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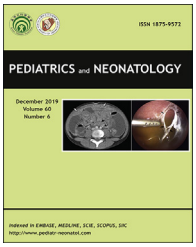

Original Article

Basal serum cortisol concentration in very low birth weight infants

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Key Words
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cortisol;
perinatal distress;
premature infants

Background: The aim of our study was to measure the basal serum cortisol concentration immediately after birth and to determine its association with perinatal factors and clinical outcomes in very low birth weight (VLBW) infants.
Methods: Basal serum cortisol level was obtained within one hour after birth in inborn VLBW infants. The association between the basal serum cortisol level and perinatal and clinical outcomes was analyzed by comparing the groups with high versus low cortisol levels.
Results: In total, 80 infants were included. The median concentration of basal serum cortisol was 167 nmol/L with an interquartile range of 98–298 nmol/L. The basal serum cortisol concentration positively correlated with elapsed time from the last betamethasone dose. Low serum cortisol concentration was associated with antenatal corticosteroid therapy, low lactic acid level, and low leukocyte count at birth. Basal serum cortisol level was not associated with mortality and neonatal morbidities including hypotension and severe grade intraventricular hemorrhage.
Conclusion: Both maternal corticosteroid therapy and perinatal distress may affect the basal serum cortisol concentration in VLBW infants early after birth.

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1. Introduction

Sufficient endogenous cortisol production is important for successful adaptation to extrauterine life.[1,2] Glucocorticoids have various effects such as maturation of the lung, maintenance of glucose homeostasis, control of hormonal adaptation immediately after birth, and regulation of body temperature.[3] Preterm infants have an immature hypothalamic-pituitary-adrenal (HPA) axis, which might be due to failure of hypothalamus in recognizing the stimulatory signal or ineffective adrenal steroidogenesis.[4,5] The degree of HPA axis immaturity is associated with gestational age, postnatal age,[6,7] intrauterine growth restriction,[8] and antenatal corticosteroids.[7,9,10] Acute illness such as respiratory distress syndrome, requirement of mechanical ventilation, and infection also influence the HPA response in preterm infants.[6,10] However, preterm infants' ability to produce cortisol in response to perinatal events remains unknown.

This study aimed to evaluate the effects of perinatal events on the serum cortisol concentration early after birth and to determine the association between basal serum cortisol concentration and neonatal morbidities in very low birth weight (VLBW) infants.

2. Patients and methods

2.1. Study population

This is a retrospective study based on the data obtained by local protocol. Infants with a birth weight of <1500 g hospitalized in the neonatal intensive care unit at a tertiary medical center from January 2016 to August 2017 were selected. The exclusion criteria were 1) infants with prenatally diagnosed major congenital anomalies including structural cardiac or renal diseases and 2) fetal hydrops, and 3) confirmed early onset-sepsis. Demographic and clinical data were collected from medical records after obtaining permission from the Institutional Review Board at a tertiary medical center.

2.2. Serum cortisol concentration assay

Based on the institutional policy, blood samples were obtained via indwelling arterial catheter or arterial puncture within one hour after birth to measure blood gas, glucose, lactate, complete blood count, C-reactive protein, and serum cortisol level in all inborn VLBW infants. The samples for serum cortisol (0.5 ml) were transported to the laboratory either immediately or the next morning after being stored in a refrigerator at 4 °C overnight. Serum cortisol was measured once using a commercial kit by radioimmunoassay (Asbach Medical Product GmbH, Obrigheim, Germany). A coated tube with 25 μl of calibrators, 25 μl of samples or controls, and 500 μl of tracer was prepared. The solvent was incubated for 45 min at 37 °C in a water bath. After cleansing with wash solution, contents were counted using a Cobra II gamma counter (Packard instrument Co., Meriden, CT, USA). Interassay and intraassay coefficients of cortisol variations were 4.7% and 10.4%, respectively. Detection limit for cortisol was 14 nmol/L.

2.3. Outcomes and variables

Maternal data such as mode of delivery, dosing time of antenatal betamethasone, preeclampsia, chorioamnionitis confirmed by histopathology, and ruptured membranes were collected. Intramuscular administration of two 12-mg doses of betamethasone, 24-h apart, within 1–7 days before birth was considered a complete course of antenatal steroids.[11] Infant characteristics including gestational age, birth weight, sex, small for gestational age according to the Fenton preterm growth charts,[12] 1-min and 5-min Apgar scores, resuscitation information in the delivery room, body temperature at admission, and laboratory values within one hour after birth were also obtained to determine their association with basal serum cortisol concentration. Data on neonatal outcomes included hypotension requiring catecholamine, sepsis, respiratory distress syndrome, stage 2 or higher necrotizing enterocolitis according to Bell et al.'s criteria,[13] grades 3–4 intraventricular hemorrhage (IVH) according to Papile et al.'s criteria,[14] any retinopathy of prematurity (ROP), ROP requiring laser photocoagulation and/or intravitreal bevacizumab, moderate or severe bronchopulmonary dysplasia defined by the NIH consensus definition,[15] and mortality.

2.4. Statistical analysis

Data were statistically analyzed using the Statistical Package for the Social Sciences (SPSS version 21.0, SPSS Inc., Chicago, IL, USA). Risk factors causing low serum cortisol concentration were analyzed using chi-square test, Fisher's exact test, two-sample t-test, and Mann–Whitney U-test. The correlation between serum cortisol concentration and elapsed time from the last betamethasone dose was determined using a linear regression model. Neonatal mortality and morbidity were analyzed using a logistic regression. A P-value of <0.05 was considered statistically significant.

3. Results

A total of 80 infants were included in the study, with the mean gestational age and birth weight of 29.2 ± 3.6 weeks and 1009.0 ± 283.7 g, respectively. The median basal serum cortisol concentration was 167 nmol/L with an interquartile range of 98–298 nmol/L (Fig. 1). One outlier (4276 nmol/L) was excluded from the data analysis thereafter. The cortisol concentration did not differ between patients with (331 ± 662 nmol/L) and without (221 ± 160 nmol/L) ventilator therapy ($P = 0.958$).

Based on the median serum concentration, patients were categorized into low (<167 nmol/L) or high (≥167 nmol/L) basal cortisol groups. Low serum cortisol concentration was associated with male sex, use of antenatal steroids and acidemia (pH < 7.2), low lactic acid level, and low leukocyte count on initial blood test. Gestational age did not show any significant association with cortisol concentration. Among the significant variables

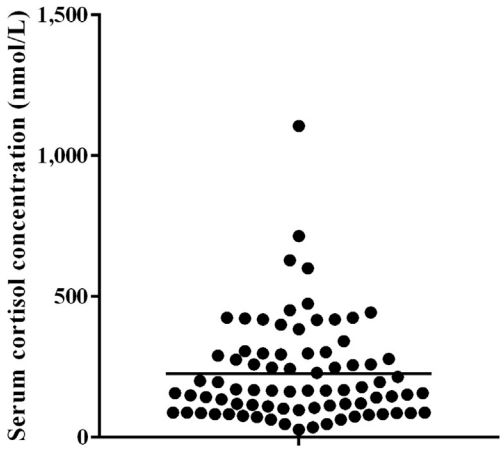

Figure 1 Distribution of basal serum cortisol concentration that were obtained immediately after birth in very low birth weight infants. One outlier (4276 nmol/L) was excluded from this figure.

in univariate analysis, use of antenatal steroids, lactic acid level, and leukocyte count were associated with basal serum cortisol concentration (Table 1). Among the 24 patients who were administered a complete course of betamethasone, the basal serum cortisol concentration was positively correlated with elapsed time from the last betamethasone dose ($R^2 = 0.37$, $P = 0.002$) (Fig. 2). This correlation was not significant in the 22 infants born to mothers with a single dose of betamethasone ($R^2 = 0.00$, $P = 0.937$).

Neonatal outcomes were compared between the group with low serum baseline cortisol levels and those with high serum baseline cortisol levels. In the univariate analyses, low serum cortisol concentration was associated with the incidence of hypoglycemia. However, after adjusting for gestational age, birth weight, use of antenatal steroids, lactic acid level, and leukocyte count, there were no significant association between the basal cortisol levels and the neonatal outcomes (Table 2).

4. Discussion

To the best of our knowledge, this is the first study to investigate the serum cortisol concentration immediately after birth in VLBW infants. Our study revealed that the first day's postnatal serum cortisol level was influenced both by maternal corticosteroid therapy and perinatal distress. In addition, our study indicates that the early postnatal cortisol level cannot be a predictor for neonatal outcomes in preterm infants.

The median value of serum cortisol concentration of our study seems to be lowest amongst previous studies.[16–19] These studies investigated the reference ranges of serum cortisol concentration in preterm infants with various postnatal ages and clinical settings. Heckmann et al. provided a reference range of 73–562 nmol/L from random samples that were obtained during the first 14 days.[16] Ng et al. measured the basal and the peak cortisol levels on day 7 and day 14 in response to corticotropin-releasing hormone (CRH) stimulation in the VLBW infants.[17] The

Table 1 Perinatal characteristics and serum basal cortisol concentration in very low birth weight infants.

Number (%) or Mean ± SD	Serum cortisol concentration < 167 nmol/L (n = 40)	Serum cortisol concentration ≥ 167 nmol/L (n = 40)	*P*-value[a]	Odds ratio[b] (95% CI)	*P*-value[b]
Infants					
Gestational age (weeks)	29.4 ± 3.2	29.1 ± 3.9	0.314	0.95 (0.77–1.16)	0.616
Birth weight (g)	1044.5 ± 287.1	973.6 ± 279.4	0.999	1.00 (1.00–1.00)	0.194
Male sex	25 (62.5)	16 (40.0)	0.044	2.04 (0.74–5.65)	0.169
SGA	14 (35.0)	14 (35.0)	1.000	1.36 (0.23–8.06)	0.736
Intrapartum					
Cesarean section	32 (80.0)	31 (77.5)	0.785	0.89 (0.27–2.97)	0.846
Antenatal steroids (any)	39 (97.5)	32 (80.0)	0.029	14.53 (1.27–165.63)	0.031
Preeclampsia	13 (62.5)	9 (22.5)	0.317	1.93 (0.61–6.06)	0.262
Chorioamnionitis	14 (35.0)	19 (47.5)	0.256	0.70 (0.25–1.94)	0.491
Ruptured membrane	14 (35.0)	18 (45.0)	0.361	0.63 (0.20–2.04)	0.443
Delivery room					
APGAR at 1 min	5.3 ± 1.5	5.0 ± 2.2	0.679	0.99 (0.71–1.37)	0.928
APGAR at 5 min	7.4 ± 1.4	6.8 ± 2.1	0.257	1.12 (0.79–1.61)	0.522
Mechanical ventilator (days)	21 (52.5)	20 (50.0)	0.823	1.26 (0.37–4.29)	0.706
Laboratory values (≤1 h)					
pH < 7.2	8 (21.1)	16 (45.7)	0.023	0.37 (0.12–1.14)	0.084
Base excess (mmol/L)	−4.8 ± 3.1	−7.6 ± 6.4	0.107	0.91 (0.80–1.03)	0.122
Lactic acid (mmol/L)	3.3 ± 1.9	5.7 ± 4.0	0.008	0.78 (0.62–0.98)	0.033
C-reactive protein ≥1 mg/dL	1 (2.5)	3 (7.5)	0.615	0.32 (0.03–3.77)	0.365
Leukocyte count (x10^3/μL)	7.1 ± 4.1	10.5 ± 7.4	0.019	0.88 (0.79–0.98)	0.021

[a] Univariate analysis.
[b] Multivariate analysis adjusted for gestational age, birth weight, sex, lactic acid level, and leukocyte count.

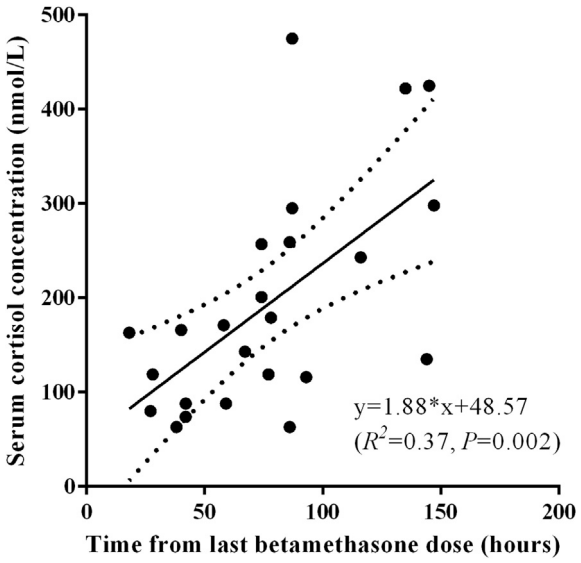

Figure 2 Correlation between serum cortisol concentration and elapsed time from the last betamethasone dose. A positive correlation ($R^2 = 0.37$, $P = 0.002$) was found in 24 patients (30.0% out of 75 population) who were administered a complete course of betamethasone.

mean basal serum cortisol concentration on day 7 and day 14 was 311 nmol/L and 219 nmol/L, respectively. Korte et al. reported that 60% of the VLBW infants had baseline cortisol concentrations on the second morning of life of 138 nmol/L or less.[18] However, in the study by Aucott, median serum cortisol concentrations were 441 nmol/L at 12–48 h.[19] The reason for the variation of reference ranges of the serum cortisol levels across the studies might be due to the difference in the patient population, stress levels and, most importantly, the time of blood sampling. For example, the basal and the post-stimulation serum cortisol levels, which were measured at postnatal day 7 and day 14, differed depending on the severity of respiratory support.[10] On the other hand, the basal cortisol levels measured immediately after birth did not differ between the patients with ventilator therapy and those without ventilator therapy.

In this study, low basal serum cortisol concentration was significantly associated with the use of antenatal steroids and well correlated with elapsed time from the last betamethasone dose of the mothers with a complete course of betamethasone. This association is generally consistent with the findings of a study by Ng et al., in which the basal and post-stimulation serum cortisol levels correlated with the time interval between the last dose of antenatal dexamethasone.[10] Interestingly, the association was found only in the group of infants born to mothers who received more than two doses of dexamethasone. Considering our results and others,[20,21] functional changes in the HPA axis in the fetus might be influenced by the cumulative doses of antenatal corticosteroid. Although antenatal steroids may provide some benefits in terms of lung maturation and subsequent clinical benefit after preterm birth,[22,23] the optimal dose of antenatal steroids regarding the biological effects on HPA axis needs to be further investigated.

In the blood samples obtained within 1 h after birth, low plasma lactic acid levels were associated with low basal serum cortisol level. This might reflect better adaptive cardiorespiratory response to perinatal stressful events in the preterm infants born to mothers treated with antenatal steroids. A trend toward the lower incidence of

Table 2 The basal serum cortisol concentration and neonatal outcomes in very low birth weight infants.

Number (%)	Serum cortisol concentration < 167 nmol/L (n = 40)	Serum cortisol concentration ≥ 167 nmol/L (n = 40)	*P*-value[a]	Odds ratio[b](95% CI)	*P*-value[b]
Body temperature at admission <36.0 °C	3 (7.5)	6 (15.0)	0.481	0.99 (0.14–6.80)	0.990
Hypoglycemia (BST <40 mg/dL)	2 (5.0)	12 (30.0)	0.003	0.18 (0.03–1.13)	0.067
Hypotension within 1 week	7 (17.5)	6 (15.0)	0.762	7.50 (0.78–72.51)	0.082
Hypotension during hospitalization	13 (32.5)	12 (30.0)	0.809	4.14 (0.76–22.60)	0.101
Late onset sepsis (>1 week)	9 (22.5)	13 (32.5)	0.317	0.41 (0.10–1.66)	0.209
RDS	22 (55.0)	26 (65.0)	0.361	1.26 (0.32–4.99)	0.739
NEC ≥ stage II	6 (15.0)	3 (7.5)	0.481	26.02 (0.66–1 × 10^3)	0.083
Severe IVH (≥grade III)	4 (10.0)	4 (10.0)	1.000	0.95 (0.11–8.43)	0.964
ROP requiring treatment	5 (13.9)	8 (20.0)	0.480	0.94 (0.07–12.17)	0.965
Moderate to severe BPD	11 (30.6)	18 (45.0)	0.196	0.99 (0.24–4.13)	0.992
Mortality	4 (10.0)	0 (0.0)	0.116	2 × 10^8 (0.00–∞)	0.997

Abbreviations: BST, blood sugar test; RDS, respiratory distress syndrome; NEC, necrotizing enterocolitis; IVH, intraventricular hemorrhage; ROP, retinopathy of prematurity; BPD, bronchopulmonary dysplasia.

[a] Univariate analysis.

[b] Multivariate analysis adjusted for gestational age, birth weight, sex, any use of antenatal steroids, lactic acid level, and leukocyte count.

hypoglycemia in the lower cortisol group also supports our speculation. Exposure to uncontrollable stress during pregnancy resulted in elevated maternal and fetal plasma cortisol levels in rat.[24] Experimental data suggest that antenatal steroid therapy augments postnatal neuroendocrine adaptation in response to hypoxic stimuli despite depression of plasma cortisol level.[25,26] The impact of antenatal steroid on the fetal plasma lactate level has not been studied in human. In pregnant ewes, dexamethasone injection acutely increased fetal plasma lactate level.[23] However, this increase dissipates beyond 24 h after injection. Accordingly, the low plasma lactate level at birth is less likely due to antenatal corticosteroid therapy in the present study.

In the present study, the basal serum cortisol concentration was also not associated with mortality and major morbidities including severe grade IVH. An association of upper quartile value of serum cortisol measured at 12—48 h with higher incidence of severe grade IVH was found in extremely low birth weight and VLBW infants.[18,19] As antenatal steroid reduces the risk of severe grade IVH,[27] the upper quartile value of serum cortisol is presumably associated with incomplete or lacking antenatal steroid treatment although data on the antenatal corticosteroid treatment were not listed in those studies.

In our study, hypotension within a week after birth or during hospitalization was not associated with the serum cortisol concentration immediately after birth. Similar to our study, serum cortisol concentration earlier than day 7 did not predict transient adrenal insufficiency.[19] Basal, peak or rise of serum cortisol level on day 7 of life is known to be the most relevant factor for catecholamine-resistant hypotension episode in VLBW infants, which is so-called transient adrenal insufficiency in premature infants.[17] Transient adrenal insufficiency is the consequence of hypocortisolemia and the response to an acute illness as well.[28] In order to confirm transient adrenal insufficiency, the CRH stimulation test is indispensable in addition to basal serum cortisol level.[29] Unfortunately, including our study, there is no study on the CRH stimulation test performed on the first day after birth.

There are several limitations to our study. First, the basal serum cortisol data were obtained at a single point, with a decision-making indicator to start hydrocortisone in cases of early pressor-resistant hypotension in VLBW infants. No local protocol was available regarding the serial follow-up of serum cortisol levels. However, the baseline serum cortisol data can be useful indicators that are reflective of maternal and perinatal condition without being contaminated by the severities of early neonatal illnesses. Second, the normal diurnal variation of cortisol levels was not considered. However, there is evidence that the normal diurnal variation of cortisol levels was not observed in preterm infants.[30,31] Third, the study population had a wide range of gestational age and varied level of respiratory support status. However, those parameters were not associated with the baseline cortisol level in the present study. Lastly, some cortisol samples were obtained by a direct puncture, which might provoke stress response to painful stimuli in preterm infants. To minimize this, a baseline salivary cortisol level could be an alternative for further research.[32]

In conclusion, basal serum cortisol concentrations, measured immediately after birth, might be influenced by the coverage of antenatal corticosteroid therapy and perinatal stressful events in VLBW infants. Early basal cortisol level was not a reliable indicator that was predictive of adverse neonatal outcomes.

Conflict of interest

The authors declare no conflict of interest.

References

1. Mesiano S, Jaffe RB. Developmental and functional biology of the primate fetal adrenal cortex. *Endocr Rev* 1997;**18**:378—403.
2. Ng PC. The fetal and neonatal hypothalamic-pituitary-adrenal axis. *Arch Dis Child Fetal Neonatal Ed* 2000;**82**:F250—4.
3. Liggins GC. The role of cortisol in preparing the fetus for birth. *Reprod Fertil Dev* 1994;**6**:141—50.
4. Lee MM, Rajagopalan L, Berg GJ, Moshang Jr T. Serum adrenal steroid concentrations in premature infants. *J Clin Endocrinol Metab* 1989;**69**:1133—6.
5. Hingre RV, Gross SJ, Hingre KS, Mayes DM, Richman RA. Adrenal steroidogenesis in very low birth weight preterm infants. *J Clin Endocrinol Metab* 1994;**78**:266—70.
6. Scott SM, Watterberg KL. Effect of gestational age, postnatal age, and illness on plasma cortisol concentrations in premature infants. *Pediatr Res* 1995;**37**:112—6.
7. Ng PC, Wong GW, Lam CW, Lee CH, Wong MY, Fok TF, et al. Pituitary-adrenal response in preterm very low birth weight infants after treatment with antenatal corticosteroids. *J Clin Endocrinol Metab* 1997;**82**:3548—52.
8. Xiong F, Zhang L. Role of the hypothalamic-pituitary-adrenal axis in developmental programming of health and disease. *Front Neuroendocrinol* 2013;**34**:27—46.
9. Ng PC, Wong GW, Lam CW, Lee CH, Fok TF, Wong MY, et al. Effect of multiple courses of antenatal corticosteroids on pituitary-adrenal function in preterm infants. *Arch Dis Child Fetal Neonatal Ed* 1999;**80**:F213—6.
10. Ng PC, Lam CW, Lee CH, Ma KC, Fok TF, Chan IH, et al. Reference ranges and factors affecting the human corticotropin-releasing hormone test in preterm, very low birth weight infants. *J Clin Endocrinol Metab* 2002;**87**:4621—8.
11. Committee on Obstetric Practice. Committee Opinion No. 713: Antenatal corticosteroid therapy for fetal maturation. *Obstet Gynecol* 2017;**130**:e102—9.
12. Fenton TR, Kim JH. A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants. *BMC Pediatr* 2013;**13**:59.
13. Walsh MC, Kliegman RM. Necrotizing enterocolitis: treatment based on staging criteria. *Pediatr Clin North Am* 1986;**33**:179—201.
14. Papile LA, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. *J Pediatr* 1978;**92**:529—34.
15. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 2001;**163**:1723—9.
16. Heckmann M, Wudy SA, Haack D, Pohlandt F. Reference range for serum cortisol in well preterm infants. *Arch Dis Child Fetal Neonatal Ed* 1999;**81**:F171—4.
17. Ng PC, Lee CH, Lam CW, Ma KC, Fok TF, Chan IH, et al. Transient adrenocortical insufficiency of prematurity and systemic

hypotension in very low birthweight infants. *Arch Dis Child Fetal Neonatal Ed* 2004;**89**:F119—26.
18. Korte C, Styne D, Merritt TA, Mayes D, Wertz A, Helbock HJ. Adrenocortical function in the very low birth weight infant: improved testing sensitivity and association with neonatal outcome. *J Pediatr* 1996;**128**:257—63.
19. Aucott SW, Watterberg KL, Shaffer ML, Donohue PK, PROPHET Study Group. Do cortisol concentrations predict short-term outcomes in extremely low birth weight infants? *Pediatrics* 2008;**122**:775—81.
20. Jobe AH, Soll RF. Choice and dose of corticosteroid for antenatal treatments. *Am J Obstet Gynecol* 2004;**190**:878—81.
21. Antonow-Schlorke I, Helgert A, Gey C, Coksaygan T, Schubert H, Nathanielsz PW, et al. Adverse effects of antenatal glucocorticoids on cerebral myelination in sheep. *Obstet Gynecol* 2009;**113**:142—51.
22. Elimian A, Figueroa R, Spitzer AR, Ogburn PL, Wiencek V, Quirk JG. Antenatal corticosteroids: are incomplete courses beneficial? *Obstet Gynecol* 2003;**102**:352—5.
23. Quaedackers JS, Roelfsema V, Fraser M, Gunn AJ, Bennet L. Cardiovascular and endocrine effects of a single course of maternal dexamethasone treatment in preterm fetal sheep. *BJOG* 2005;**112**:182—91.
24. Takahashi LK, Turner JG, Kalin NH. Prolonged stress-induced elevation in plasma corticosterone during pregnancy in the rat: implications for prenatal stress studies. *Psychoneuroendocrinology* 1998;**23**:571—81.
25. Ervin MG, Padbury JF, Polk DH, Ikegami M, Berry LM, Jobe AH. Antenatal glucocorticoids alter premature newborn lamb neuroendocrine and endocrine responses to hypoxia. *Am J Physiol Regul Integr Comp Physiol* 2000;**279**:R830—8.
26. Kauffman KS, Seidler FJ, Slotkin TA. Prenatal dexamethasone exposure causes loss of neonatal hypoxia tolerance: cellular mechanisms. *Pediatr Res* 1994;**35**:515—22.
27. Ballabh P. Pathogenesis and prevention of intraventricular hemorrhage. *Clin Perinatol* 2014;**41**:47—67.
28. Helbock HJ, Insoft RM, Conte FA. Glucocorticoid-responsive hypotension in extremely low birth weight newborns. *Pediatrics* 1993;**92**:715—7.
29. Iwanaga K, Yamamoto A, Matsukura T, Niwa F, Kawai M. Corticotrophin-releasing hormone stimulation tests for the infants with relative adrenal insufficiency. *Clin Endocrinol (Oxf)* 2017;**87**:660—4.
30. Neu M, Goldstein M, Gao D, Laudenslager ML. Salivary cortisol in preterm infants: validation of a simple method for collecting saliva for cortisol determination. *Early Hum Dev* 2007;**83**:47—54.
31. Al Saedi S, Dean H, Dent W, Cronin C. Reference ranges for serum cortisol and 17-hydroxyprogesterone levels in preterm infants. *J Pediatr* 1995;**126**:985—7.
32. Davis EP, Townsend EL, Gunnar MR, Georgieff MK, Guiang SF, Ciffuentes RF, et al. Effects of prenatal betamethasone exposure on regulation of stress physiology in healthy premature infants. *Psychoneuroendocrinology* 2004;**29**:1028—36.