Sex-related differences in the associations between maternal dioxin-like compounds and reproductive and steroid hormones in cord blood: the Hokkaido Study

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Abbreviations: AA/GG, adrenal androgen/glucocorticoid (sum of dehydroepiandrosterone and androstenedione)/(sum of cortisol and cortisone); AHR, aromatic hydrocarbon receptor; B, regression coefficient; CI, confidence interval; DHEA, dehydroepiandrosterone; LOD, limit of detection; DLC, dioxin-like compound; E2, estradiol; ER, estrogen receptor; FSH, follicle-stimulating hormone; HRGC/HRMS, chromatography/high-resolution mass spectrometry; INSL3, insulin-like factor 3; LC-MS/MS, Liquid chromatography-tandem mass spectrometry; LH, luteinizing hormone; LOQ, limit of quantification; PCB, polychlorinated biphenyl; PCDD, polychlorinated dibenzo-*p*-dioxins; PCDF, polychlorinated dibenzofuran; SHBG, sex hormone-binding globulin; T, testosterone; TEQ, toxic equivalent.

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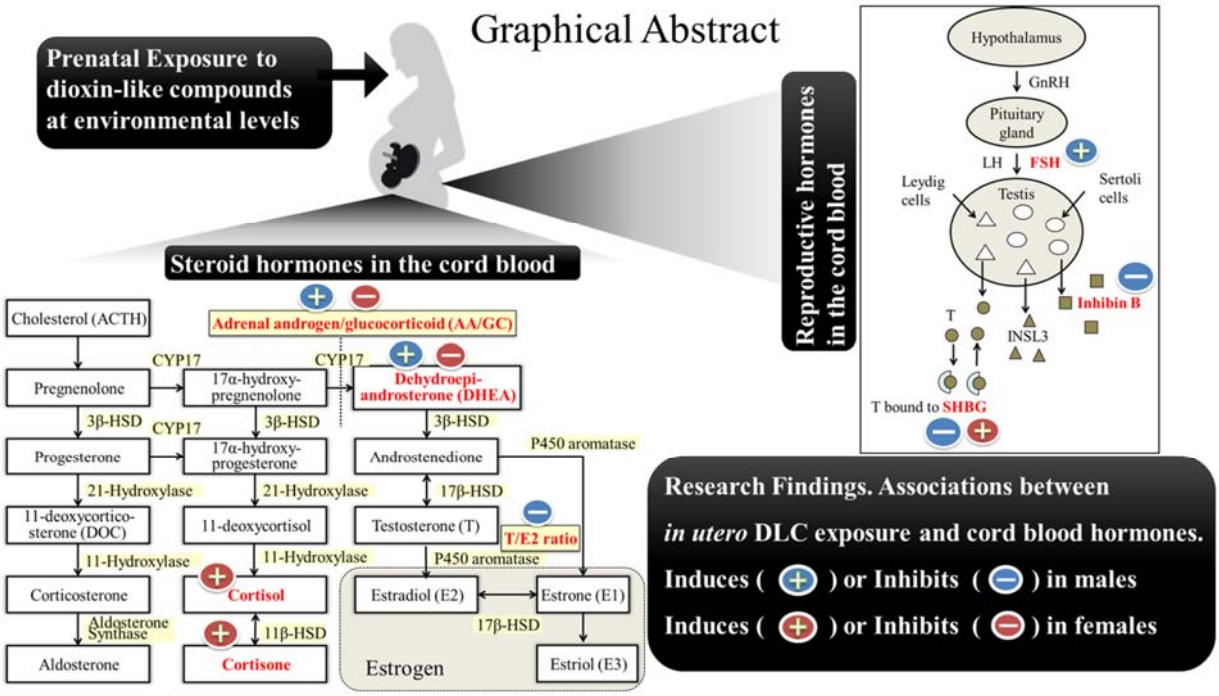
Running title: Maternal dioxins and cord blood hormones


Graphical Abstract
Prenatal Exposure to dioxin-like compounds at environmental levels
Steroid hormones in the cord blood
Cholesterol (ACTH)
Adrenal androgen/glucocorticoid (AA/GC)
Pregnenolone
17α-hydroxy-pregnenolone
Dehydroepi-androsterone (DHEA)
Progesterone
17α-hydroxy-progesterone
Androstenedione
11-deoxycortico-sterone (DOC)
11-deoxycortisol
Testosterone (T)
T/E2 ratio
Corticosterone
Cortisol
Estradiol (E2)
Estrone (E1)
Aldosterone
Cortisone
Estriol (E3)
Estrogen
CYP17
3β-HSD
21-Hydroxylase
11-Hydroxylase
17β-HSD
P450 aromatase
Aldosterone Synthase
11β-HSD
Reproductive hormones in the cord blood
Hypothalamus
GnRH
Pituitary gland
LH
FSH
Leydig cells
Testis
Sertoli cells
Inhibin B
T
INSL3
T bound to SHBG
Research Findings. Associations between
in utero DLC exposure and cord blood hormones.
Induces (⊕) or Inhibits (⊖) in males
Induces (⊕) or Inhibits (⊖) in females

Abstract

Background: Prenatal exposure to dioxin-like compounds (DLCs) irreversibly affect fetal reproductive and steroid hormone synthesis. **Objective**: This study aimed to assess the relationships between maternal DLCs and cord blood reproductive and steroid hormones. **Methods**: Participants in this study were pregnant women who enrolled in the Sapporo Cohort of the Hokkaido Study between 2002 and 2005. We quantified 29 DLCs during the 2nd and 3rd trimesters in maternal blood. Additionally, we measured the concentrations of progesterone, estradiol (E2), testosterone (T), androstenedione, dehydroepiandrosterone (DHEA), cortisol, cortisone, sex hormone-binding globulin (SHBG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, inhibin B, and insulin-like factor-3 (INSL3) in cord blood samples. **Results**: Data from 183 mother-child pairs were analyzed. We observed sex-dependent associations of DLCs on T/E2 ratios, DHEA, cortisol, cortisone, adrenal androgen/glucocorticoid (AA/GC: sum of DHEA and androstenedione)/(sum of cortisol and cortisone) ratios and SHBG. An increase in maternal DLCs related to decreased T/E2 ratios and SHBG and inhibin B levels, and increased AA/GC ratios and FSH and DHEA levels in male cord blood samples. However, an increase in maternal mono-ortho polychlorinated biphenyls related to increased cortisol, cortisone, and SHBG levels, and decreased DHEA levels and AA/GC ratios in female cord blood samples. **Conclusions**: Prenatal exposure to DLCs alters steroidogenesis and suppresses the secretion of inhibin B in male cord blood. Relationships between maternal DLCs and cord blood hormones differ between boys and girls. Further studies are required to clarify whether the effects of *in utero* exposure to DLCs on adrenal hormones extend into infancy and puberty.

Keywords: Dioxin-like compounds, reproductive hormones, cord blood, prenatal exposure.

1. Introduction

Human exposure to persistent organic pollutants, including polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs), is widespread from environmental sources and daily food intake (Todaka et al., 2008). Seventeen PCDDs/PCDFs and 12 PCBs have been categorized as dioxin-like compounds (DLCs) (Van den Berg et al., 2006). Aromatic hydrocarbon receptors (AHRs) regulate xenobiotic metabolism, cell proliferation, and cell cycle control in fetal tissues. DLCs bind to AHRs and disrupt normal fetal development (Van den Berg et al., 2006). Exposure to DLCs *in utero* inhibits fetal reproductive development and steroid hormone synthesis (Cao et al., 2008; Hsu et al., 2005). Consequently, the immature endocrine system results in dyshomeostatic endocrine and reproductive development during adolescents (WHO. 2012). Sex-related differences in the susceptibility to DLC toxicity on birth size, immunity, and neurobehavioral disorders are reported (Kishi et al., 2017). Therefore, it is worthwhile to identify the underlying mechanisms.

In 1979, several pregnant women were accidently exposed to high levels of DLCs after consuming DLC-contaminated rice oil; this accident was called the Yucheng accident. Increased free testosterone (fT) levels and elevated fT/estradiol (E2) ratios in the serum of boys (aged 8–12 years), born to these women, were observed compared to those in age-matched boys born to women who were not exposed to DLC during their pregnancy (Hsu et al., 2005). In a birth cohort from Duisburg (Germany), fT and E2 concentrations in the cord blood inversely correlated with maternal PCDD/PCDF concentrations. The decrease in fT levels was more pronounced among females, whereas that of E2 levels was more pronounced among males (Cao et al., 2008). These studies suggest that *in utero* exposure to

DLCs may be associated with the sex-dependent adverse effects of DLCs on reproductive and steroid hormone levels.

The fetal adrenal gland produces adrenal androgenic steroids, including dehydroepiandrosterone (DHEA) and sulfoconjugated-DHEA, which are cortisol antagonists and major precursors for placental estrogen production during pregnancy (Kuijper et al., 2013). Therefore, its development and functioning are critical for fetal maturation. An inverse relationship between maternal PCDD/PCDFs and DHEA levels in their offspring was observed among a specific Vietnamese population residing in a PCDD/PCDF-contaminated area (Kido et al., 2016). A cross-sectional study has revealed that Chinese children living in an electronic waste dismantling area had higher levels of PCDDs/PCDFs and cortisol in their blood than control children (Xu et al., 2014). These studies have reported inconsistent and limited associations between exposure to high levels of DLCs and androgenic and glucocorticoid hormones in children (Kido et al., 2016; Xu et al., 2014).

However, human studies focusing on the possible effects of *in utero* exposure to DLCs on reproductive and steroid hormones at birth are scarce. Therefore, this study aimed to evaluate the sex-related differences in the associations of *in utero* DLC exposure with reproductive and steroid hormones in the cord blood.

2. Materials and Methods

2.1. Study population and data collection

Participants in this prospective study were pregnant women who were enrolled in the Sapporo Cohort of the Hokkaido Study on Environment and Children's health (Kishi et al., 2017). Protocol details regarding the participant demographics, data collection, sampling of biological specimens, and preparation of questionnaire items have been previously described

(Kishi et al., 2017). They were 23–35 weeks pregnant native Japanese women living in and around Sapporo, who gave informed consent to participate in the study between July 2002 and October 2005, at an obstetrics and gynecology hospital in Sapporo, Hokkaido, Japan. Among 1,347 pregnant women, 514 were enrolled in this study (participation rate, 38.2%). The participants completed a basic questionnaire regarding maternal age at delivery, smoking behavior, alcohol consumption, educational background, annual household income, and medical history. Additional information on maternal height, pre-pregnancy weight, maternal complications during pregnancy and delivery, gestational duration, infant sex, parity, and infant birth size of 504 mother-children pairs was collected from medical records. The participant recruitment flowchart in shown in Fig. 1.

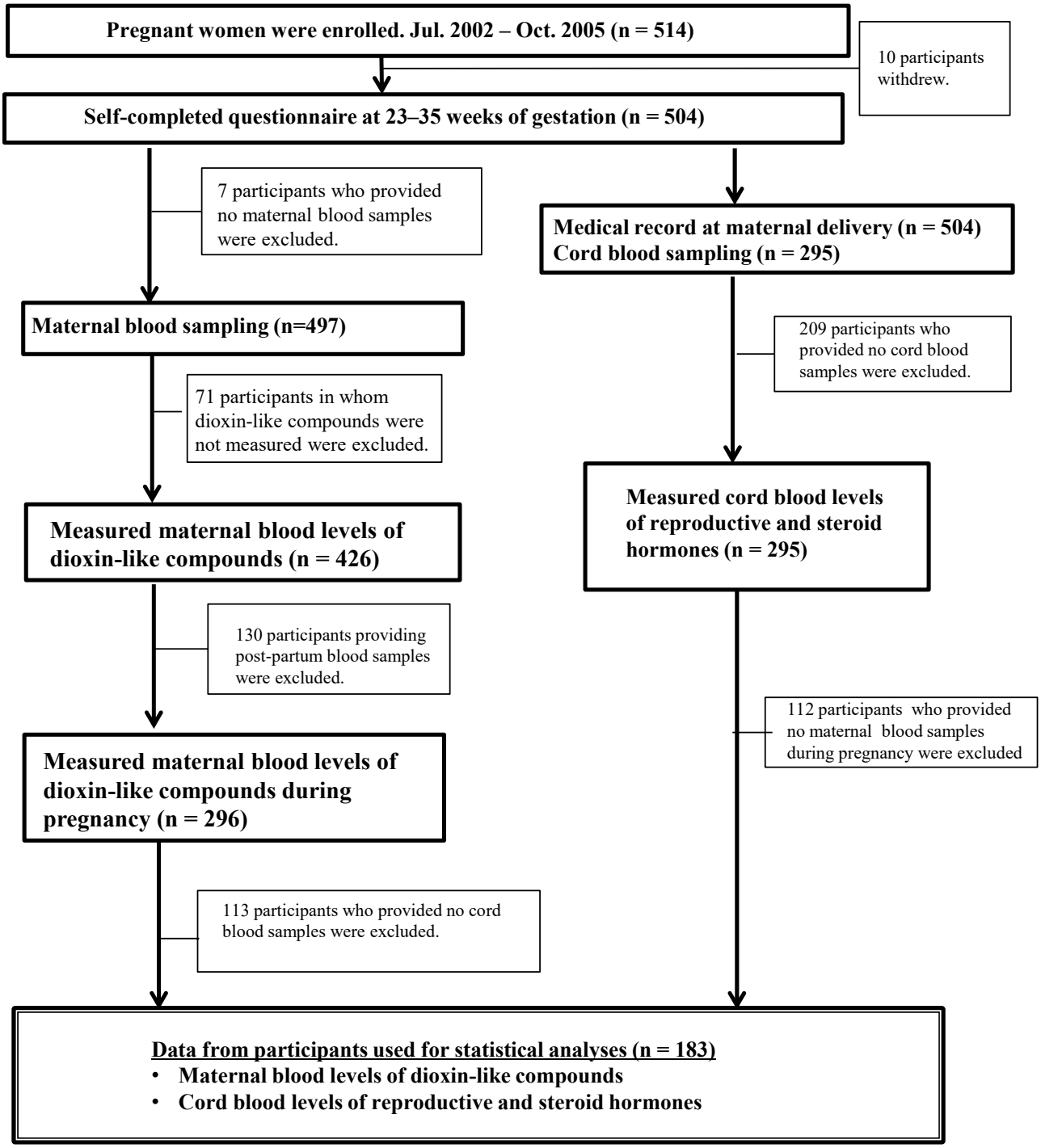


Fig. 1 Participant recruitment flowchart

2.2. Assessment of exposure

Collection and storage of blood samples and measurement of DLCs were performed as previously described (Todaka et al., 2008). Briefly, peripheral blood samples were obtained from 497 participants during the second or third trimester of pregnancy, or immediately following delivery. DLC concentrations were measured in 426 maternal blood samples; the remaining 71 samples were not analyzed because of an inadequate sample volume, which were collected from pregnant women with poor physical conditions. Of the 426 samples, 296 samples were collected during the second or third trimester of pregnancy when the participants underwent routine check-ups at the hospital, whereas the remaining 130 samples were collected from hospitalized participants within a week after delivery because blood sampling during pregnancy was not possible due to their poor health conditions.

DLC concentrations in maternal whole blood samples were detected using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) equipped with a solvent-cut large-volume injection system at the Fukuoka Institute of Health and Environmental Sciences (Iidai and Todaka, 2003; Todaka et al., 2003). Due to their lipid nature, detected DLC levels were divided by the total lipid content (pg/g lipids). We determined the concentrations of 29 individual DLC congeners (categorized into 4 groups of DLCs, including 7 PCDDs, 10 PCDFs, 4 non-ortho PCBs, and 8 mono-ortho PCBs) as shown in Table 2. When DLC blood levels were below limits of detection (LODs), we considered them to be half of the individual LODs. Furthermore, to evaluate the biological effects of individual DLC congeners, toxic equivalent (TEQ) values were calculated by multiplying the concentration of individual DLC congeners by the corresponding toxic equivalency factors (Van den Berg et al., 2006). Among the 426 blood samples analyzed for

29 DLC congeners, we excluded 130 maternal blood samples, which were collected after delivery, to evaluate *in utero* DLC exposure.

2.3. Outcome measures

The collection and storage of cord blood samples after delivery and measurement of hormones in these samples were performed as previously described (Goudarzi et al., 2016; Ito et al., 2016). Briefly, concentrations of six reproductive hormones, including luteinizing hormone (LH), follicle-stimulating hormone (FSH), sex hormone-binding globulin (SHBG), prolactin, inhibin B, and insulin-like factor-3 (INSL3), and seven steroid hormones, including E2, total T, progesterone, cortisol, cortisone, DHEA, and androstenedione, were measured at Aska Pharma Medical Co., Ltd. (Kanagawa, Japan). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to measure the concentrations of the seven steroid hormones. An immunoradiometric assay was used to measure the concentrations of four reproductive hormones (LH, FSH, prolactin, and SHBG). An enzyme-linked immunosorbent assay and an enzyme immunoassay were used to measure the concentrations of inhibin B and INSL3, respectively. The limit of quantification (LOQ) values are shown in Table 3. Because LH and inhibin B were detected only in 1 and 24.1% female cord blood samples, respectively, and FSH was undetected, only data from male cord blood samples were statistically analyzed. In addition, INSL3 was measured only in 20 female cord blood samples; therefore, these values were excluded. Finally, data from 295 cord blood samples in both males and females were included for progesterone, E2, T, DHEA, androstenedione, cortisol, cortisone, SHBG, and prolactin, whereas data from 135 male cord blood samples were included for LH, FSH, inhibin B, and INSL3 quantification. When concentrations were below LOQs, we considered them to be half of the individual LOQs.

2.4. Statistical analyses

As shown in Fig. 1, data for 183 mother-child pairs were included in the statistical analysis. For a preliminary data analysis, relationships between continuous variables (concentrations of maternal DLCs and cord blood hormones) and continuous variables (maternal age, pre-pregnancy BMI, gestation week at which blood was collected during pregnancy, gestational age, and infant birth weight) were analyzed using Spearman's correlation coefficient. Relationships between the concentrations of maternal DLCs or cord blood hormones and categorical valuables, including maternal parity, smoking and alcohol consumption during pregnancy, educational background, and annual household income, were analyzed using Mann–Whitney U tests (Table S2).

We assessed the relationships between maternal DLC concentrations and infant cord blood hormone levels by multivariate linear regression models, and the significance ($p < 0.05$) of individual regression coefficients was checked. The regression models were adjusted for potential confounding factors, which were previously reported in human studies (Cao et al., 2008; Kido et al., 2016; Miyashita et al., 2015; Mocarelli et al., 2011; Warembourg et al., 2016). Potentially confounding factors included maternal age at delivery (continuous), parity (≥1 or 0), smoking behavior during pregnancy (yes or no), alcohol consumption during pregnancy (yes or no), annual household income (< or ≥ 5 million yen), and gestation week at which blood was sampled during pregnancy (continuous). The distributions of DLC concentrations and outcome variables of cord blood hormone levels were right-skewed; therefore, they were $\log_e$ transformed to approximate normal distributions and used in multivariate linear regression analyses. To identify sex-related differences in the relationships between DLC levels and the concentrations of hormones, we conducted multivariate linear

regression after adding sex of the cord blood sample as an interaction term along with other predefined predictor variables. When the interactions between sex and DLC concentrations were statistically significant, we conducted multivariate linear regression analyses stratified by sex.

Further, when significance ($p < 0.05$) was established in the sex-stratified multivariate linear regressions, we assessed the dose-response relationship between DLCs and cord blood hormones after adjusting for maternal age, parity, smoking and alcohol consumption during pregnancy, blood sampling week during pregnancy, and annual household income. DLC levels were divided into four quartiles; Table 2 shows the median concentrations of DLCs and the interquartile range (IQR) as TEQ (pg/g lipids) in male and female cord blood samples. The marginal means and 95% confidence intervals (CIs) of log-transformed cord blood hormone levels were calculated after adjusting for maternal age, parity, smoking, and alcohol consumption during pregnancy, blood sampling week during pregnancy, and annual household income, and back-transformed to absolute values. To evaluate the dose-response relationship, p-values for the trend were calculated using four categorical quartiles of DLC concentrations, which were analyzed as ordinal variables, and linear contrast coefficients of – 3, − 1, + 1, and + 3 were assigned to the 1st, 2nd, 3rd, and 4th quartiles, respectively. To evaluate the change in the concentrations of cord blood hormones for each increase in the quartile DLC levels, the 1st quartile was compared to the 2nd, 3rd, and 4th quartiles, and the p-values were adjusted using Bonferroni's correction ($p < 0.0166$) (Araki et al., 2014, 2017). When the interaction terms between sex and DLCs were statistically significant ($p < 0.05$), congener-specific analyses stratified by sex were performed only for selected congeners that were detected in > 80% of the samples. This eliminated the possibility of underestimating or overestimating the toxicity of DLC

congeners (Table S1). All statistical analyses were performed with the JMP Clinical 5.0 software (SAS Institute Inc., NC, USA).

2.5. Ethics approval

The study was approved by the institutional ethical board for epidemiological studies at Hokkaido University Graduate School of Medicine, Hokkaido University Center for Environmental and Health Sciences, in accordance with the Declaration of Helsinki. Informed consent was obtained from all study participants before enrollment in this study.

3. Results

The demographics of participants included in the present study (n = 183) are shown in Table 1.

Table 1. Maternal and infant demographics (n = 183)

Characteristic	Mean ± SD or Median (IQR)	n (%)
Maternal characteristics:		
Age at delivery (years)	30.3 ± 4.7	
Pre-pregnancy BMI (m^2/kg)	21.2 ± 3.1	
Blood sampling week during pregnancy	33.1 (31.3, 37.1)	
Parity		
0		100 (54.6)
≥ 1		83 (45.4)
Education level (years)		
≤ 12		78 (42.6)
≥ 12		105 (57.4)
Tobacco smoking during pregnancy		
Nonsmoker		148 (80.9)
Smoker		35 (19.1)
Alcohol consumption during pregnancy		
No		119 (65.0)
Yes		64 (35.0)
Annual household income (million yen)		
< 5		124 (67.8)
≥ 5		59 (32.2)
Infant characteristics:		

Sex		
Male		85 (46.4)
Female		98 (53.6)
Gestational age at birth (weeks)	39.4 ± 1.0	
Birth weight (g)		
Male	3162.8 ± 292.5	
Female	3107.5 ± 355.3	

IQR, interquartile range

Table 2 shows the concentrations of 29 DLCs categorized into four subgroups. We found no significant difference in median maternal DLC levels between male and female infants. However, maternal DLC level was significantly associated with maternal age, parity, and annual household income (Table S2).

Table 3 shows the distribution and percentage above LOQ of reproductive and steroid hormones for all children as well as for male and female cord blood samples separately. We found significant differences in the levels of T and DHEA as well as the T/E2 ratio between male and female cord blood samples. Progesterone, E2, T/E2, cortisol, cortisone, LH, and FSH levels were significantly associated with maternal age at delivery. Cortisol, cortisone, AA/GC ration, and SHBG were significantly associated with parity. T/E2 ratio and SHBG level were significantly associated with tobacco smoking. Cortisol level and AA/GC ratio were significantly associated with alcohol consumption during pregnancy. T/E2 ratio was significantly associated with annual household income. T, cortisol, SHBG, and inhibin B levels were significantly associated with gestational age (Table S2).

Table 2. Concentrations of DLCs in maternal blood (TEQ pg/g lipid).

	All (n = 183)	Male (n = 85)	Female (n = 98)	p-value[a]
	Median (IQR)			
Sub-total PCDDs	7.05 (5.17, 9.34)	7.24 (5.32, 9.44)	6.95 (4.86, 9.36)	0.424
Sub-total PCDFs	2.56 (1.96, 3.18)	2.56 (1.98, 3.25)	2.56 (1.80, 3.04)	0.208
Sub-total non-*ortho* PCBs	4.23 (2.81, 6.18)	4.35 (3.26, 5.81)	3.92 (2.60, 6.36)	0.428
Sub-total mono-*ortho* PCBs	0.35 (0.24, 0.49)	0.37 (0.24, 0.46)	0.33 (0.23, 0.52)	0.464
Total DLCs	14.5 (10.4, 18.6)	14.7 (11.1, 18.8)	14.3 (10.0, 18.2)	0.457

TEQs were calculated using toxic equivalency factor values (Van den Berg et al. 2006). IQR, interquartile range; DLCs, dioxin-like compounds; TEQ, toxic equivalent; PCDD, polychlorinated dibenzo-p-dioxins; PCDF, polychlorinated-dibenzofuran; PCB, polychlorinated biphenyl.

[a]p-values were calculated using the Mann-Whitney U test.

Table 3. Detection proportion and distribution of reproductive and steroid hormones in cord blood samples from male and female infants.

			All (n = 183)	Male (n = 85)	Female (n = 98)	
Reproductive hormone	LOQ	＞LOQ (%)	Median (IQR)	Median (IQR)	Median (IQR)	p-value[b]
Progesterone (ng/mL)	0.01	100	223 (182, 276)	234 (186, 289)	217 (170, 269)	0.166
E2 (ng/mL)	0.01	100	5.03 (3.57, 7.28)	5.02 (3.57, 7.51)	5.07 (3.48, 7.02)	0.587
T (pg/mL)	0.01	100	84.2 (59.8, 116)	96.7 (77.3, 124)	69.8 (52.5, 98.8)	＜0.001
T/E2			16.5 (12.4, 22.4)	18.2 (13.5, 23.9)	15.0 (11.8, 21.2)	0.027
Androstenedione (ng/mL)	0.01	100	0.46 (0.36, 0.58)	0.47 (0.38, 0.57)	0.45 (0.35, 0.59)	0.689
DHEA (ng/mL)	0.01	100	2.28 (1.80, 3.14)	2.08 (1.60, 2.73)	2.55 (1.99, 3.41)	0.001
Cortisol (ng/mL)	0.25	96.7	41.2 (23.0, 67.1)	38.8 (21.3, 66.2)	45.2 (25.4, 68.0)	0.187
Cortisone (ng/mL)	0.1	94.0	96.8 (69.9, 125)	96.8 (71.3, 123)	98.3 (69.5, 126)	0.912
Adrenal androgen/glucocorticoid ratio[a]			0.02 (0.01, 0.03)	0.02 (0.01, 0.03)	0.02 (0.01, 0.03)	0.278
SHBG (nmol/L)	1.1	99.5	15.6 (13.5, 18.6)	15.9 (13.8, 19.1)	15.4 (13.1, 18.2)	0.257
Prolactin (ng/mL)	1	98.8	85.9 (61.5, 116)	81.6 (63.2, 116)	88.6 (58.3, 120)	0.634
LH (mIU/mL)	0.5	38.8		0.25 (0.25, 0.88)		
FSH (mIU/mL)	0.5	49.4		0.25 (0.25, 0.67)		
Inhibin B (pg/mL)	11	100		43.6 (33.6, 58.5)		
INSL3 (ng/mL)	0.1	100		0.28 (0.25, 0.32)		

E2, estradiol; FSH, follicle stimulating hormone; INSL3, insulin like factor 3; LH, luteinizing hormone; SHBG, sex hormone-binding globulin; T, testosterone; LOQ, limit of quantification; DHEA, dehydroepiandrosterone; IQR, interquartile range.

[a]Adrenal androgen/glucocorticoid ratio = (DHEA+ androstenedione)/(cortisol and cortisone)

[b]p-values were calculated using the Mann-Whitney U test.

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Table 4 shows that an increase in the concentration of maternal subtotal non-ortho PCBs was significantly associated with an increase in DHEA (B: 0.27, 95% CI: 0.01, 0.54). Because interaction terms between sex and DLCs were significant for T/E2 ratios, DHEA, cortisol, cortisone, and SHBG levels and AA/GC [(sum of DHEA and androstenedione)/(sum of cortisol and cortisone) ratio], we conducted adjusted linear regression analyses stratified by sex for the above cord blood hormones (Table 5). Additionally, we conducted adjusted linear regression analyses for LH, FSH, inhibin B, and INSL3 in male cord blood samples (Table 6). An increase in the levels of maternal subtotal non-ortho PCBs was significantly associated with a decrease in the T/E2 ratio, and an increase in the levels of maternal subtotal non-ortho and total DLCs was significantly associated with a decrease in the SHBG, whereas an increase in the levels of maternal subtotal PCDDs, PCDFs, non-ortho, mono-ortho PCBs, and total DLCs was significantly associated with a decrease in inhibin B levels (Table 5 and 6). Moreover, an increase in the levels of maternal subtotal PCDDs, non-ortho PCBs, and total DLCs was significantly associated with an increase in DHEA levels, an increase in the levels of maternal subtotal non-ortho PCBs was significantly associated with an increase in AA/GC ratio, and an increase in the levels of maternal subtotal non-ortho PCBs was significantly associated with an increase in FSH in male cord blood samples (Table 5 and 6). However, an increase in the levels of maternal subtotal mono-ortho PCBs was significantly associated with a decrease in DHEA levels as well as a decrease in AA/GC ratios in female cord blood samples (Table 5). In contrast, an increase in the levels of maternal subtotal mono-ortho PCBs was significantly associated with an increase in cortisol, cortisone, and SHBG levels (Table 5).

Based on adjusted marginal means, we found that non-ortho PCBs correlated inversely and significantly with T/E2; non-ortho PCBs and total DLCs correlated positively

and significantly with DHEA; non-ortho PCBs correlated positively and significantly with AA/GC ratios; PCDDs, PCDFs, non-ortho PCBs, and total DLCs correlated inversely and significantly with inhibin B in male cord blood samples (Fig. 2). Based on adjusted marginal means, we found that mono-ortho PCBs correlated positively and significantly with cortisone; mono-ortho PCBs correlated inversely and significantly with AA/GC ratios in female cord blood samples (Fig. 2). After applying the Bonferroni correction ($p < 0.0166$), marginal means and 95% CIs of SHBG significantly decreased in the 4th quartile (marginal means = 23.4, 95% CI = 16.2, 28.2) compared to those of mono-ortho PCBs in the 1st quartile (marginal means = 14.9, 95% CI = 11.4, 19.3) in female cord blood samples (Fig. 2). Table S3 and S4 show results of congener-specific analyses stratified by infant sex.

Table 4. Adjusted linear regression coefficients (B) and 95% confidence intervals (CI) for reproductive and steroid hormone levels in cord blood in relation to total concentrations of dioxin-like compounds (DLCs) in maternal blood.

	Progesterone		E2		T		T/E2		Androstenedione		DHEA	
	B (95% CI)	p for interaction	B (95% CI)	p for interaction	B (95% CI)	p for interaction	B (95% CI)	p for interaction	B (95% CI)	p for interaction	B (95% CI)	p for interaction
Sub-total PCDDs	-0.18 (-0.62, 0.26)	0.471	0.31 (-0.05, 0.68)	0.464	0.15 (-0.28, 0.57)	0.531	-0.17 (-0.51, 0.17)	0.121	0.04 (-0.33, 0.40)	0.759	0.41 (-0.02, 0.83)	0.269
Sub-total PCDFs	-0.27 (-0.74, 0.20)	0.446	0.13 (-0.25, 0.51)	0.683	-0.05 (-0.50, 0.40)	0.134	-0.18 (-0.54, 0.18)	0.154	-0.10 (-0.48, 0.28)	0.215	0.35 (-0.10, 0.80)	0.471
Sub-total Non-ortho PCBs	-0.17 (-0.45, 0.10)	0.281	0.16 (-0.07, 0.38)	0.142	-0.03 (-0.29, 0.24)	0.522	-0.18 (-0.39, 0.03)	0.018	-0.02 (-0.24, 0.21)	0.772	0.27 (0.01, 0.54)*	0.057
Sub-total Mono-ortho PCBs	-0.17 (-0.51, 0.18)	0.105	0.14 (-0.14, 0.43)	0.231	0.01 (-0.33, 0.34)	0.724	-0.14 (-0.41, 0.13)	0.085	0.02 (-0.27, 0.30)	0.972	0.22 (-0.11, 0.56)	0.025
Total DLCs	-0.23 (-0.64, 0.18)	0.343	0.25 (-0.09, 0.59)	0.451	0.03 (-0.37, 0.43)	0.349	-0.22 (-0.54, 0.10)	0.049	-0.02 (-0.36, 0.32)	0.59	0.40 (0.00, 0.79)	0.143

Cord hormone and DLC concentrations were $\log_e$-transformed and included in the model separately. Therefore, B (95% CI) in the Table is expressed as an e-fold increase in the cord blood hormone concentration for each e-fold increase in dioxin concentration. DHEA, dehydroepiandrosterone; E2, estradiol; T, testosterone.

[a]Adjusted for maternal age, parity, smoking during pregnancy, alcohol consumption during pregnancy, blood sampling week during pregnancy, annual income, infant sex, and interaction of sex and sub-total or total DLCs.

*$p < 0.05$

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Table 4. (continued)

	Cortisol		Cortisone		Adrenal androgen/glucocorticoid ratio[b]		SHBG		Prolactin	
	B (95% CI)	p for interaction	B (95% CI)	p for interaction	B (95% CI)	p for interaction	B (95% CI)	p for interaction	B (95% CI)	p for interaction
Sub-total PCDDs	-0.42 (-1.25, 0.40)	0.277	-0.65 (-1.74, 0.44)	0.236	0.89 (-0.36, 2.14)	0.241	-0.08 (-0.36, 0.20)	0.172	-0.10 (-0.44, 0.25)	0.972
Sub-total PCDFs	-0.61 (-1.48, 0.26)	0.317	-0.84 (-1.99, 0.31)	0.249	1.02 (-0.30, 2.34)	0.327	-0.04 (-0.34, 0.25)	0.34	-0.14 (-0.51, 0.22)	0.814
Sub-total Non-*ortho* PCBs	-0.43 (-0.94, 0.08)	0.11	-0.56 (-1.24, 0.11)	0.083	0.72 (-0.05, 1.49)	0.074	-0.08 (-0.25, 0.09)	0.052	-0.20 (-0.41, 0.01)	0.468
Sub-total Mono-*ortho* PCBs	-0.30 (-0.94, 0.34)	0.033	-0.45 (-1.29, 0.40)	0.022	0.57 (-0.40, 1.54)	0.019	-0.06 (-0.27, 0.16)	0.035	-0.13 (-0.40, 0.14)	0.404
Total DLCs	-0.52 (-1.29, 0.24)	0.179	-0.75 (-1.76, 0.27)	0.131	0.97 (-0.20, 2.13)	0.137	-0.09 (-0.35, 0.17)	0.098	-0.19 (-0.51, 0.13)	0.644

Cord hormone and DLC concentrations were $\log_e$-transformed and included in the model separately. Therefore, B (95% CI) in the Table is expressed as an e-fold increase in the cord blood hormone concentration for each e-fold increase in dioxin concentration. SHBG, sex hormone-binding globulin.

[a]Adjusted for maternal age, parity, smoking during pregnancy, alcohol consumption during pregnancy, blood sampling week during pregnancy, annual income, infant sex, and interaction of sex and sub-total or total DLCs.

[b]Adrenal androgen/glucocorticoid ratio = (DHEA and androstenedione)/(cortisol and cortisone)

*$p < 0.05$

Table 5. Adjusted linear regression coefficients (B) and 95% confidence intervals (CI) for reproductive and steroid hormone levels in cord blood in relation to total concentrations of dioxin-like compounds (DLCs) in maternal blood stratified by sex (TEQ pg/g lipid).

		T/E2	DHEA	Cortisol	Cortisone	Adrenal androgen/glucocorticoid ratio[b]	SHBG
		B (95% CI)	B (95% CI)	B (95% CI)	B (95% CI)	B (95% CI)	B (95% CI)
Male	Sub-total PCDDs	-0.23 (-0.56, 0.10)	0.47 (0.07, 0.86)*	-0.46 (-1.29, 0.37)	-0.68 (-1.76, 0.40)	0.97 (-0.24, 2.18)	-0.14 (-0.29, 0.01)
	Sub-total PCDFs	-0.26 (-0.62, 0.10)	0.40 (-0.04, 0.84)	-0.66 (-1.56, 0.24)	-0.87 (-2.03, 0.30)	1.08 (-0.23, 2.39)	-0.10 (-0.27, 0.07)
	Sub-total Non-*ortho* PCBs	-0.22 (-0.42, -0.03)*	0.30 (0.07, 0.54)*	-0.46 (-0.96, 0.04)	-0.59 (-1.23, 0.06)	0.78 (0.05, 1.50)*	-0.11 (-0.20, -0.02)*
	Sub-total Mono-*ortho* PCBs	-0.23 (-0.49, 0.03)	0.28 (-0.04, 0.60)	-0.37 (-1.03, 0.29)	-0.52 (-1.37, 0.33)	0.68 (-0.28, 1.64)	-0.09 (-0.21, 0.03)
	total DLCs	-0.29 (-0.60, 0.02)	0.46 (0.09, 0.83)*	-0.58 (-1.35, 0.20)	-0.80 (-1.80, 0.21)	1.07 (-0.06, 2.20)	-0.15 (-0.29, -0.01)*
Female	Sub-total PCDDs	0.19 (-0.15, 0.53)	0.06 (-0.38, 0.50)	0.19 (-0.63, 1.02)	0.23 (-0.87, 1.34)	-0.13 (-1.41, 1.14)	0.21 (-0.12, 0.54)
	Sub-total PCDFs	0.16 (-0.15, 0.46)	0.14 (-0.25, 0.54)	-0.07 (-0.81, 0.67)	-0.01 (-1.01, 0.98)	0.22 (-0.92, 1.37)	0.16 (-0.14, 0.45)
	Sub-total Non-*ortho* PCBs	0.17 (-0.07, 0.41)	-0.14 (-0.45, 0.17)	0.19 (-0.40, 0.77)	0.34 (-0.45, 1.12)	-0.36 (-1.26, 0.55)	0.18 (-0.05, 0.42)
	Sub-total Mono-*ortho* PCBs	0.19 (-0.09, 0.46)	-0.37 (-0.72, -0.02)*	0.73 (0.08, 1.39)*	1.04 (0.16, 1.91)*	-1.18 (-2.19, -0.18)*	0.29 (0.03, 0.55)*
	total DLCs	0.21 (-0.11, 0.53)	-0.04 (-0.45, 0.38)	0.20 (-0.59, 0.98)	0.33 (-0.72, 1.38)	-0.26 (-1.47, 0.95)	0.23 (-0.08, 0.55)

Cord hormone and DLC concentrations were $\log_e$-transformed and included in the model separately. Therefore, B (95% CI) in the Table is expressed as an e-fold increase in the cord blood hormone concentration for each e-fold increase in dioxin concentration. DHEA, dehydroepiandrosterone; E2, estradiol; T, testosterone; TEQ, toxic equivalent; PCDDs, polychlorinated dibenzo-p-dioxins; PCBs, polychlorinated biphenyls; PCDFs, polychlorinated-dibenzofurans; SHBG, sex hormone-binding globulin.

[a]Adjusted for maternal age, parity, smoking during pregnancy, alcohol consumption during pregnancy, blood sampling week during pregnancy, and annual income.

[b]Adrenal androgen/glucocorticoid ratio = (DHEA and androstenedione)/(cortisol and cortisone)

*$p < 0.05$

Table 6. Adjusted linear regression coefficients (B) and 95% confidence intervals (CI) for reproductive and steroid hormone levels in cord blood in relation to total concentrations of dioxin-like compounds (DLCs) in males (TEQ pg/g lipid).

		LH	FSH	Inhibin B	INSL3
		B (95% CI)	B (95% CI)	B (95% CI)	B (95% CI)
Male	Sub-total PCDDs	-0.19 (-0.68, 0.30)	0.04 (-0.32, 0.41)	-0.34 (-0.61, -0.07)*	-0.11 (-0.32, 0.10)
	Sub-total PCDFs	0.08 (-0.46, 0.61)	0.35 (-0.03, 0.74)	-0.35 (-0.64, -0.06)*	-0.07 (-0.30, 0.15)
	Sub-total Non-*ortho* PCBs	-0.11 (-0.41, 0.19)	0.12 (-0.10, 0.34)	-0.26 (-0.41, -0.10)**	-0.09 (-0.21, 0.04)
	Sub-total Mono-*ortho* PCBs	0.08 (-0.31, 0.47)	0.29 (0.01, 0.57)*	-0.24 (-0.45, -0.02)*	-0.10 (-0.27, 0.06)
	total DLCs	-0.13 (-0.59, 0.34)	0.17 (-0.17, 0.51)	-0.36 (-0.61, -0.11)**	-0.11 (-0.31, 0.08)

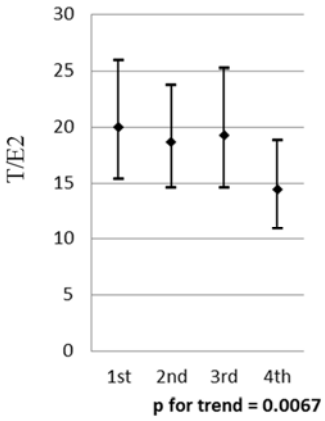
Cord hormone and DLC concentrations were $\log_e$-transformed and included in the model separately. Therefore, B (95% CI) in the Table is expressed as an e-fold increase in the cord blood hormone concentration for each e-fold increase in dioxin concentration.

FSH, follicle stimulating hormone; INSL3, insulin like factor 3; LH, luteinizing hormone; TEQ, toxic equivalent; PCDDs, polychlorinated dibenzo-p-dioxins; PCBs, polychlorinated biphenyls; PCDFs, polychlorinated-dibenzofurans.

[a]Adjusted for maternal age, parity, smoking during pregnancy, alcohol consumption during pregnancy, blood sampling week during pregnancy, and annual income.

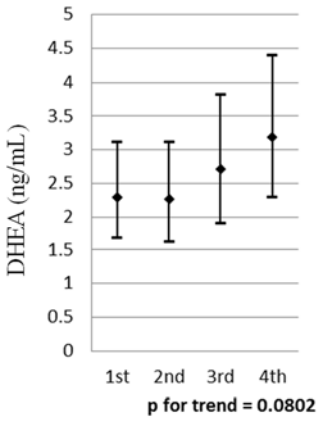
*$p < 0.05$, **$p < 0.01$

(A)



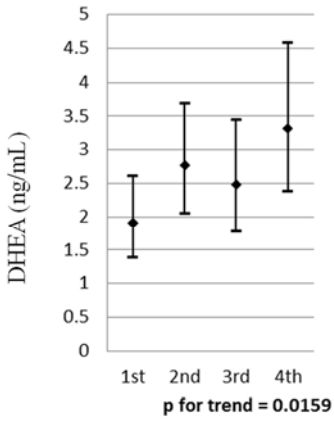
Sub-total Non-ortho PCBs (male)

(B)



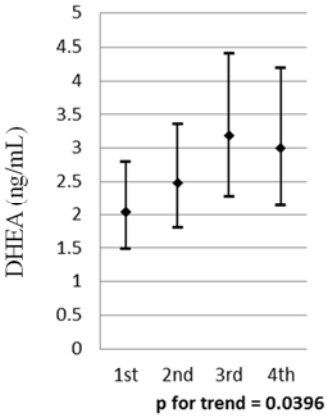
Sub-total PCDDs (male)

(C)



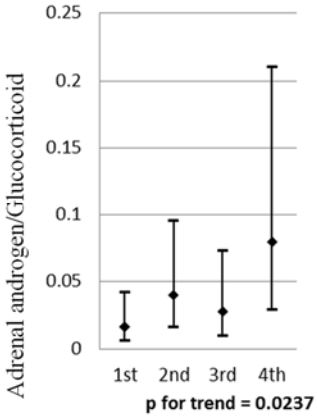
Sub-total Non-ortho PCBs (male)

(D)



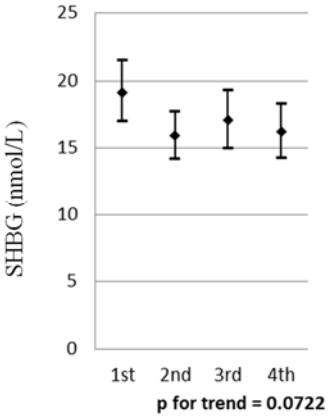
Total DLCs (male)

(E)



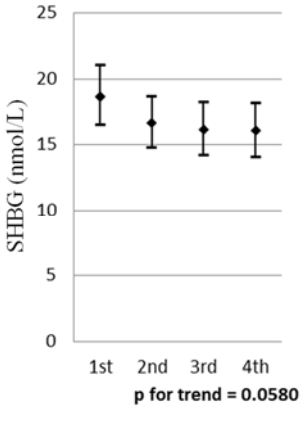
Sub-total Non-ortho PCBs (male)

(F)



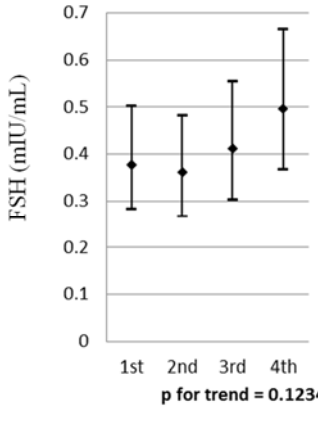
Sub-total Non-ortho PCBs (male)

(G)



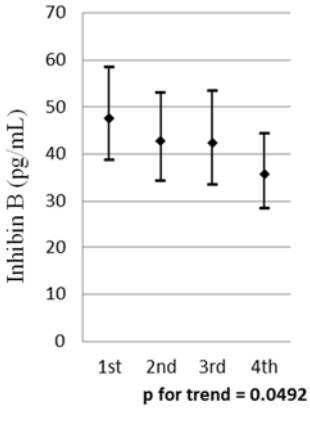
Total DLCs (male)

(H)



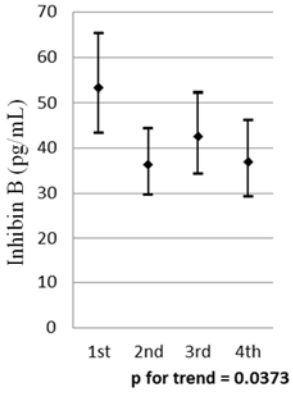
Sub-total Mono-ortho PCBs (male)

(I)



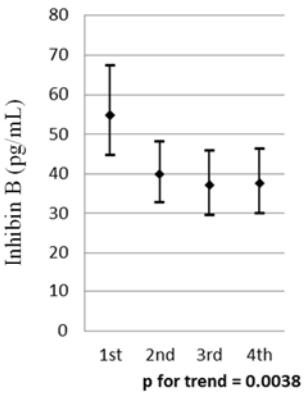
Sub-total PCDDs (male)

(J)



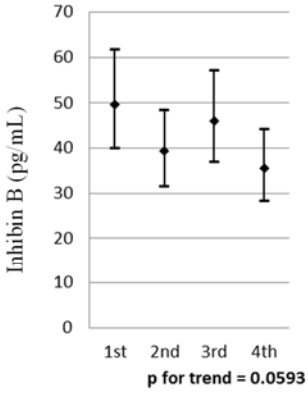
Sub-total PCDFs (male)

(K)



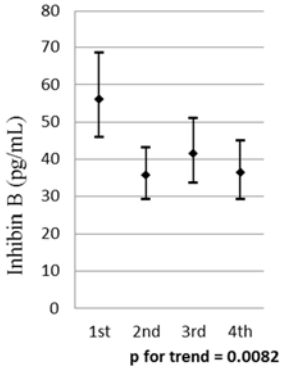
Sub-total Non-ortho PCBs (male)

(L)



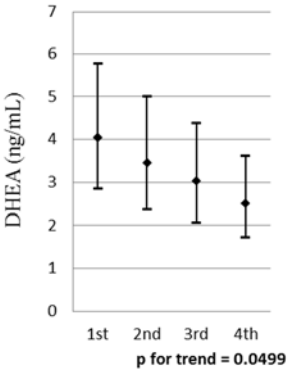
Sub-total Mono-ortho PCBs (male)

(M)



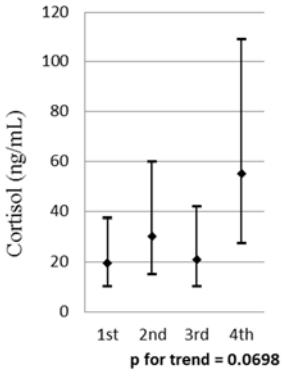
Total DLCs (male)

(N)



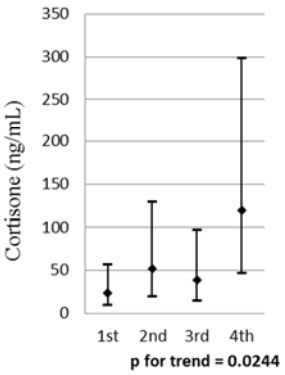
Sub-total Mono-ortho PCBs (female)

(O)



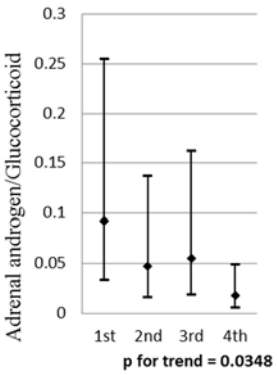
Sub-total Mono-ortho PCBs (female)

(P)



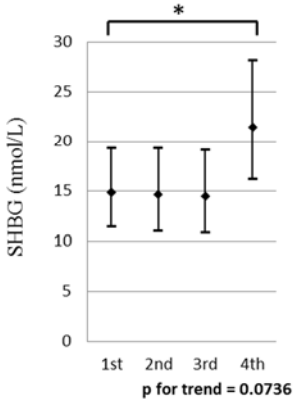
Sub-total Mono-ortho PCBs (female)

(Q)



Sub-total Mono-ortho PCBs (female)

(R)



Sub-total Mono-ortho PCBs (female)

Fig. 2 Relationships between quartiles of dioxin-like compounds (DLCs) (X-axes) and cord blood hormones (Y-axes) in male and female infants; adjusted for maternal age at delivery, parity, smoking behavior during pregnancy, alcohol consumption during pregnancy, gestation week at which blood was sampled during pregnancy, and annual household income. Marginal means and 95% confidence intervals (CIs) of log-transformed hormone levels were calculated and back-transformed. Significance in trend correlation (p-value for trend; statistical significance at $p < 0.05$) was calculated using 4 categorical quartiles of DLC concentrations, which were analyzed as ordinal variables. *$p < 0.0166$ compared with the 1st quartile, after applying the Bonferroni correction. DHEA, dehydroepiandrosterone; DLC, dioxin-like compound; E2, estradiol; PCB, polychlorinated biphenyl; PCDD, polychlorinated dibenzo-p-dioxins; PCDF, polychlorinated-dibenzofuran; T, testosterone. (A), (B), (C), (D), (E), (F), (G), (H), (I), (J), (K), (L), and (M) relationships between DLCs and cord blood hormones in male infants. (N), (O), (P), (Q), and (R) show relationships between DLCs and cord blood hormones in female infants.

4. Discussion

To the best of our knowledge, this is the first study to report the effects of sex on susceptibility to maternal DLC-related changes in cord blood T/E2 ratio, DHEA, cortisol, cortisone, AA/GC ratio, and SHBG levels. We observed that in male cord blood samples, T/E2 ratios and SHBG levels decreased, whereas DHEA levels and AA/GC ratios increased with an increase in maternal DLCs (Table 5). In addition, p-values for trends of dose-response relationship were significant between maternal non-ortho PCBs and T/E2 ratios, maternal non-ortho PCBs, and DLCs, and DHEA levels and non-ortho PCBs and AA/GC ratios in male samples in quartile models (Fig. 2). However, we observed that in female cord blood samples, DHEA levels and AA/GC ratios decreased, whereas cortisol, cortisone, and SHBG levels increased with an increase in maternal DLCs (Table 5). In addition, p-values for trends of dose-response relationship were significant between maternal mono-ortho PCBs and DHEA, cortisone, and AA/GC ratios in female samples in quartile models (Fig. 2). Therefore, maternal DLC concentrations correlated with cord blood DHEA, AA/GC ratios, and SHBG levels in the opposite direction stratified by sex (Table 5).

Cord blood represents a mixture of hormones synthesized and secreted by the placenta and the fetus. The placental steroid metabolizing system converts fetal adrenal DHEA-S to DHEA, and facilitates estrogen biosynthesis via aromatization from T, which is metabolized from DHEA. T/E2 ratio indicates activities of the aromatase enzyme (Warembourg et al., 2016). AA/GC ratio indicates the steroidogenesis balance of androgenic and glucocorticoid hormones produced by fetal adrenal cortex. Androgenic hormones are the major precursors for placental estrogen production (Greaves et al., 2014; Kuijper et al., 2013). In addition, androgenic hormones are antagonistic to glucocorticoids. Glucocorticoids play a crucial role in the fetal programming of hypothalamic–pituitary–adrenal axis function. In

addition, glucocorticoids regulate the maintenance of homeostasis as well as fetal growth, and lung and heart development (Kapoor et al., 2008). Our results suggest that steroidogenesis shifts to androgenic hormones in males and glucocorticoid hormones in females. Although the critical time window during pregnancy is not completely clear, DLC levels can be used as an exposure indicator for the whole period of pregnancy because DLCs have a long half-life in the body. Sex-related differences in adrenal fetal steroidogenesis after *in utero* DLC exposure has been demonstrated (Li and Wang 2005; Takeda et al., 2013). In addition, cytochrome P450C17 (CYP17) plays a key role in glucocorticoid and androgen biosynthesis (Takeda et al., 2013). Therefore, it is possible that DLC exposure modulates CYP17 activities differently in males and females. However, further studies are required to clarify whether the effects of *in utero* exposure to DLCs on adrenal hormones extend into infancy and puberty.

In our previous study conducted on the same cohort, we found a significant association between maternal DLCs and birth weight (Kishi et al., 2017), as well as birth weight and adrenal steroid hormones, including androstenedione, DHEA, cortisol, and cortisone, in cord blood (Mitsui et al., 2018). In mediation analysis stratified by children sex, we evaluated the mediatory effect of adrenal steroid hormones in cord blood on the association between maternal DLCs and birth weight. However, we found no significant mediatory effect of adrenal steroid hormones in male and female cord samples possibly because the sample sizes were not enough (data not shown). We propose to evaluate the mediatory effect of reproductive and steroid hormones in cord blood on the association between maternal DLCs and health outcomes after birth using larger sample sizes.

We found SHBG levels at the 4th quartile of mono-ortho PCBs to be significantly higher than those at the 1st quartile in quartile models in female samples (Fig. 2). This

indicates that female SHBG levels are more vulnerable upon DLC exposure *in utero* compared with that of male SHBG levels. T facilitates binding to SHBG compared with E2, and an increase in SHBG level suppresses T activity. In our study, maternal mono-ortho PCBs were positively associated with SHBG level, which is related to T activity; two previous studies have indicated the possibility that decreased cord blood T levels related with prenatal exposure to PCDD/PCDFs were more pronounced among females (Boda et al., 2018; Hsu et al., 2005). Therefore, our results were partly consistent with results of these two previous studies. In our previous study conducted on the same cohort, we reported that sex-specific effects of maternal DLCs associated with birth weight, prevalence of infections at 18 months of age, and indices of the BSID-II Mental Developmental Index at 6 and 18 months of age (Kishi et al., 2017; Nakajima et al., 2017). Therefore, results of the present study provide additional evidence of sex-related differences in the negative effects of in utero DLC-exposure on outcomes of fetal development.

PCDD/PCDFs congeners in maternal breast milk inversely correlated with DHEA levels and salivary AA/GC ratios in 3-year-old children born to these Vietnamese mothers residing in DLC-contaminated areas (Kido et al., 2016). However, we found positive relationships between the maternal concentrations of non-ortho PCBs and cord blood levels of DHEA (Table 4). Our contrasting findings may be explained by the difference in exposure levels; these Vietnamese women lived in areas with high levels of PCDD/PCDFs contamination due to the spraying of Agent Orange, a potent herbicide used by the US military forces during the Vietnam war (Kido et al., 2016). In our previous study conducted on the same cohort, maternal DLC levels (median PCDD/PCDF: 6.1 TEQ pg/g lipids in the breast milk) (Todaka et al., 2010) are relatively lower than those reported in a study on Vietnamese females living in DLC-contaminated areas (median PCDD/PCDF: 11.0 TEQ

pg/g lipids in the breast milk) (Kido et al., 2016). This exposure to DLCs at environmental levels from daily food intake possibly resulted in the low maternal DLCs reported earlier (Todaka et al., 2010). Additionally, the timing of our outcome assessments was different; the enhanced fetal adrenal function rapidly declines after birth (Kuijper et al., 2013).

We observed that a decrease in inhibin B and an increase in FSH in male cord blood samples were associated with an increase in maternal DLCs (Table 6). Additionally, p-values for trends of dose-response relationship were significant between PCDDs, PCDFs, non-ortho PCBs, DLCs, and inhibin B in male cord blood samples (Fig. 2). Inhibin B is synthesized in the testis of the male fetus and the fetal placenta during pregnancy (Luisi et al., 2005). Inhibin B is produced by Sertoli cells and provides negative feedback control to pituitary FSH secretion. FSH promotes Sertoli cell function, increasing sperm count, sperm motility, and spermatogenic status. Inhibin B is a marker of testicular function, including semen quality (Luisi et al., 2005; Wan et al., 2013). Human and animal studies suggest that *in utero* exposure to DLCs may be one of the main causes of poor reproductive function, including semen quality (Mocarelli et al., 2011) as well as epididymal and ejaculated sperm numbers in males (Foster et al., 2011). Additionally, these studies suggest that the developing reproductive tract is most sensitive to effects of DLC when exposure occurs *in utero* and early after birth (Foster et al., 2011; Mocarelli et al., 2011). Epidemiological study suggests that *in utero* and lactational exposure of children to relatively low DLC doses could permanently reduce sperm quality as occurred in the Seveso accident (Mocarelli et al., 2011). Consistent with results of this previous study, our results suggest that testicular function is attenuated by *in utero* exposure to DLCs at environmental levels. Therefore, further studies are required to clarify whether *in utero* exposure to DLCs affects testicular function in adolescence. Moreover, inhibin B regulates FSH secretion, and indirectly changes estrogen

levels (Luisi et al., 2005). We consider that exposure to DLCs *in utero* could alter inhibin B production and estrogen biosynthesis in the placenta in the male fetus.

PCDDs, including 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin and 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin, are the most toxic DLC congeners, and have been shown to adversely affect the mammalian reproductive system (Van den Berg et al., 2006; Xu et al., 2014). The PCDF, 2,3,4,7,8-pentachlorodibenzofuran, is reported to have negatively affected infant health more than other DLC congeners during the Yusho poisoning incident in Japan (Masuda, 2001). Furthermore, the negative effects of 2,3,4,7,8-pentachlorodibenzofuranon on birth weight, neurodevelopment, and immune function in male infants have been reported in the Sapporo Cohort of the Hokkaido Study (Kishi et al., 2017). Non-ortho PCBs, including PCB-126 and PCB-169, have stronger estrogenic activities than other PCBs, and have been shown to exert toxic effects on adrenal secretion (Li and Wang 2005; Van den Dungen et al., 2015). Therefore, we propose that PCDD/PCDFs and non-ortho PCBs significantly related to a change in the levels of T/E2 ratio, DHEA, cortisol, AA/GC ratio, SHBG, and inhibin B in the cord blood (Table S3 and S4) through complex mechanisms involving AHRs, estrogen receptors, and steroidogenic enzymes. Moreover, we found relationships between mono-ortho PCBs and T/E2 ratio, DHEA, cortisol, cortisone, AA/GC ratios, SHBG, FSH, and inhibin B in the cord blood regardless of sex (Table S3 and S4). Mono-ortho PCBs, including PCB-123, PCB-118, PCB-105, PCB-167, and PCB-189, are reported to cause partial AHR activation and CYP1A1 induction in an *in vitro* model; however, details of their toxic properties are not known (Van den Berg et al., 2006), suggesting that further studies are needed in this direction.

The strengths of this study include the accurate quantification of 29 DLC congeners (using HRGC/HRMS) as well as of seven steroid hormones in the cord blood (using LC-MS/MS, which has a very high sensitivity and specificity compared with conventional immunoassays) (Yamashita et al., 2007a; Yamashita et al., 2007b). Moreover, measuring these hormones during pregnancy are essential to reasonably evaluate and interpret the effects of prenatal exposure to DLCs (Hollier et al., 2014).

However, our study also has some limitations. Firstly, the concentrations of steroid and reproductive hormones greatly change immediately before and after birth (Kuijper et al., 2013), and unexpected factors, such as placental weight and preeclampsia, may affect these hormones (Hollier et al., 2014). Therefore, we only included women with no pregnancy complications during their vaginal singleton deliveries. Additionally, we adjusted our regression models for maternal smoking and alcohol consumption, blood sampling week during pregnancy, and annual household income to minimize the effects of these confounding factors. Secondly, our results are not free from selection bias; the participants included in this study presented with increased gestational ages, were sampled for blood at a later week during gestation, gave birth to heavier infants, and were mostly primipara women compared with those who were excluded from the study (Table S5). Therefore, we may have underestimated the associations of DLCs with levels of cord blood hormones as the participants included in this study were expected to be in a healthier state than those excluded from the study. Thirdly, a time gap in assessment may have occurred; maternal DLCs were measured in the 2nd and 3rd trimesters, whereas the critical window of possible exposure effect on fetal hormones exists during early pregnancy. However, DLCs have a long half-life in the body and the range from 5 to 20 years (Todaka et al., 2010). The body burden of substantial DLCs accumulated in daily life does not change with temporary events during

pregnancy. Thus, DLC levels can be used as an exposure indicator for the whole time period of pregnancy, including early pregnancy. In addition, we controlled blood sampling week during pregnancy as adjusted factors in regression analysis to reduce bias with a change in the detected DLC levels accompanying gestational age. Finally, measurements below LODs were assigned values of one-half of the LODs; such an assignment approach may have introduced a bias in the association between total DLCs and SHBG, as well as PCDFs, mono-ortho PCBs, and FSH. We cannot exclude the possibility of false positive results because their dose-response relationships in quartile models (Fig. 2) or DLC congeners were not significant in congener-specific analyses (Table S3 and S4).

5. Conclusions

A decrease in T/E2 ratios, SHBG, and inhibin B levels, and an increase in DHEA levels and AA/GC ratios in male cord blood samples were associated with an increase in maternal DLCs. However, a decrease in DHEA levels and AA/GC ratios, and an increase in cortisol, cortisone, and SHBG levels in female cord blood samples were associated with an increase in maternal DLCs. Therefore, sex-related differences were observed in the relationships between maternal DLCs and cord blood hormones. These results suggest that *in utero* exposure to DLCs modifies steroidogenesis and suppresses the secretion of inhibin B. However, further studies are required to evaluate its long-term effects on the onset and progression of puberty.

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Conflicts of interests

None.

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