Effects of Antenatal Betamethasone on Fetal Doppler Indices and Short Term Fetal Heart Rate Variation in Early Growth Restricted Fetuses

Introduction

In human pregnancy, a single course of antenatal corticosteroids reduces the occurrence of respiratory distress syndrome and other complications following preterm birth [1]. However, antenatal betamethasone treatment has been reported to induce transient effects on fetal behavior, including a decrease in fetal heart rate and reduced short term fetal heart rate variation without decelerations, observed up to 4 days after administration [2]. Doppler studies in small cohorts of human pregnancies complicated by fetal growth restriction (FGR) have observed a temporary decrease in the pulsatility index (PI) of the umbilical artery (UA), the reappearance of end-diastolic flow in this vessel, and/or a decrease in the ductus venosus PI (DVPIV) [3-5]. Other studies did not observe such changes [6,7]. Therefore, there is ongoing discussion as to whether such effects of betamethasone represent ominous clinical signs of worsening of the fetal condition or simply transient effects with no significant sequelae for the developing infant [2]. This discussion is further compounded by the current paucity of data derived from large human cohorts, particularly those involving pregnancies with FGR.

The recent Trial of Umbilical and Fetal Flow in Europe (TRUFFLE) multi-centre study on the management of severe early onset FGR has reported a more favorable neurological outcome among those fetuses who were delivered based on both Doppler assessment of the ductus venosus (DV) and cardiotocography (CTG) [8]. In this context, the possible effects of glucocorticoids on changes in DVPIV and fetal CTG short term variability (CTG-STV) might have played a significant role that would benefit from clarification.

The aim of this study was to investigate the effects of antenatal betamethasone administration on fetal CTG-STV, DV Doppler waveform and umbilico-cerebral ratio (UC ratio, calculated as the ratio between the pulsatility index of the umbilical artery and the pulsatility index of the middle cerebral artery) in the TRUFFLE cohort.

Methods

We conducted a post-hoc analysis of data from the TRUFFLE study, a prospective, multicentre, randomized management trial that took place in 20 European tertiary care centres in five countries [8]. Eligible participants for the TRUFFLE trial were women over 18 years of age, with a singleton pregnancy at 26^{+0} to 31^{+6} weeks of gestation diagnosed with early isolated FGR, defined as fetal

abdominal circumference below the 10th percentile and umbilical artery Doppler PI above the 95th percentile. Details about inclusion and exclusion criteria have been previously reported [8]. The intervention was delivery of the fetus according to the criteria of the randomization group, determined by CTG indices of reduced short-term variation (CTG-STV; CTG-STV<3.5 ms at <29 weeks of gestation or CTG-STV <4 ms at ≥29 weeks of gestation), early abnormalities of the DV waveform (DVPIV ≥95th centile) or late DV changes (absent or negative A-wave). In cases where corticosteroids had been given to accelerate fetal lung maturation, no decision regarding delivery was made on the grounds of reduced STV from 24 h to 72 h after the first intramuscular injection. Following 32^{+0} weeks, the timing of delivery was according to local management protocol and the decision to deliver was based on local criteria as DV waveforms were no longer taken into consideration [8]. In all groups, delivery could be undertaken based on maternal indications, such as severe pre-eclampsia. The study protocol was approved by the institutional ethics committee, and patients provided written informed consent. For the current post-hoc study we selected women who had been treated with corticosteroids for improving fetal maturity because it was expected that delivery was imminent due to deterioration of the fetal condition in association with FGR. The timing of maternal prophylactic steroid administration was according to local protocols. Two doses of intramuscular 12 mg betamethasone were given, the second 12–24 h after the first. Repeated doses of steroids were never administered. To qualify for this post hoc study women should have a CTG-STV recorded within 48 hours before the first corticosteroid administration and at least 2 CTG-STV recorded in the following week. CTG-STV values were categorized as pre-corticosteroids and in 24-hour periods after corticosteroids up to 10 days. Because it was expected that in this group of women with a pregnancy complicated by severe FGR the fetal wellbeing would deteriorate at a certain moment, necessitating delivery, and that at that moment CTG-STV would be low, and DVPIV and UC ratio would be high, as a result of fetal condition, in order to avoid possible bias, we excluded from the current analysis CTG-STV and Doppler values recorded within 48 hours from delivery. The Oxford Sonicaid 8002 system or an equivalent Dawes-Redman software based algorithm were used for STV calculation. The recordings were at least 45 min in duration. CTG-STV measurements were not necessarily performed at the same time intervals following betamethasone administration nor at the same time during the day. For all the fetuses included, repeated measurements between the first betamethasone dose (day +1) and 10 days after (+10) were considered. According to the study protocol UA pulsatility index (PI), middle cerebral artery (MCA) PI, DVPIV and CTG-STV were assessed in each fetus before randomization. We evaluated, for each different measurement in each fetus, between +1 and + 10, the relative change from the last recording within 48 hours before the first dose of betamethasone (basal value) for CTG-STV (i.e. $CTG\text{-}STV_n\text{-}CTG\text{-}STV_0/CTG\text{-}STV_0$), DVPIV, and UC ratio. After randomization the TRUFFLE protocol recommended measurement of

UA PI, MCA PI, CTG-STV and DVPIV, (if randomized to the DV-groups) to be performed at least once weekly, but assessment could be more frequent according to local policies. Median and interquartile ranges (IQR) were used for descriptive statistics. The Kruskal–Wallis test was used to test if a difference in the medians of CTG-STV and Doppler indices was detectable for 10 days after steroid administration. Multiple comparisons were subsequently performed using Dunn's test with Bonferroni correction. Based on literature we expected a small increase of STV during the first day and a decrease on day 2 or 3 [9]. Multilevel analysis was performed to verify such a pattern and to evaluate the course of CTG-STV, DVPIV, and UC ratio longitudinally. We assessed whether there was a significant time effect on the Doppler and CTG-STV values for 10 days from steroids administration and whether changes of these biophysical parameters were significantly affected by other clinical variables. Multilevel analysis allows for the dependency of measurements and can be used if there are missing data and/or when measurements have been made at different time points. Repeat Doppler and CTG assessment in the same fetus at different gestational ages belong to the first level and the different fetuses in the study belong to the second level [10]. For further analysis of CTG-STV and Doppler indices, a mixed model based on clinical predictors was constructed, removing items from the initial model in a stepwise manner when the t-test p value was > 0.15. The variables evaluated in the models were: the ratio between the estimated fetal weight (EFW) and the 50th centile for EFW at that gestational age (EFW p50 ratio) , fetal gender, presence of fetal heart rate decelerations at cardiotocography; CTG-STV; DVPIV; umbilical artery end diastolic flow (UAEDF, present versus absent or reverse); and days from delivery. The dependent variables were the individual Doppler indices and CTG-STV (the dependent variable was of course removed from the variables tested in the model). Since the main aim of the study was to evaluate the longitudinal course of fetal Doppler waveforms and CTG-STV after corticosteroids administration, we never removed from the model the time variable expressed as the number of days elapsed from the first dose of corticosteroids. Since the decision to administer steroids is usually made on the basis of worsening fetal or maternal conditions in view of a decision to terminate the pregnancy, we performed an analysis forcing the steroid-to-delivery interval (expressed in days) into the model. The Wald test was used to assess significance of parameter estimates. Values of $p < 0.05$ were considered statistically significant. Statistical analysis was performed with Stata 13.1 (StataCorp. 2013. Stata: Release 13. Statistical Software. College Station, TX: StataCorp LP) [11].

Results

The TRUFFLE trial [8] included 503 women, 476 (95%) of these received corticosteroids to improve fetal maturity. Most of these women (n=361; 72%) did not have a computerized CTG within 48 hours before the first corticosteroid administration recorded in the study database, because corticosteroids

had been given before referral from a secondary care center, or before study inclusion was considered. A total of 115 women (23 %) qualified for this secondary analysis (Fig. 1, Tab. 1). Tab. 2 shows the number of observations and the change from baseline (median, IQR), recorded in each day of the study interval for CTG-STV and Doppler parameters. An increase of CTG-STV from basal value was found on day +1 (p=0.019), after the first dose of corticosteroids (Tab. 2, Fig. 2). DVPIV was not significantly different from basal in any of the 10 days following the first dose of betamethasone (p=0.167), although a slight, non-significant decrease could be observed on day +1 (Tab. 3, Fig. 3). During the 10-day period of observation there were no significant changes from baseline in the UC ratio (Tab. 4, Fig. 4).

Multilevel analysis applying the steroid-to-delivery interval into the model demonstrated that after stepwise removal of less significant predictors, the time elapsed from antenatal administration of betamethasone was associated with a decrease in CTG-STV in the following 10 days (p=0.045; Fig. 2, Tab. S1). In addition, the time elapsed from the first dose of steroids was associated with an increase in the DVPIV (p=0.001, Tab. S2) and in the UC ratio ($p < 0.001$, Tab. S3). However, all the regression coefficients were of small magnitude (ranging from -0.01 to 0.02), suggesting no clinical significance [12].

Discussion

In a cohort of early FGR fetuses with abnormal umbilical artery Doppler, we showed that antenatal administration of betamethasone is associated with a clinically detectable increase in CTG-STV the first day after the first dose of steroids followed by a small decrease in CTG-STV afterwards. Multilevel analysis further revealed that although the time elapsed from steroid administration was statistically significantly associated with CTG-STV, the DVPIV and the UC ratio relative changes, the magnitude of these associations was clinically irrelevant. The change we observed in CTG-STV, a short increase after the first dose of betamethasone, followed by a small decrease in STV in the days afterwards was similar to the effect described by Mulder et al. [13]. The current data show that the effect of betamethasone in FGR fetuses is similar to what seen in pregnancies with preterm premature rupture of the membranes or premature labour. This strongly suggests that the change in CTG-STV is independent of placental function.

Previous research has described a possible effect of antenatal maternal administration of corticosteroids on fetal heart rate variables. A transient increase in fetal arterial blood pressure, induced by glucocorticoids [14], will increase the vagal drive to the fetal heart by recruiting baroreceptor afferent fiber discharge as part of a baroreflex [15]. The withdrawal of sympathetic outflow will lower the low frequency/high frequency ratio of the fetal heart rate power spectrum,

thereby in keeping with transient depressive effects of glucocorticoids on fetal heart rate variation [9]. Clinical studies observing an effect of steroids on CTG-STV, however, were never performed on a large population of well-defined early severe FGR fetuses such as the TRUFFLE cohort [2]. In FGR, high placental vascular resistance leads to an increase in fetal cardiac afterload, myocardial dysfunction and ductus venosus dilatation, all possibly associated with CTG-STV decrease and DVPIV increase [16]. It is possible that the severe progressive chronic hypoxia characterizing TRUFFLE fetuses might limit further compensatory responses to steroids.

With regards to short-term changes in Doppler indices, our findings are in agreement with an earlier study in 57 early FGR fetuses [7] and with a recent prospective study reporting no change in DVPIV in the preterm FGR fetus following maternal treatment with betamethasone [17]. However, in the latter study this was observed in FGR fetuses with a mean gestational age of 34 weeks, significantly later than in our cohort. During the 10-day period of observation the course of CTG-STV, the DVPIV and the UC ratio are consistent with a progressive deterioration of the fetal condition due to worsening chronic fetal hypoxemia [16] rather than the use of betamethasone. In agreement with some previous studies both the UC ratio and DVPIV changes shortly after maternal betamethasone administration were not clinically significant [7,18,19]. Our observations did not confirm previous reports of a DVPIV reduction after betamethasone [5]. We highlight that a progressive deterioration of the fetal condition, due to chronic fetal hypoxia, is likely to be the main determinant of longitudinal CTG-STV and Doppler changes in early FGR fetuses [16] even after betamethasone exposure. Chronic fetal hypoxia leads to a circulatory compromise that accounts for increased ductus venosus shunting [20], myocardial dysfunction and fetal acidosis [21,22]. This can be documented as a progressive increase in DVPIV, manifested initially as a deepening and, ultimately, reversal of the A-wave [23]. Deferring delivery until absent or reverse flow during the A-wave, unless delivery is mandated earlier by the CTG safety net criteria, compared with delivery based only on CTG-STV, is a safe management strategy with regard to intact survival without impairment at 2 years of age, corrected for prematurity [8,24-27]. According to our data, any CTG-STV decrease [8,28] within 72 hours from the first dose of steroids might be interpreted as a sign of fetal deterioration due to progressing chronic fetal hypoxia. At the same time, the occurrence of spontaneous FHR decelerations, included in the TRUFFLE study as a safety net criterion to deliver the fetus, does not seem to be induced by corticosteroids [2] and remains therefore important for fetal surveillance after steroid administration, just as the assessment of the ductus venosus.

The main strength of our analysis is that effects of antenatal steroids on Doppler indices and CTG-STV were evaluated in a large population of early severe FGR fetuses followed up longitudinally until delivery. We analysed the UC ratio rather than the cerebroplacental ratio (CPR), as we previously demonstrated that the former allows for a better discrimination in the abnormal range [29].

A limitation is that evaluation of the effects of betamethasone on fetal Doppler and CTG-STV was not the aim of the TRUFFLE study [8]. Therefore, the number of Doppler and CTG-STV measurements per day is relatively small, moreover Doppler and CTG-STV measurements were not necessarily performed at the same time intervals following betamethasone administration nor at the same time during the day; however we can assume that such intervals varied randomly. Transient reductions in STV and fetal movements following betamethasone are not found in the morning, most likely since betamethasone abolishes diurnal rhythms, i.e. increases in these variables that normally occur during the course of the day [30].

In conclusion, we demonstrate that in early FGR fetuses betamethasone administration is not associated with a decrease in CTG-STV after adjusting for confounders. The data available at the inception of the TRUFFLE trial [8], although not specific for an early FGR population, suggested a short term effect of steroids on CTG-STV [2]. Therefore, according to the original study protocol no decision regarding delivery was to be made on the grounds of reduced CTG-STV from 24 h to 72 h after the first dose of corticosteroids. The current study suggests that, given the lack of clinically significant effect of betamethasone on CTG-STV in early FGR, CTG-STV assessment remains valid even following steroid administration. Therefore, delivery for fetal indication should be considered if the CTG-STV falls below the safety net criteria of the TRUFFLE protocol, spontaneous repeated persistent and unprovoked FHR decelerations occur or if the DVPIV becomes significantly abnormal.

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Table 1. Characteristics of the TRUFFLE Fetuses included in the post hoc analysis compared to those of TRUFFLE fetuses not included.

	TRUFFLE Fetuses included in the post hoc analysis (n=115)	**TRUFFLE fetuses not included in the post hoc analysis (n=388)**	**P**
Gestational age at randomization, weeks	28.9 (27.7 to 30.0)	29.3 (27.9 to 30.3)	0.09
Gestational age at the 1st dose of glucocorticoids	29.0 (28.0 to 30.7)	28.6 (27.1 to 29.7)	0.00
Gestational age at delivery, weeks	30.7 (29.1 to 32.6)	30.6 (29.1 to 32.0)	0.46
EFW at inclusion	825 (677 to 1017)	876 (708 to 1047)	0.20
EFW p50 ratio at inclusion	0.66 (0.59 to 0.72)	0.65 (0.58 to 0.71)	0.47
Randomization to delivery interval, days	11 (4 to 18)	7 (3 to 17)	0.01
Day of the 1st dose of glucocorticoids to delivery interval, days	7 (3 to 16)	12 (5 to 24)	0.00
UA PI at randomization	1.8 (1.6 to 2.2)	1.9 (1.7 to 2.2)	0.44
UA PI at at the 1st dose of glucocorticoids	1.9 (1.7 to 2.2)	---	---
MCA PI at randomization	1.5 (1.3 to 1.7)	1.4 (1.2 to 1.7)	0.23
MCA PI at the 1st dose of glucocorticoids	1.5 (1.2 to 1.8)	---	---
DVPIV at randomization	0.60 (0.47 to 0.70)	0.58 (0.49 to 0.70)	0.88
DVPIV at the 1st dose of glucocorticoids	0.57 (0.46 to 0.70)	---	---
CTG-STV at randomization, ms	6.5 (5.0 to 8.4)	6.2 (5.0 to 7.6)	0.43
CTG-STV at the 1st dose of glucocorticoids, ms	6.3 (5.0 to 8.3)	---	---
Fetuses with UA ARED at randomization	38%	43%	0.45
Fetuses with UA ARED at the 1st dose of glucocorticoids	46 %	---	---
Fetuses with UA ARED within 1 week before delivery	61%	55%	0.29
Male fetuses	56 (49%)	196 (50%)	0.75
Female fetuses	59 (51%)	192 (50%)	0.75
Birthweight, g	970 (800 to 1170)	980 (792 to 1179)	0.98
Birthweigth p50 ratio	0.58 (0.53 to 0.64)	0.59 (0.52 to 0.66)	0.63

Median and in parenthesis interquartile range (IQR) are shown for continuous variables. P values are calculated by Mann-Whitney of Fisher exact test
Abbreviations: CTG-STV, cardiotocography short-term variation; DVPIV, ductus venosus pulsatility index; EFW p50 ratio, ratio between the estimated fetal weight (EFW) and the 50th centile of the estimated fetal weight at the correspondent gestation; MCA PI, middle cerebral artery pulsatility index; UA PI umbilical artery pulsatility index; UA ARED umbilical artery absent or reverse end diastolic flow.

Table 2. Number of observations recorded and deliveries in each day of the study interval for CTG-STV.

Days from the 1st dose of glucocorticoids	CTG-STV		Number of pregnancies	
	Observations (n) #	***Change from basal (%) (median [IQR])#***	**Ongoing**	**Delivered**
Before corticosteroids	***102/115***	*0*	113	**2**
+1	***50/81***	*23% ** *(-2% to 42%)*	74	**7**
+2	***55/89***	*8%* *(-11% to 29%)*	77	**12**
+3	***56/86***	*-5%* *(-23% to 16%)*	74	**12**
+4	***42/62***	*0%* *(-19% to 25%)*	53	**9**
+5	***42/59***	*-13%* *(-20% to 16%)*	56	**3**
+6	***41/60***	*-12%* *(-28% to 4%)*	50	**10**
+7	***28/45***	***-11%*** ***(-31% to 12%)***	41	**4**
+8	***32/45***	***-7%*** ***(-19% to 13%)***	38	**7**
+9	***29/41***	***-15%*** ***(-29% to -3%)***	35	**6**
+10	***31/37***	***-5%*** ***(-17% to 5%)***	34	**3**

Abbreviations: cardiotocography short-term variation (CTG-STV), interquartile range (IQR), number (n). Measurements taken within 48 hours before delivery were excluded#. Values significantly different from before corticosteroids were marked with *. The number of observations recorded and the number of fetuses delivered in each day of the study interval is shown for CTG-STV. Measurements were not necessarily taken on each day for all ongoing pregnancies, moreover measurements taken within 48 hours before delivery were excluded.

Table 3. Number of observations recorded and deliveries in each day of the study interval for DV.

Days from the 1st dose of glucocorticoids	DVPIV		Number of pregnancies	
	Observations (n#)	*Change from basal (%) (median [IQR])#*	Ongoing	Delivered
Before corticosteroids	***61/64***	***0***	64	**0**
+1	***15/24***	***-28% (-46% to 13%)***	24	**0**
+2	***20/30***	***-3% (-11% to 9%)***	25	**5**
+3	***19/33***	***-5% (-33% to 18%)***	30	**3**
+4	***12/20***	***-7% (-31% to 21%)***	18	**2**
+5	***15/24***	***-9% (5% to 28%)***	23	**1**
+6	***13/19***	***4% (-14% to 22%)***	18	**1**
+7	***13/22***	***3% (-7% to 25%)***	21	**1**
+8	***9/16***	***2% (-2% to 5%)***	13	**3**
+9	***8/13***	***7% (-9% to 23%)***	12	**1**
+10	***14/20***	***7% (0% to 41%)***	18	**2**

Abbreviations: ductus venosus pulsatility index (DVPIV). Measurements taken within 48 hours before delivery were excluded#

The number of observations recorded and the number of fetuses delivered in each day of the study interval is shown for DVPIV. Measurements were not necessarily taken on each day for all ongoing pregnancies, moreover measurements taken within 48 hours before delivery were excluded.

Table 4. Number of observations recorded and deliveries in each day of the study interval for UC ratio.

Days from the 1st dose of glucocorticoids	UC ratio		Number of pregnancies	
	Observations (n#)	*Change from basal (%) (median [IQR])#*	Ongoing	Delivered
Before corticosteroids	***83/93***	***0***	93	0
+1	***19/34***	***-5% (-20% to 5%)***	34	0
+2	***21/36***	***-2% (-30% to 11%)***	30	6
+3	***22/30***	***4% (-24% to 23%)***	29	1
+4	***16/25***	***-2% (-17% to 28%)***	22	3
+5	***15/25***	***-7% (-23% to 25%)***	23	2
+6	***14/20***	***22% (-21% to 44%)***	17	3
+7	***13/21***	***9% (-10% to 31%)***	21	0
+8	***10/20***	***11% (-0.5% to 28%)***	15	5
+9	***13/17***	***32% (0.5% to 43%)***	16	1
+10	***14/21***	***20% (-0.1% to 28%)***	19	2

Abbreviations: Umbilical cerebral pulsatility index ratio (UC). Measurements taken within 48 hours before delivery were excluded#

The number of observations recorded and the number of fetuses delivered in each day of the study interval is shown for UC ratio. Measurements were not necessarily taken on each day for all ongoing pregnancies, moreover measurements taken within 48 hours before delivery were excluded.

Figure 1. Study design and population. The population of this post hoc analysis were 115 women who received antenatal glucocorticoids for respiratory distress syndrome prophylaxis after being randomized in the TRUFFLE trial.

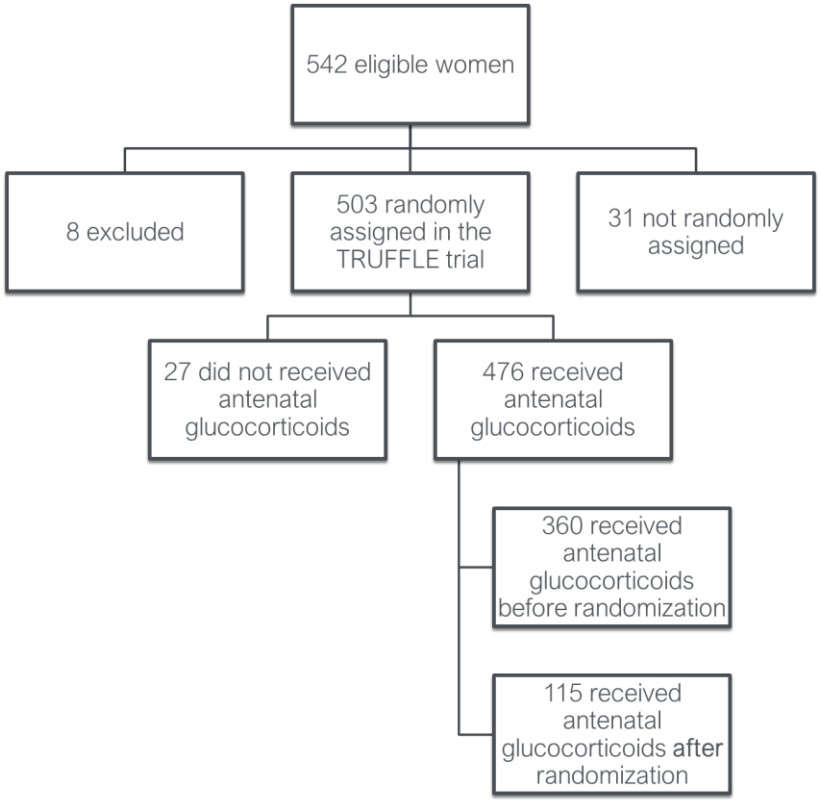


Figure 2:

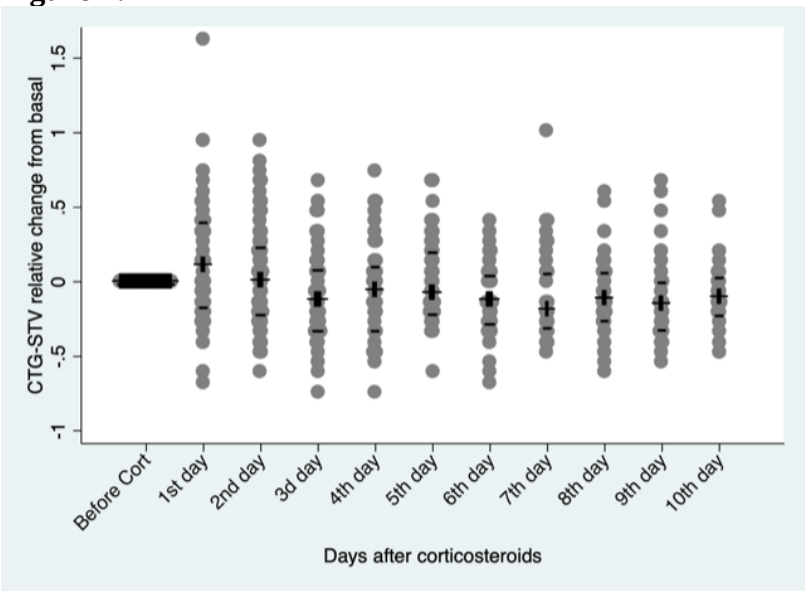


Figure 3:

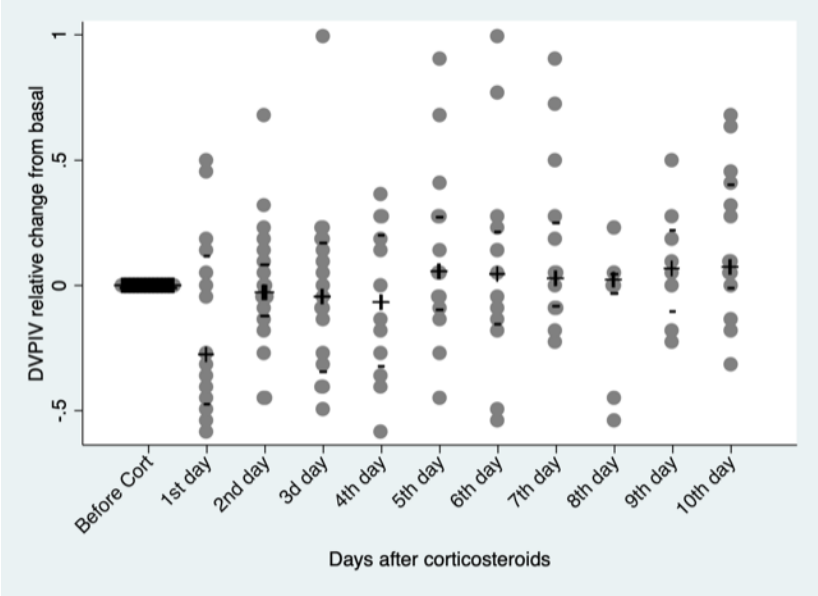
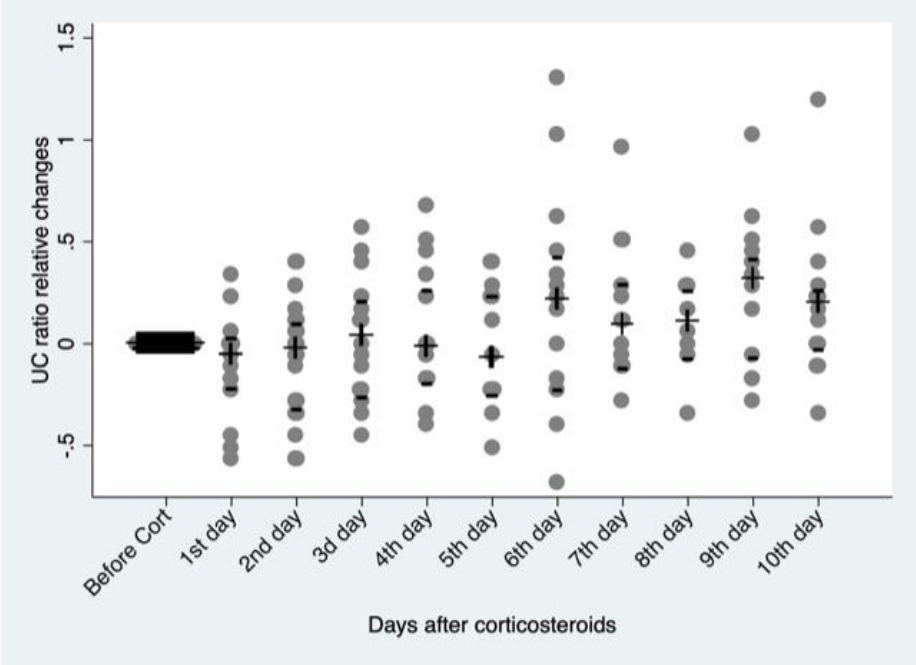


Figure 4:



Supplementary Material

Table S1. Summary of multilevel stepwise regression for variables predicting CTG-STV relative change from the day of the first dose of betamethasone. Measurements taken within 48 hours before delivery were excluded.

CTG-STV relative change from day 0	
	STEP 1
	Coeff. **(SE)**
Days from Betamethasone	-0.01057* (0.00429)
Decelerations	-0.10066 (113.854)
EFWp50 ratio	0.00138 (0.00234)
Gender	-0.02220 (0.04240)
UA EDF at assessment	-0.02981 (0.02279)
DVPIV at assessment	0.01016 (0.07726)
Days from delivery	-0.00041 (0.00166)
Constant	0.09655 (113.8541)
	VARIANCE **(SE)**
LEVEL 1	0 .03286 (0.00384)
LEVEL 2 (fetuses)	0.01908 (0.00548)
Observations N	218
Fetuses N	83

+ p<0.15, * p<0.05, ** p<0.01, *** p<0.001

Coeff: regression coefficient; DVPIV: ductus venosus pulsatility index; EFWp50 ratio: ratio between the estimated fetal weight (EFW) and the 50th centile of the estimated fetal weight at the correspondent gestation; Gender (0,male ; 1,female); SE: standard error; UA EDF: umbilical artery end diastolic flow (present, absent/reverse.

Table S2. Summary of multilevel stepwise regression for variables predicting DVPIV relative change from the day of the first dose of betamethasone. Measurements taken within 48 hours before delivery were excluded.

DV PIV relative change from day 0			
	STEP 1	**STEP 2**	**STEP 3**
	Coeff. (SE)	**Coeff. (SE)**	**Coeff. (SE)**
Days from Betamethasone	0.01766 * (0.00516)	0.01732** (0 .00518)	0.01732** (0.00518)
Decelerations	208.2963** (77.41966)	-0.00122 (45.44634)	
UA EDF	0.021124 (0.02883)		
CTG-STV at assessment	0.01793* (0.00941)	0.01773* (0.00947)	0. 01773* (0.00947)
Gender	0.11112* (0.04577)	0.10963** (0.04657)	0.10963* (0.04657)
EFWp50 ratio	-0.00297 (0.00258)		
Constant	-208.4527 (77.41935)	-0.31888 (45.44633)	-0.32011 (0.09940)
	VARIANCE (SE)	**VARIANCE (SE)**	**VARIANCE (SE)**
LEVEL 1	0.04451 (0.00539)	0.04461 (0.00534)	0.04461 (0.00534)
LEVEL 2 (fetuses)	0.01350 (0.00495)	0.01454 (0.00489)	0.01454 (.00489)
Observations N	180	180	180
Fetuses N	69	69	69

+ p<0.15, * p<0.05, ** p<0.01, *** p<0.001
CTG-STV: cardiotocography short-term variation; EFWp50 ratio: ratio between the estimated fetal weight (EFW) and the 50th centile of the estimated fetal weight at the correspondent gestation; Gender (0,male ; 1,female);; UA EDF: umbilical artery end diastolic flow (present, absent/reverse).

Table S3. Summary of multilevel stepwise regression for variables predicting UC ratio relative change from the day of the first dose of betamethasone. Measurements taken within 48 hours before delivery were excluded.

UC ratio relative change from day 0			
	STEP 1	**STEP 2**	
	Coeff. (SE)	**Coeff. (SE)**	
Days from Betamethasone	0 .01652** (0 .00529)	0.01883*** (0.00485)	
Decelerations	-0.54526 (104.3978)		
DVPIV at assessment	0 .00358 (0.10659)		
CTG-STV at assessment	-0.01981+ (0.01017)	-0.01073+ (0.00804)	
EFWp50 ratio	0.00199 (0.00260)		
Gender	0 .03773 (0.04646)		
Constant	0.45763 (104.3977)	-0. 05154 (0 .06116)	
	VARIANCE (SE)	**VARIANCE (SE)**	
LEVEL 1	0 .04035 (0.00501)	0.04692 (0.005)	
LEVEL 2 (fetuses)	0 .01291 (0.00461)	0.01487 (0.00461)	
Observations N	167	227	
Fetuses N	65	83	

+ p<0.15, * p<0.05, ** p<0.01, *** p<0.001

Coeff: regression coefficient; CTG-STV: cardiotocography short-term variation; DVPIV: ductus venosus pulsatility index; EFWp50 ratio: ratio between the estimated fetal weight (EFW) and the 50th centile of the estimated fetal weight at the correspondent gestation.