  $^{d}$  squint n=3 of 1.6%; Poor eyesight monocular vision test with image boards or eye test n=11 of 5.9%, unspecified n=2 of 1.1%; e.g., restricted bladder and bowel control; pronounced difficulty falling asleep and falling asleep; disorders of social behavior such as aggression, no friends, does not play with peers; cannot dress; does not play; n.a.=prevalence was <5% and was therefore no evaluated.

Table 2A: The effect of antenatal betamethasone doses on neonatal outcome.

Neonatal period	≤ <b>16</b> r	ng	=24	mg	>24	mg	p-Value <sup>e</sup>	
- categorial outcome parameters with ≥5% prevalence -	n	%	n	%	n	%		
Neonatology admission (118 of 198, 59.6%)	59 of 96	61.5	45 of 78	57.7%	14 of 24	58.3%	0.873	
Non-invasive ventilation >2 continuous hrs	32 of 64	50.0	28 of 52	53.8	7 of 16	43.8	0.768	
(67 of 132, 50.8%)								
Non-invasive ventilation ≥24 continuous hrs (52 of 132, 39.4%)	25 of 64	39.1	21 of 52	40.4	6 of 16	37.5	0.976	
Antibiotic treatment	18 of 65	27.7	18 of 53	34.0	8 of 16	50.0	0.271	
(52 of 134, 38.8%)								
RDS	20 of 66	30.3	25 of 57	43.9	7 of 16	43.8	0.258	
(52 of 139, 37.4%)								
Congenital infection	18 of 66	27.3	15 of 53	28.3	<b>11 of 16</b> <sup>b</sup>	68.8 <sup>b</sup>	0.004 <sup>a</sup>	
(44 of 135, 32.6%)								
Apnoea-bradycardia-syndrome	23 of 66	34.8	13 of 53	24.5	5 of 16	31.3	0.475	
(41 of 135, 30.4%)								
Endotracheal intubation	16 of 64	25.0	11 of 53	20.8	5 of 16	31.3	0.670	
(32 of 132, 24.1%)								
Coffein application	15 of 63	23.8	9 of 52	17.3	4 of 16	25.0	0.651	
(289 of 131, 21.4%)								
Wet lung	11 of 66	16.7	9 of 51	17.6	2 of 16	12.5	0.889	
(22 of 133, 16.5%)								
Surfactant	10 of 65	15.4	6 of 52	11.5	3 of 16	18.8	0.724	
(19 of 133, 14.3%)								
Respiratory insufficiency	8 of 66	12.1	9 of 53	17.0	2 of 16	12.5	0.737	
(19 of 135, 14.1%)								
Sepsis	4 of 66	6.1	3 of 53	5.7	7 of 16 <sup>d</sup>	43.8 <sup>d</sup>	<0.001°	
(14 of 134, 10.5%)								
Intraventricular hemorrhage (IVH) (7 of 134, 5.2%)	5 of 65	7.7	2 of 53	3.8	0 of 16	0	0.385	

 $^{a}\chi^{2}(2)=10.815$ , p=0.004,  $\phi$ =0.285 (>24 mg, p=0.001 Bonferroni post hoc test);  $^{b}$ subgroup analysis >24 vs.  $\leq$ 24 mg  $\chi^{2}(2)=10.801$ , p=0.001,  $\phi$ =0.283, OR 5.733 (95% CI 1.851-17.762), all expected cell frequencies were greater than five;  $\chi^2(2)$ =21.763, p<0.001,  $\phi$ =0.402 (>24 mg, p<0.001 Bonferroni post hoc test); <sup>d</sup>subgroup analysis <24 vs.  $\leq$ 24 mg  $\chi^2(2)=12.444$ , p<0.001,  $\phi=0.401$ , OR 12.444 (95% CI 3.571–43.369), 25.0% of expected cell frequencies were not greater than 5. Significant differences are indicated in bold. eA chi-square test with post-hoc Bonferroni correction was used to compare the effect of dosis on neonatal outcome parameters. If significant, subgroup analysis between doses were performed.

pairs (OR 4.895, 95% CI 1.447-16.560; Table 3B). In early childhood (60-64 months), male-male pairs presented with the highest rates of motor clumsiness (OR 4.33, 95% CI 1.684-11.149), female-female pairs with the lowest

rates (OR 0.081, 95% CI 0.011-0.618; Table 3C). In mixedpairs, males did worse compared to females with significantly higher rates of pronunciation disorders (OR 0.082, 95% CI 0.010-0.697; Table 3C).

Table 2B: The effect of antenatal betamethasone doses on infant outcome.

Infancy (21–24 months)	≤ <b>16</b> r	ng	=24 r	ng	>24 n	p-Value <sup>a</sup>	
- categorial outcome parameters with ≥5% prevalence -	n	%	n	%	n	%	
No first free steps after 15months (181 of 194, 93.3)	88 of 95	92.6	71 of 76	93.4	22 of 23	95.7	0.872
Overall impression: no age-appropriate development (n=31 of 194, 16.0%)	16 of 95	16.8	13 of 76	17.1	2 of 23	16.0	0.597
Health trouble (14 of 194, 7.2%)	5 of 95	5.3	7 of 76	5.5	2 of 23	1.7	0.586

<sup>&</sup>lt;sup>a</sup>A chi-square test was used to compare the effect of dosis on neonatal outcome parameters.

**Table 2C:** The effect of antenatal betamethasone doses on early childhood outcome.

Early childhood (60–64 months)	≤ <b>16</b> r	ng	=24 r	ng	>24	p-Value <sup>a</sup>	
<ul> <li>categorial outcome parameters with ≥5% prevalence –</li> </ul>	n	%	n	%	n	%	
Pronunciation disorders	15 of 87	17.2	16 of 77	20.8	3 of 23	13.0	0.667
(34 of 187, 18.2%)							
Health disorder	12 of 87	13.8	16 of 77	12.8	3 of 23	13.0	0.432
(31 of 187, 16.6%)							
Speech disorders	9 of 87	10.3	13 of 77	16.9	3 of 23	13.0	0.470
(25 of 187, 13.4%)							
Motor clumsiness	13 of 87	14.9	7 of 76	9.2	1 of 23	4.3	0.273
(21 of 186, 11.3%)							
Eye abnormalities	8 of 87	9.2	4 of 77	5.2	4 of 23	17.4	0.178
(16 of 187, 8.6%)							
Does not paint or tinker or reluctantly	5 of 87	5.7	6 of 77	7.8	1 of 23	4.3	0.790
(12 of 187, 5.9%)							
Behavioral problems	4 of 87	4.6	6 of 76	7.9	1 of 23	4.3	0.635
(11 of 186, 5.9%)							

<sup>&</sup>lt;sup>a</sup>A chi-square test was used to compare the effect of dosis on neonatal outcome parameters.

Table 3A: The effect of twin pair structure on neonatal outcome.

Neonatal period  — categorial outcome parameters with ≥5%	Male-male		Female- female		Mixed		p- Value <sup>e</sup>	Mixed <sup>x</sup>					
prevalence –	n	%	n	%	n	%		Male		Female		p-	
								n	%	n	%	<b>Value</b> <sup>d</sup>	
Neonatology admission	35 of	53.0	43 of	65.2	40 of	60.6	0.358	19 of	57.6	21 of	63.6	0.614	
(118 of 198, 59.6%)	66		66		66			33		33			
Non-invasive ventilation $\geq 2$ continuous hrs	26 of	61.9	19 of	41.3	22 of	50.0	0.154	12 of	57.1	9 of 21	42.9	0.355	
(67 of 132, 50.8%)	42		46		44			21					
Non-invasive ventilation ≥24 continuous hrs	20 of	47.6	14 of	30.4	18 of	40.9	0.249	10 of	47.6	7 of 21	33.3	0.346	
(52 of 132, 39.4%)	42		46		44			21					
Antibiotic treatment	19 of	45.2	16 of	33.3	17 of	38.6	0.512	9 of 21	42.9	8 of 21	38.1	0.763	
(52 of 134, 38.8%)	42		48		44								
RDS	15 of	40.0	15 of	30.0	19 of	43.2	0.381	10 of	47.6	9 of 21	42.9	0.757	
(52 of 139, 37.4%)	45		50		44			21					
Congenital infection	18 of	41.9	12 of	25.0	14 of	31.8	0.228	8 of 21	38.1	6 of 21	28.6	0.531	
(44 of 135, 32.6%)	43		48		44								
Apnoea-bradycardia-syndrome	19 of	14.6	5 of	<b>10.4</b> <sup>b</sup>	17 of	38.6	<b>0.001</b> <sup>a</sup>	10 of	47.6	7 of 21	33.3	0.346	
(41 of 135, 30.4%)	43		<b>48</b> <sup>b</sup>		44			21					
Endotracheal intubation	15 of	34.9	9 of 48	18.8	8 of 42	19.0	0.130	6 of 21	28.6	2 of 19	10.5	0.154	
(32 of 133, 24.1)	43												
Coffein application	12 of	30.0	7 of 47	14.9	9 of 44	20.5	0.227	6 of 21	28.6	3 of 21	14.3	0.259	
(289 of 131, 21.4%)	40												
Wet lung	7 of 41	17.1	6 of 48	12.5	9 of 44	20.5	0.587	4 of 21	19.0	4 of 21	19.0	1.000	
(22 of 133, 16.5%)													
Surfactant	9 of 42	21.4	4 of 47	8.5	6 of 44	13.6	0.218	4 of 21	19.0	2 of 21	9.5	0.378	
(19 of 133, 14.3%)													
Respiratory insufficiency	8 of 43	18.6	4 of 48	8.3	7 of 44	15.9	0.340	4 of 21	19.0	3 of 21	14.3	0.679	
(19 of 135, 14.1%)													
Sepsis	6 of 43	14.0	4 of 48	8.3	4 of 44	9.1	0.642	3 of 21	14.3	1 of 21	4.8	0.293	
(14 of 134, 10.5%)													
Intraventricular hemorrhage (IVH) (7 of 134, 5.2%)	2 of 42	4.8	0 of 48	0	5 of 44	11.4	0.049 <sup>c</sup>	2 of 21	9.5	3 of 21	14.3	0.634	

 $^{a}\chi^{2}(2)=14.340$ , p=0.001,  $\phi$ =0.326 (female-female p<0.001 Bonferroni post hoc test);  $^{b}$  subgroup analysis female-female vs. others  $\chi^{2}(2)$ =14.024, p<0.001,  $\phi$ =0.322, OR 0.165 (95% CI 0.059-0.457), all expected cell frequencies were greater than five;  ${}^{c}\chi^{2}(2)$ =6.014, p=0.049, φ=0.212 (Bonferroni post hoc test failed to be significant), 50% of cell frequencies were lower than 5. X=only mixed pairs included with complete data sets. Significant differences are indicated in bold. dA chi-square test was used to compare the effect of sex on neonatal outcome parameters in mixed-pairs. eA chi-square test with post-hoc Bonferroni correction was used to compare the effect of twin pair structure on neonatal outcome parameters. If significant, subgroup analysis between doses were performed.

**Table 3B:** The effect of twin pair structure on infant outcome.

Infancy (21–24 months)	Male-n	nale	Female-female		Mixed		p-Value <sup>d</sup>	Mixed <sup>x</sup>					
<ul> <li>categorial outcome parameters</li> <li>with ≥5% prevalence –</li> </ul>	n	%	n	%	% n	%		Male		Female		p-Value <sup>c</sup>	
								n	%	n	%		
No first free steps after 15 months (181 of 194, 93.3)	57 of 66 <sup>b</sup>	86.4 <sup>b</sup>	61 of 62	98.4	63 of 66	95.5	0.017 <sup>a</sup>	2 of 33	6.1	1 of 33	3.0	0.555	
Overall impression: no age-appropriate development (n=31 of 194, 16.0%)	14 of 66	21.2	5 of 62	8.1	12 of 66	18.2	0.107	7 of 33	21.2	5 of 33	15.2	0.523	
Health trouble (14 of 194, 7.2%)	5 of 66	7.6	6 of 62	9.7	3 of 66	4.5	0.528	3 of 33	9.1	0 of 33	0	0.076	

 $^{a}\chi^{2}(2)=8.136$ , p=0.017,  $\phi$ =0.205 (male-male p=0.006 Bonferroni post hoc test);  $^{b}$ subgroup analysis male-male vs. others  $\chi^{2}(2)=7.969$ , p=0.006,  $\phi$ =0.199, OR 4.895 (95% CI 1.447-16.560), 25% of cell frequencies were lower than 5. X=only mixed pairs included with complete data sets. Significant differences are indicated in bold. A chi-square test was used to compare the effect of sex on neonatal outcome parameters in mixed-pairs. dA chi-square test was used to compare the effect of dosis on neonatal outcome parameters.

**Table 3C:** The effect of twin pair structure on early childhood outcome.

Early childhood (60–64 months)  — categorial outcome parameters with ≥5%	Male-male		Female- female		Mixed		p- Value <sup>j</sup>	Mixed <sup>x</sup>				
prevalence –	n	%	n	%	n	%		Male		Female		p-Value <sup>i</sup>
								n	%	n	%	
Pronunciation disorders	15 of	25.4	9 of 64	14.1	10 of	15.6	0.213	9 of	28.1	1 of	3.1	0.006 <sup>f</sup>
(34 of 187, 18.2%)	59				64			32		32		
Health disorder	16 of	27.1	6 of 64	9.4	9 of 64	14.1	$0.024^{a}$	8 of	25.0	1 of	3.1	$0.012^{g}$
(31 of 187, 16.6%)	59							32		32		
Speech disorders	12 of	20.3	5 of 64	7.8	8 of 64	12.5	0.121	7 of	21.9	1 of	3.1	0.023 <sup>h</sup>
(25 of 187, 13.4%)	59							32		32		
Motor clumsiness	13 of	22.4°	1 of	<b>1.6</b> <sup>d</sup>	7 of 64	10.9	<b>0.001</b> <sup>b</sup>	5 of	15.6	2 of	6.3	0.230
(21 of 186, 11.3%)	<b>58</b> <sup>c</sup>		<b>64</b> <sup>d</sup>					32		32		
Eye abnormalities	6 of 59	10.2	5 of 64	7.8	5 of 64	7.8	0.866	4 of	12.5	1 of	3.1	0.162
(16 of 187, 8.6%)								32		32		
Does not paint or tinker or reluctantly	6 of 59	10.2	0 of 64	0	6 of 64	9.4	0.035 <sup>e</sup>	5 of	15.6	1 of	3.1	0.086
(12 of 187, 5.9%)								32		32		
Behavioral problems	4 of 58	6.9	2 of 64	3.1	5 of 64	5.9	0.494	4 of	12.5	1 of	3.1	0.162
(11 of 186, 5.9%)								32		32		

 $^{a}\chi^{2}(2)=7.434$ , p=0.024,  $\phi$ =0.199 (Bonferroni post hoc test failed to be significant).  $^{b}\chi^{2}(2)=13.220$ , p=0.001,  $\phi$ =0.267 (male-male p=0.001; female-female p=0.002 Bonferroni post hoc test); subgroup analysis male-male vs. others  $\chi^2(2)$ =10.412, p=0.001,  $\varphi$ =0.237, OR 4.33 (95% CI 1.684–11.149), all cell frequencies were greater than five;  $^{d}$ subgroup analysis female-female vs. others  $\chi^{2}(2)$ =9.219, p=0.002,  $\varphi$ =0.223, OR 0.081 (95% CI 0.011–0.618), all cell frequencies were greater than five.  ${}^{\rm e}\chi^2(2)=6.704$ , p=0.035,  $\phi$ =0.189 (Bonferroni post hoc test failed to be significant).  $\sqrt[6]{2}$ (2)=7.585, p=0.006,  $\phi$ =0.344, OR 0.082 (95% CI 0.010–0.697), all cell frequencies were greater than five.  $\sqrt[6]{2}$ (2)=6.335, p=0.012,  $\phi$ =0.315, OR 0.097 (95% CI 0.011–0.828), 50% of cell frequencies were lower than five.  $^{h}\chi^{2}(2)$ =5.143, p=0.023,  $\phi$ =0.283, OR 0.115 (95% CI 0.013-0.999), 50% of cell frequencies were lower than 5. X=only mixed pairs included with complete data sets. Significant differences are indicated in bold. <sup>1</sup>A chi-square test was used to compare the effect of sex on neonatal outcome parameters in mixed-pairs. <sup>1</sup>A chi-square test was used to compare the effect of dosis on neonatal outcome parameters.

#### Dose effect and twin pair structure

Dosage escalation of >24 mg did not improve neonatal, infant or early childhood outcome, independent of twin pair structure (Supplementary Material S2A-C). In malepairs, antenatal betamethasone dose of =24 mg was associated with lowest rates of congenital infections (OR 0.115 (95% CI 0.022-0.611)) and apnea-bradycardiasyndrome (OR 0.512 (95% CI 0.323-0.813), S2A). In early childhood, male-male pairs with ≤16 mg betamethasone dose presented with significantly higher rates of motor clumsiness as compared to >16 mg dose (OR 4.044 (95% CI 1.108-14.766), S2C). In mixed-pairs, lowest rates of congenital infections (U1) were observed in those who had received ≤16 mg betamethasone OR 0.583 (95% CI 0.366-0.792).

# **Discussion**

The growth restricting effects of antenatal betamethasone treatment reported previously in this follow up study of our original twin-preterm birth cohort, persisted beyond birth and resulted in an impairment in infant and early childhood growth up to 5.3 years of age, independent of possible confounding factors [18]. For the first time we now demonstrate that betamethasone dosage escalation >24 mg in twin pregnancies born between 23<sup>+5</sup> and 33<sup>+6</sup> weeks of gestation, obviously does not improve neonatal, infant or early childhood morbidity. In contrast, higher doses of betamethasone >24 mg resulted even in significantly higher rates of congenital infections and, therefore, cannot be recommended.

ANS treatment in preterm twin pregnancies for the induction of lung maturation is still under discussion as to whether equivalent to the dosage in singleton pregnancies, it has been sufficient to promote lung maturation in twins [3-5, 8, 9, 24-27]. Different pharmacokinetics of betamethasone in multiple pregnancies when compared to singletons are evident [3]. Clearance of betamethasone in twins appeared to be greater when compared to singletons and raises the question of whether the same ANS dose as recommended in singletons is subtherapeutic for lung maturation in twins [3-5, 8, 9, 28]. Some guidelines therefore even recommend repeat dosing of antenatal corticosteroids [29].

We have recently shown in this follow up study of our original twin-preterm birth cohort, that the fetal growth restricting effects of ANS treatment, independent of possible confounding factors, persisted beyond birth and resulted in a dose-dependent and sex-specific alteration in infant and early childhood growth up to 5.3 years of age [18], potentially indicating an ANS associated increased risk for later life diseases. Follow up studies of antenatal glucocorticoid treatment and its impact on long-term development and morbidity, considering the special needs in twin pregnancies, are missing.

In multiple pregnancy, a significant decrease in the incidence of RDS after ANS treatment has been previously reported [11], but the effect decreased with increasing plurality [4]. In a Cochrane meta-analysis published in 2017, however, it was shown that the current recommended treatment regimen for antenatal ANS with a total dose of 24 mg cannot significantly reduce in multiples the occurrence of RDS (four studies, total n=320), the occurrence of IVH (one study, n=137) and the rate of neonatal mortality (two studies, total n=236) [2]. Others demonstrated that ANS in preterm twin pregnancies significantly decrease neonatal mortality, short-term respiratory morbidity, and severe neurological injury that is similar in magnitude to that observed among singletons [15].

In the current study, the overall prevalence rates in twins of RDS (37.4%) and mechanical ventilation (24.1%) after ANS treatment were similar to preterm born singletons after ANS [2, 30] and therefore indicate a positive ANS effect on lung maturation also in twin pregnancies. However, dose escalation did not significantly improve the neonatal outcome with regard to the rate of neonatal admission to NICU, RDS, wet lung, apnea bradycardia syndrome or respiratory insufficiency as well as the need for surfactant or caffeine treatment, mechanical ventilation therapy or CPAP treatment over two as well as over 24 h. In contrast, higher doses of betamethasone >24 vs. ≤24 mg were associated with significantly higher rates of congenital infections. However, this might reflect increased latency between the onset of premature contractions with cervical shortening or premature rupture of the membranes and the actual date of delivery, prompting repeat dosages of betamethasone.

In addition, a lower BET total dose (≤16 mg) does not seem to lead to a worse outcome, but might be associated with less side effects of ANS known from singleton pregnancies, such as a transient decrease in fetal heart rate variability, fetal respiratory excursions and fetal movements [31] as well as lower rates of a reduction in birth weight and body length and head circumference in newborns depending on the dose applied [16, 32–34].

For the first time, now we could also demonstrate in twins, that dosage escalation >24 mg did not significantly improve infant or early childhood outcome, independent of twin-pair structure. Higher doses of antenatal BET did not significantly improve long-term postnatal development in twin pregnancies in this follow up study up to the 5.3 years.

Sex differences as reported previously could be observed here as well [35]. Male sex was obviously a factor for lower rates of apnea-bradycardia-syndrome in neonates (OR 0.165, 95% CI 0.059–0.457), for higher rates of no free steps after 15 months in infancy (OR 4.895, 95% CI 1.447-16.560) and for highest rates of motor clumsiness in early childhood (OR 4.33, 95% CI 1.684-11.149). In mixed-pairs, males did worse compared to females with higher rates of pronunciation disorders in early childhood (OR 0.082, 95% CI 0.010-0.697). A total does of 24 mg in males was associated with lowest rates of congenital infections (OR 0.115, 95% CI 0.022-0.611) and apnea-bradycardia-syndrome (OR 0.512, 95% CI 0.323–0.813) and therefore suggestive of the appropriate dose for male preterm twins.

There are some limitations of our study, as discussed previously [18]. Uncontrolled data collection and selection bias may be present in the analyses through its retrospective character. However, due our large sample size, these effects are unlikely to account for the overall observations and differences observed. Furthermore, the validity of the standardized examinations carried out be pediatricians and general practitioners without supervision has not been established yet. In addition, there is uncertainty on how to correct results for prematurity and multilingual families. Unique strengths of our study is the large sample size; the exclusion of fetuses with EFW <10th centile at the time of betamethasone treatment; our ability to differentiate between same-sex and mixed-sexed pairs; a broad gestational age range, including very early and early preterm infants, and the analysis of different betamethasone dosage regimens.

## **Conclusions**

Antenatal betamethasone treatment in preterm twin pregnancies to induce lung maturation and improve neonatal morbidity and mortality was associated with similar morbidity rates compared to singletons who received ANS for preterm birth treatment. Of note, dose escalation >24 mg was associated with significantly higher rates of congenital infections while no difference emerged for other neonatal, infant or early childhood morbidity. This argues against dosages exceeding 24 mg in twin pregnancies while 24 mg was associated with the lowest morbidity in male twins. An individualized and gender-specific approach for ANS treatment in preterm birth might help to improve neonatal and long-term outcome.

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