Placental distribution of endogenous and exogenous substances: A pilot study utilizing cryo-sampled specimen off delivery room

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Abstract

***Introduction*:** Reliability in the use of placentome (including placenta, umbilical cord, and cord blood) biomarkers requires an understanding of their distributions. Here we aim to develop a simple and proper placenta sampling scheme, and to evaluate the placental distributions of biomarkers.

***Methods*:** We developed a continuous cooling chain protocol off delivery room and cryo-subsampling method for placenta sampling. The levels of thyroid hormones (THs), elements, persistent organic pollutants (POPs), monoamines, and vitamin E were measured using UPLC-Q-TOF-MS, HPLC-ICP-MS, HPLC-EcD, and HRGC-HRMS, respectively. The distributions of biomarkers were assessed.

***Results*:** In human placentome, $_{L}$-thyroxine (T_4), Cd, Se, Zn, Cu, Fe, Ca, K, Mg, α-tocopherol, β-tocopherol, and β-tocotrienol levels were higher in placenta than in umbilical cord, while Pb and Mn were concentrated in human cord. In porcine placentome, T_4, 3,3′,5′-triiodo-$_{L}$-thyronine (rT_3), 3,3′-diiodo-$_{L}$-thyronine, Cd, Pb, Zn, K, and Al levels were higher in the cord. The intraclass correlation coefficient (ICC) was <0.4 for 3,3′,5-triiodo-$_{L}$-thyronine, rT_3, α-tocopherol, and 7 elements in human basal plate, indicating low reliability. rT_3, Cd, Zn, Mn, and Cu were significantly concentrated in the central region in human placenta, while higher levels of As, Cd, Cr, and Al were found in the periphery region in porcine placenta. Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) showed moderate reliability (ICC: 0.40–0.98) except PCB-81, -126, and BDE-208, while polychlorinated dibenzo-*p*-doixins/furans (PCDD/Fs) showed poor reliability (ICC: 0.07–0.31).

***Discussion*:** These results highlight the complexity of placenta sampling. This study provides a novel and simple sampling approach in investigating placental exposomics.

Keywords: Placental distribution; Thyroid hormones; Elements; Persistent organic pollutants; Monoamines; Vitamin E

1 Introduction

Placenta, umbilical cord, and cord blood work as a regulator and transport system for nutrients and essential hormones [1,2]. Passive diffusion, active transport, facilitated diffusion, filtration, and pinocytosis are involved in this process [3]. Some environmental contaminants can also be transferred across the placenta due to chemical similarities with nutrients, or simply as a result of passive diffusion. The developing fetus is particularly susceptible to these abnormal stimuli because the organs, excretory system and blood-brain barrier are not fully developed. Recent studies indicate that *in-utero* exposure may disrupt the homeostasis of hormones and elements in the fetus [4–6], resulting in severe repercussions for newborns and late adult deleterious effects [7,8].

Consequently, placentome tissues, which represent the whole group of fetal and maternal tissues that are involved in placentation, have been increasingly used to examine the intrauterine environment [9,10]. These samples are readily accessible, require no invasive procedure, and offer possibilities for monitoring the exposures exerted on both the mother and the fetus [11]. Due to their sizes, these tissues are particularly suitable for exposomic evaluations, which explores the totality of exposures associated with disease prevalence [12–14]. For example, >20 mL serum is required to measure the 250–300 currently biomonitored chemicals, which can be limiting for certain age groups under study [15].

The value of costly biomonitoring is diminished when unrepresentative samples lead to biased inferences regarding exposure-effect interactions. Obtaining a representative biospecimen from placentome is therefore crucial considering the potential heterogeneity of chemical distributions. Previous studies observed correlations between the locations of the sampling sites and the levels of Fe, Zn, Cu, Ca, Cd, Hg, Pb, and gene expression [16–21], while progesterone was found to be homogeneously distributed in placenta [22]. These results suggest

that different mechanisms might be involved in the chemical distribution. To avoid potential bias, some studies homogenized the entire placenta [23–25]. Some other studies collected the same part of placenta, or random samples from unspecified sites within the placenta [26,27]. Other studies did not provide details in sampling [9, 28]. Placenta sample storage is another crucial problem considering the potential degradation of biomarkers. Ideally, the placenta should be kept at −80 °C immediately after delivery. This is normally not practically available, especially in large birth cohort studies. The sampling and storage differences may engender different observations [5,6], resulting in difficulties in the interpretation of previous studies.

To address this gap in knowledge we developed a cryo-sampling procedure off delivery room until analysis to obtain homogenous cryopowder of placenta suitable for repetitive analysis of a variety of cryo-stable biomarkers of exposure and effect. The placental distribution of these biomarkers was evaluated using the powder-subsamples. The biomarkers include thyroid hormones (THs), elements, persistent organic pollutants (POPs), monoamines, and vitamin E (VE), which generally represent prominent essential and toxic constituents in intrauterine environment. Both human and porcine placentome tissues were examined. The regional distribution and reliability of biomarkers were evaluated. Finally, the placenta cryosampling optimization for reducing bias and gaining logistic simplicity was discussed.

2 Methods

2.1 Data collection

This study was approved by the local ethic boards. Informed written consent was obtained from each participant. Five anonymous human placentas with umbilical cords and cord blood (infant gender: female) were collected from the Women's Hospital of Tübingen University, Germany. The average age of the women was 28.4 years (range: 21–34 years). The average gestational age was 271 days (range: 267–272 days). The delivery mode was vaginal. Three porcine placentas with umbilical cords were obtained from the Technical University of Munich, Germany. The porcine placentas were collected at 115 days of pregnancy. The delivery was a natural, vaginal mode. The type of chemical distribution in human and porcine placenta might be different because human placenta is hemochorial while porcine placenta is epitheliochorial. We included placentomes with different structures to compare the results. Appropriate precautions were taken to avoid contamination. Placenta and umbilical cord were separated immediately after delivery and specifically placed and frozen at < −20 °C in a two-chambered food-safe aluminum container, transported to the Helmholtz Center Munich, Germany (<−20 °C), and stored at < −80 °C until further processing.

A cryo-drilling approach was developed for sampling. Complete details are given in Supplementary method and Figs. S1–S3 **(Supplementary Data)**. Briefly, a drilling machine was used to collect finely powdered subsamples across the basal plate. At the temperature of < −150 °C in liquid nitrogen, 11–15 and 3–6 subsamples were collected from the basal plate of each placenta and umbilical cord, respectively. The number of subsamples generated was determined by the size of the tissues, which were 67, 17, 30, and 17 in human placenta, human cord, porcine placenta, and porcine cord, respectively (Table S1). The wet weight of each powdered subsample was <2 g. Seven THs, 15 elements, 9 monoamines, and 9 VEs were measured in these subsamples. At least 10 g sample was needed for POP analysis, thus the residual placenta was cut into 4 parts, each part was homogenized, and 74 POPs were measured (see Tables 1, 2, 3 and Table S3 for the analytes). About 10 mL of cord blood was collected from each umbilical cord, in which Heparin was doped and THs and monoamines were determined.

alt-text: Table 1

Table 1

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Descriptive statistics and intraclass correlation coefficients (ICCs) of THs, elements, and VEs in human placentome.

	Human basal plate			Human umbilical cord				
	Mean (SD)	Median (Range)	ICC[a]	Mean (SD)	Median (Range)	ICC[a]	C_c/C_p (mean (SD))	p [b]
THs								
T_4 (ng/g)	42.3 (7.2)	42.4 (24.3–57.6)	0.52	19.1 (11.2)	15.9 (5.79–47.2)	0.06	0.46 (0.11)	**<0.001**
T_3 (ng/g)[c]	0.66 (0.20)	0.65 (0.34–1.31)	0.06	0.52 (0.11)	0.49 (0.40–0.68)	–	–	–
rT_3 (ng/g)	2.74 (1.23)	2.97 (0.48–4.86)	0.26	2.41 (0.85)	2.42 (1.16–4.45)	0.31	0.88 (0.41)	0.151
Elements								
As (ng/g)	3.27 (3.21)	2.03 (0.55–15.4)	0.37	3.34 (2.27)	2.48 (1.51–11.0)	0.21	1.45 (1.14)	0.142
Cd (ng/g)	4.66 (2.70)	4.16 (1.01–15.1)	0.04	1.40 (0.97)	1.04 (0.46–4.14)	0.39	0.46 (0.37)	**<0.001**
Cr (μg/g)	9.46 (12.7)	3.86 (0.08–60.9)	0.36	4.46 (2.97)	3.70 (1.06–12.5)	0.07	0.72 (0.41)	0.854
Hg (ng/g)	8.86 (8.13)	6.42 (0.38–37.2)	0.68	8.08 (6.32)	5.30 (2.14–22.6)	0.62	1.51 (0.72)	0.699
Pb (ng/g)	8.57 (4.39)	7.47 (2.36–22.8)	0.10	12.9 (4.67)	13.5 (6.03–21.6)	0.15	6.80 (10.0)	**<0.001**
Se (ng/g)	159 (22)	163 (96.1–201)	0.42	65.3 (15.7)	61.1 (43.9–96.0)	0.12	0.43 (0.06)	**<0.001**
Zn (μg/g)	9.21 (1.91)	8.84 (6.57–16.5)	0.38	5.35 (1.39)	4.82 (4.27–9.76)	0.40	0.62 (0.17)	**<0.001**
Mn (μg/g)	0.30 (0.34)	0.15 (0.59–1.67)	0.37	0.37 (0.22)	0.29 (0.11–0.87)	0.60	1.75 (0.80)	**0.01**
Cu (μg/g)	1.06 (0.49)	0.93 (0.68–3.01)	0.29	0.49 (0.07)	0.48 (0.37–0.64)	0.04	0.50 (0.09)	**<0.001**
Fe (μg/g)	200 (97)	180 (67.8–517)	0.46	135 (105)	101 (31.2–371)	0.35	0.62 (0.29)	**<0.01**
Ca (mg/g)	0.79 (0.80)	0.51 (0.13–4.02)	0.61	0.09 (0.01)	0.09 (0.08–0.10)	0.41	0.15 (0.08)	**<0.001**

K (mg/g)	1.99 (0.20)	1.97 (1.64–2.38)	0.47	1.04 (0.28)	0.99 (0.63–1.55)	0.19	0.52 (0.04)	**<0.001**
Mg (μg/g)	91.2 (36.0)	80.5 (60.5–253)	0.59	37.0 (4.0)	36.8 (29.6–46.9)	0.18	0.44 (0.10)	**<0.001**
Na (mg/g)	1.83 (0.27)	1.82 (0.21–2.23)	0.76	1.85 (0.25)	1.86 (1.39–2.25)	0.28	1.02 (0.16)	0.763
Al (μg/g)	0.50 (0.34)	0.39 (0.09–1.68)	0.43	0.67 (0.44)	0.55 (0.17–1.94)	0.15	1.99 (1.13)	0.090
Σ heavy metals (μg/g)[d] ~~d~~	8.50 (12.4)	3.49 (0.02–60.9)	0.04	4.48 (2.97)	3.72 (1.07–12.5)	0.61	0.85 (0.49)	0.776
Σ elements (mg/g)[e]	4.84 (0.90)	4.66 (3.43–8.34)	0.31	3.17 (0.56)	2.99 (2.34–4.23)	0.11	0.65 (0.10)	**<0.001**
VE [f]								
α-tocopherol (μg/g)	15.0 (4.6)	14.6 (5.83–34.8)	0.36	1.20 (0.50)	0.98 (0.71–2.69)	0.57	0.08 (0.01)	**<0.001**
β-tocopherol (ng/g)	94.8 (27.9)	87.7 (47.0–203)	0.95	7.89 (4.31)	6.02 (<LOD–21.4)	0.42	0.08 (0.01)	**<0.001**
γ-tocopherol (ng/g)	321 (123)	285 (161–621)	0.97	–	–	–	–	–
β-tocotrienol (ng/g)	6.47 (3.61)	5.66 (<LOD–20.7)	0.80	0.68 (0.31)	0.63 (<LOD–1.68)	0.46	0.11 (0.01)	**<0.001**
γ-tocotrienol-	–	–	–	2.18 (1.52)	1.60 (<LOD–6.11)	0.34	–	–
α-tocopherylquinone (ng/g)	456 (223)	409 (74.2–1098)	0.94	79.4 (57.9)	66.9 (14.8–254)	0.76	0.16 (0.13)	0.14
Σ VE (μg/g)	15.9 (4.7)	15.5 (6.70–35.8)	0.28	1.30 (0.58)	1.05 (0.73–3.10)	0.55	0.07 (0.03)	**<0.001**

Abbreviations: ICC, intraclass correlation coefficient. C_c, concentration in human umbilical cord. C_p, concentration in human placenta. ~~IQR, interquartile range showing the 25th and 75th percentile values~~[Instruction: IQR is not shown in the Table 1. It should be removed]. VE, vitamin E. [a]Variances estimated for each chemical using a one-way random-effects ANOVA model; calculations were performed on log10-transformed data; ICC = between-tissue variance/total variance. [b]Difference between human placenta and umbilical cord analyzed by Mann-Whitney *U* test. [c]The ICC value of T_3 in human umbilical cord was not calculated due to the low detection frequency. [d]The sum of As, Cd, Cr, Hg, and Pb. [e]The sum of all the elements measured. [f]Only the VEs with DF > 80% were shown in this table.

alt-text: Table 2

Table 2

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Descriptive statistics and intraclass correlation coefficients (ICCs) of THs, elements, and VEs in porcine placentome.

	Porcine basal plate			Porcine umbilical cord				
	Mean (SD)	Median (Range)	ICC[a]	Mean (SD)	Median (Range)	ICC[a]	C_c/C_p (mean SD))	p [b]
THs								
T_4 (ng/g)	11.3 (6.8)	9.71 (4.20–36.9)	0.50	25.5 (5.9)	24.5 (17.4–35.4)	0.56	2.85 (1.46)	**<0.001**
T_3 (ng/g)	0.70 (0.52)	0.59 (0.19–2.50)	0.36	0.63 (0.21)	0.55 (0.37–1.07)	0.19	1.18 (0.60)	0.722
rT_3 (ng/g)	1.23 (0.43)	1.16 (0.64–3.08)	0.21	2.08 (0.37)	2.02 (1.58–2.64)	0.66	1.84 (0.30)	**<0.001**
3,3′-T_2 (ng/g)	2.34 (1.13)	2.06 (0.76–4.76)	0.23	3.55 (0.86)	3.48 (2.00–5.07)	0.24	1.70 (0.45)	**<0.001**
Elements								
As (ng/g)	2.95 (3.30)	1.67 (0.34–14.3)	0.10	3.48 (2.98)	2.08 (0.84–11.2)	0.42	1.38 (0.62)	0.211
Cd (ng/g)	0.98 (1.72)	0.63 (0.08–9.56)	0.24	10.3 (11.6)	4.85 (0.47–39.5)	0.14	14.2 (8.4)	**<0.001**
Cr (μg/g)	10.0 (14.5)	3.69 (0.28–62.7)	0.05	4.20 (5.80)	1.62 (0.50–2.22)	0.56	0.55 (0.40)	0.189
Hg (ng/g)	5.81 (6.55)	4.35 (0.32–33.8)	0.06	3.54 (2.06)	3.29 (0.57–8.43)	0.11	0.87 (0.29)	0.145
Pb (ng/g)	3.40 (2.27)	2.98 (0.98–12.6)	0.03	23.9 (16.9)	19.0 (5.22–72.3)	0.02	7.69 (4.22)	**<0.001**
Se (ng/g)	125 (41)	112 (82.4–252)	0.37	96.9 (13.4)	96.8 (74.5–125)	0.61	0.84 (0.13)	**0.01**
Zn (μg/g)	4.76 (0.82)	4.90 (3.07–6.13)	0.32	7.17 (1.90)	7.11 (4.94–12.6)	0.04	1.52 (0.05)	**<0.001**
Mn (μg/g)	0.69 (0.49)	0.51 (0.27–2.30)	0.03	0.69 (0.65)	0.46 (0.20–2.21)	0.60	1.41 (1.16)	0.38
Cu (μg/g)	0.70 (0.31)	0.74 (0.11–1.30)	0.30	0.49 (0.23)	0.53 (0.12–0.91)	0.52	0.63 (0.21)	**<0.01**
Fe (μg/g)	122 (139)	71.5 (20.8–659)	0.01	100 (56)	108 (18.7–242)	0.04	1.07 (0.48)	0.854
Ca (mg/g)	0.81 (0.17)	0.79 (0.51–1.21)	0.13	0.44 (0.12)	0.47 (0.15–0.59)	0.02	0.57 (0.11)	**<0.001**
K (mg/g)	0.89 (0.13)	0.89 (0.65–1.12)	0.02	1.04 (0.18)	1.04 (0.78–1.47)	0.33	1.16 (0.17)	**<0.01**
Mg (μg/g)	0.10 (0.02)	0.09 (0.07–0.15)	0.48	0.11 (0.02)	0.11 (0.06–0.14)	0.10	1.11 (0.14)	0.393

Na (mg/g)	1.95 (0.23)	2.03 (1.51–2.43)	0.73	1.74 (1.86)	1.71 (1.32–1.99)	0.34	0.91 (0.05)	**<0.01**
Al (μg/g)	0.43 (0.42)	0.29 (0.08–2.30)	0.07	1.92 (1.74)	1.78 (0.04–7.25)	0.07	5.05 (2.33)	**<0.001**
Σ heavy metals (μg/g)[c]	9.69 (14.3)	3.64 (0.009–62.7)	0.07	4.24 (5.81)	1.65 (0.53–22.2)	0.57	0.57 (0.39)	0.179
Σ elements (mg/g)[d]	3.88 (0.32)	3.85 (3.43–4.74)	0.30	3.44 (0.25)	3.39 (3.07–3.90)	0.42	0.85 (0.09)	**<0.001**
VE								
α-tocopherol (μg/g)	1.91 (0.88)	1.72 (0.38–3.70)	0.39	0.23 (0.05)	0.23 (0.15–0.31)	0.46	0.12 (0.01)	**<0.001**
α-tocotrienol (ng/g)	12.9 (19.0)	7.19 (<LOD–82.9)	0.61	1.90 (0.62)	1.88 (<LOD–2.93)	0.42	0.15 (0.21)	**<0.01**
β-tocotrienol (ng/g)	7.75 (16.1)	2.86 (<LOD–85.7)	0.28	0.86 (0.67)	0.60 (<LOD–2.63)	0.61	0.11 (0.06)	0.08
γ-tocotrienol (ng/g)	4.99 (6.23)	1.36 (<LOD–20.6)	0.17	7.39 (3.87)	6.96 (<LOD–13.7)	0.43	1.48 (1.09)	0.14
α-tocopherylquinone (ng/g)	38.1 (46.7)	23.4 (<LOD–197)	0.22	–	–	–	–	–
Σ VE (μg/g)	1.96 (0.88)	1.73 (0.47–3.77)	0.53	0.24 (0.05)	0.24 (0.16–0.32)	0.82	0.12 (0.05)	**<0.001**

Abbreviations: C_c, concentration in porcine umbilical cord. C_p, concentration in porcine placenta. ~~IQR, interquartile range showing the 25th and 75th percentile values.~~[Instruction: IQR is not shown in Table 2. It should be removed] VE, vitamin E. [a]Variances estimated for each chemical using a one-way random-effects ANOVA model; calculations were performed on log10-transformed data; ICC = between-tissue variance/total variance. [b]Difference between porcine placenta and porcine umbilical cord analyzed by Mann-Whitney *U* test. [c]The sum of As, Cd, Cr, Hg, and Pb. [d]The sum of all the elements measured.

alt-text: Table 3

Table 3

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Descriptive statistics and measures of reliability of POPs with detection frequencies ≥60% in human placenta.

POPs	Detection frequency	LOD (pg/g fw)	Mean (SD) (pg/g fw)	Median (range) (pg/g fw)	ICC
PCDD/Fs					
1,2,3,4,6,7,8-HpCDD	80	0.001–0.004	0.09 (0.05)	0.08 (0.04–0.24)	0.07
2,3,4,7,8-PeCDF	90	0.001–0.002	0.10 (0.04)	0.08 (0.03–0.20)	0.16
1,2,3,4,7,8-HxCDF	65	0.001–0.004	0.05 (0.01)	0.05 (0.03–0.07)	0.17

1,2,3,6,7,8-HxCDF	65	0.001–0.004	0.03 (0.01)	0.02 (0.01–0.04)	0.31
1,2,3,4,6,7,8-HpCDF	65	0.001–0.002	0.05 (0.04)	0.03 (0.02–0.15)	0.08
Σ PCDD/Fs	100	–	0.32 (0.22)	0.29 (0.00–0.84)	0.16
PCDD/F_TEQ_1998	100	–	0.06 (0.04)	0.05 (0–0.20)	0.13
PCDD/F_TEQ_2005	100	–	0.04 (0.04)	0.03 (0–0.17)	0.15
PCBs					
PCB-28	100	≤0.1	8.62 (2.66)	8.91 (4.14–15.0)	0.58
PCB-52	60	4.8–6.6	7.99 (2.09)	7.79 (4.91–13.5)	0.58
PCB-81	60	0.004–0.01	0.07 (0.02)	0.07 (0.05–0.12)	0.06
PCB-105	100	≤0.1	5.30 (3.30)	4.15 (1.73–14.9)	0.75
PCB-114	100	≤0.1	0.52 (0.25)	0.49 (0.19–0.96)	0.65
PCB-118	100	≤0.1	13.0 (4.70)	12.8 (5.34–23.9)	0.72
PCB-123	95	≤0.35	0.88 (0.40)	0.84 (0.30–1.77)	0.46
PCB-126	100	≤0.1	0.25 (0.09)	0.25 (0.14–0.58)	0.20
PCB-138	100	≤0.1	78.4 (29.6)	87.2 (25.5–123)	0.91
PCB-153	100	≤0.1	47.4 (18.0)	51.7 (16.1–72.4)	0.92
PCB-156	100	≤0.1	5.08 (2.99)	4.42 (1.26–11.7)	0.93
PCB-157	100	≤0.1	0.83 (0.49)	0.70 (0.25–2.02)	0.76
PCB-167	100	≤0.1	1.99 (0.48)	2.04 (1.02–3.01)	0.70
PCB-180	100	≤0.1	42.5 (43.8)	23.1 (9.66–140)	0.98
Σ PCBs	100	–	212 (93)	211 (71.8–391)	0.92
PCB_TEQ_1998	100	–	0.03 (0.01)	0.03 (0.02–0.07)	0.44
PCB_TEQ_2005	100	–	0.03 (0.01)	0.03 (0.02–0.07)	0.43
BFRs					
BDE-153	75	0.6–0.7	3.71 (1.86)	4.24 (0.76–6.57)	0.94
BDE-201	75	0.2	0.96 (0.61)	1.06 (0.20–2.46)	0.65
BDE-206	65	1.1–1.2	4.52 (5.82)	2.91 (1.29–23.0)	0.40
BDE-208	65	1.0	3.36 (5.58)	1.50 (0.99–21.6)	0.34
Σ BFRs	100	–	79.5 (154)	43.0 (1.81–96.3)	0.67

Abbreviations: LOD, limit of detection. ~~l~~fw, ~~lipid~~fresh weight. ICC, intraclass correlation coefficient. TEQ, the World Health Organization Toxic Equivalent values. BFR, brominated flame retardants.

THs, elements, monoamines, VE, and POPs were measured using UPLC-Q-TOF-MS, HPLC-ICP-MS, HPLC-EcD, and HRGC-HRMS, respectively. The sample preparation, quantification, and quality control were

performed according to the methods outlined before [29–32], a brief description is given in the Supplementary Data.

2.2 Data analysis

The distribution variability of chemicals in placentome tissues was assessed by intraclass correlation coefficient (ICC), which is defined as the percent of total variance explained by between-tissue variance. We used a one-way random-effects ANOVA model to calculate the ICC for each chemical independently. Values of the ICC <0.40, 0.40–0.75, and ≥0.75 suggest poor, fair to good, and excellent reliability, respectively [33,34]. To improve statistical robustness, only chemicals with detection frequencies (DFs) of ≥60% were analyzed. Concentrations below the limit of detection (LOD) were substituted with LOD divided by square root of 2. All analyses were conducted on log10-transformed values due to their non-normal distributions.

According to their locations on placenta, the subsamples were categorized into three groups: peri-insertion (<2 cm), mid-disc (2–4 cm), and peripheral region (>4 cm). THs, elements, and VEs in these regions were compared using Kruskal-Wallis test.

The levels of THs, VE, and elements in basal plate and umbilical cord tissues were compared using Mann-Whitney *U* test. The concentration ratio between paired umbilical cord and placenta (C_c/C_p) was calculated to estimate the likelihood of bioaccumulation. Monoamines were measured in human placenta, umbilical cord and cord blood to investigate their origin and *trans*~~trans~~-placental properties.

All statistical analyses in this study were executed using R (version 3.4.2; R Foundation for Statistical Computing, Vienna, Austria). A p-value < 0.05 was considered as significant.

3 Results

Tables 1 and 2 present the results of TH, element, and VE analyses in human and porcine placentome, respectively. The DFs of T_4, T_3, and rT_3 in human placentome were 100% except T_3 in umbilical cords (27.8%). The mean levels were 42.3 ng/g fresh weight (fw) T_4, 0.66 ng/g fw T_3, and 2.74 ng/g fw rT_3, and <LOD for other THs in human basal plate, and 19.1 ng/g fw T_4, 0.52 ng/g fw T_3, 2.41 ng/g fw rT_3, and <LOD for other THs in human umbilical cord. T_4, T_3, and rT_3 were quantified in human cord blood with concentrations of 28.7 ± 7.4, 0.53 ± 0.26, 0.91 ± 0.18 ng/mL (mean ± SD), respectively. The DFs of T_4, T_3, rT_3, and 3,3′-T_2 in porcine placentome were >96%. The mean levels were 11.3 ng/g fw T_4, 0.70 ng/g fw T_3, 1.23 ng/g fw rT_3, 2.34 ng/g fw 3,3′-T_2, and <LOD for other THs in porcine basal plate, and 25.5 ng/g fw T_4, 0.63 ng/g fw T_3, 2.08 ng/g fw rT_3, and 3.55 ng/g fw 3,3′-T_2 in porcine umbilical cord. The DFs of elements were >96% in all subsamples.

As shown in Tables 1 and 2, the concentrations of elements in human basal plate, human cord, porcine basal plate, and porcine cord were 3.27 ng/g fw (As)–1.99 mg/g fw (K), 1.40 ng/g fw (Cd)–1.85 mg/g fw (Na), 0.98 ng/g fw (Cd)–1.95 mg/g fw (Na), and 3.48 ng/g fw (As)–1.74 mg/g fw (Na), respectively. The mean (SD) levels of Σ VEs were 15.9 (4.7), 1.30 (0.58), 1.96 (0.88), and 0.24 (0.05) μg/g fw in human placenta, human cord, pig placenta, and pig cord, respectively (Table 1 and 2).

In total, 5 polychlorinated dibenzo-*p*-doixins and furans (PCDD/Fs), 14 polychlorinated biphenyls (PCBs), and 4 brominated flame retardants (BFRs) had DFs of >60%. The mean concentrations were 0.03–0.10, 0.07–78.4, and 0.96–4.52 pg/g fw for PCDD/Fs, PCBs, and BFRs, respectively. The World Health Organization toxic equivalents (WHO-TEQ) of PCDD/Fs and PCBs were 0.04–0.06 and 0.03 pg/g fw, respectively[Instruction:

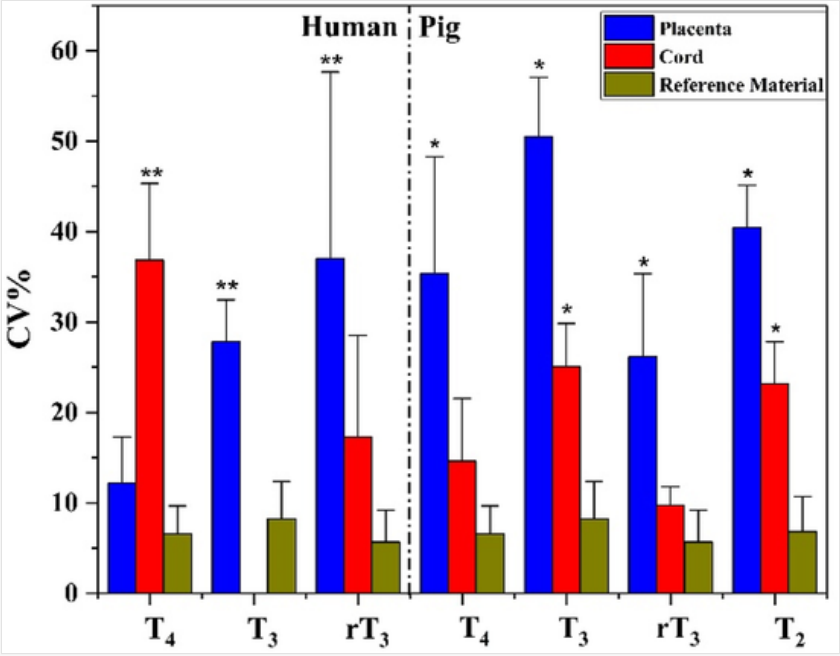
Please insert "(Table 3)" here.]. Norepinephrine (NE) and dopamine (DA) were quantified in human cord blood with concentrations of 0.21 ± 0.10 and 1.78 ± 0.74 ng/mL (mean ± SD), respectively. Other monoamines were < LOD in all human samples of basal plate, umbilical cord and cord blood.

As shown in Table 1, the levels of rT_3, As, Cr, Hg, Na, Al, and Σ heavy metals (the sum of As, Cd, Cr, Hg, and Pb) were similar between human basal plate and umbilical cord. T_4 ($p < 0.001$), Cd ($p < 0.001$), Se ($p < 0.001$), Zn ($p < 0.001$), Cu ($p < 0.001$), Fe ($p < 0.01$), Ca ($p < 0.001$), K ($p < 0.001$), Mg ($p < 0.001$), Σ elements (the sum of all the elements measured) ($p < 0.001$), α-tocopherol ($p < 0.001$), β-tocopherol ($p < 0.001$), β-tocotrienol ($p < 0.001$), and Σ VE ($p < 0.001$) levels were significantly higher in human basal plate than in umbilical cord, while Pb ($p < 0.001$) and Mn ($p = 0.01$) showed higher levels in umbilical cord with a C_c/C_p value of 6.80 and 1.75, respectively. In porcine tissues, T_3, As, Cr, Hg, Mn, Fe, Mg, and Σ heavy ~~element~~metals showed similar concentrations between basal plate and umbilical cord. Se ($p = 0.01$), Cu ($p < 0.01$), Ca ($p < 0.001$), Na ($p < 0.01$), α-tocopherol ($p < 0.001$), α-tocotrienol ($p < 0.01$), and Σ VE ($p < 0.001$) showed higher levels in basal plate. T_4 ($p < 0.001$), rT_3 ($p < 0.001$), 3,3′-T_2 ($p < 0.001$), Cd ($p < 0.001$), Pb ($p < 0.001$), Zn ($p < 0.001$), K ($p < 0.01$), and Al ($p < 0.001$) showed higher levels in umbilical cord. The C_c/C_p values of these chemicals were 1.16–14.2 (Table 2).

As shown in Tables 1 and 2, in human basal plate, the ICC measure of reliability was good (0.42–0.97) for T_4, 8 elements, and 4 VEs. ICC was poor (0.04–0.38) for T_3, rT_3, α-tocopherol, and remaining 7 elements. In human umbilical cord, reliability was moderate (0.40–0.76) for 4 elements and 5 VEs, while other chemicals showed low reliability (0.04–0.35). In porcine basal plate, the reliability was good (0.48–0.73) for T_4 and 2 elements (Mg, and Na). In porcine umbilical cord, ICCs was good (0.42–0.66) for T_4, rT_3, 4 VE, and 5 elements. Fig. 1 compares the biological and measurement variations of THs, the variances of T_3 ($p < 0.01$) and rT_3 ($p < 0.01$) in human basal plate, and T_4 ($p < 0.01$) in human umbilical cord were significantly higher than the measurement variances. The variances of T_4 ($p < 0.05$), T_3 ($p < 0.05$), rT_3 ($p < 0.05$), and 3,3′-T_2 ($p < 0.05$) in porcine basal plate, as well as T_3 ($p < 0.05$) and 3,3′-T_2 ($p < 0.05$) in porcine umbilical cord were higher than the measurement variances.

alt-text: Fig. 1

Fig. 1

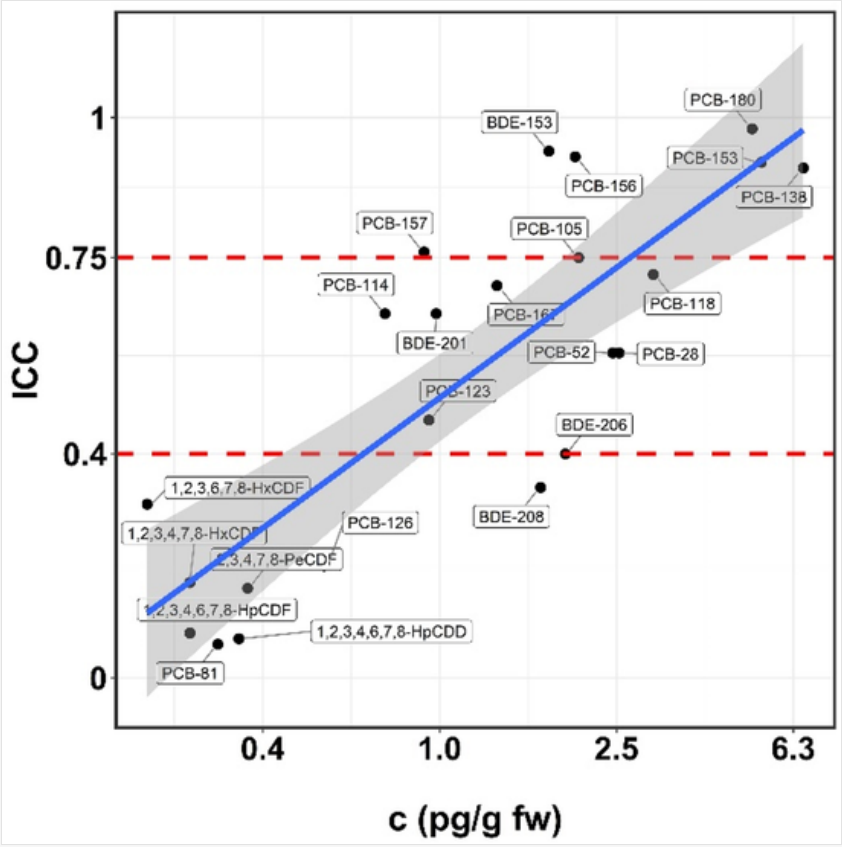


Comparison of biological variation against measurement variation in human basal plate, human umbilical cord, porcine basal plate, and porcine umbilical cord. Variation was estimated by coefficient of variation (CV%). A pooled placenta spiked with 30 ng/g of T_3, rT_3, 3,3′-T_2, 3,5-T_2, T_1, and T_1AM was used as reference material and measured repeatedly to estimate the measurement variances. The detection frequency of T_3 in human cord was <30%, and therefore not included in this analysis. *$p < 0.05$. **$p < 0.01$.

As shown in Table 3, the ICCs were low for all PCDD/Fs (0.078–0.31). PCBs showed good to excellent reliability (0.43–0.98) except PCB-81 and PCB-126 (0.06–0.20). ICCs were good to excellent (0.40–0.94) for most BFRs while was poor for BDE-208 (0.34). As shown in Fig. 2 and Fig. S5, the ICCs of POPs were linearly related with their concentrations, while the ICCs of THs and elements showed poor linearity with their concentrations.

alt-text: Fig. 2

Fig. 2



Correlation of the ICCs and concentrations of POPs with DF > 60%.

Table 4 compares the TH, element, and VE levels in the peri-insertion, mid-disc and periphery regions of human basal plate. The concentrations of T_4, T_3, As, Cr, Hg, Pb, Se, Fe, Ca, K, Mg, Na, Al, α-tocopherol, β-tocopherol, γ-tocopherol, β-tocotrienol, α-tocopherylquinone, Σ heavy metals, Σ elements, and Σ VEs were similar across the three regions. rT_3 ($p < 0.05$), Cd ($p < 0.05$), Zn ($p < 0.05$), Mn ($p < 0.05$), and Cu ($p < 0.05$) showed significant differences. Their levels in the periphery regions were 27.6%, 24.7%, 5.43%, 38.9%, and 5.32% lower than in peri-insertion regions, respectively. Table S6 shows the THs, elements, and VEs in these regions of porcine placenta. T_4, T_3, rT_3, 3,3′-T_2, Hg, Pb, Se, Zn, Mn, Cu, Fe, Ca, K, Mg, Na, α-tocopherol, α-tocotrienol, β-tocotrienol, γ-tocotrienol, Σ elements, and Σ VEs were similar across the different regions. As ($p < 0.05$), Cd ($p < 0.05$), Cr ($p < 0.05$), Al ($p < 0.05$) and Σ heavy metals ($p < 0.05$) showed significant differences. Their levels in the periphery regions were 4.12, 7.10, 2.64, 1.80 and 6.80 times higher compared with the peri-insertion region, respectively.

alt-text: Table 4

Table 4

The table layout displayed in this section is not how it will appear in the final version. The representation below is solely purposed for providing corrections to the table. To preview the actual presentation of the table, please view the Proof.

Comparison of TH and element levels among peri-insertion, mid-disc and periphery regions of the basal plate of human placenta.

	Peri-insertion[a]	Mid-disc[a]	Periphery[a]	*p*-value[b]
THs				
T_4 (ng/g)	41.1 (37.9–44.9)	42.1 (38.7–45.8)	44.6 (41.0–51.2)	0.192
T_3 (ng/g)	0.59 (0.48–0.75)	0.65 (0.52–0.83)	0.68 (0.55–0.78)	0.377
rT_3 (ng/g)	3.77 (2.97–3.98)	2.90 (1.79–3.83)	2.73 (1.43–3.18)	**<0.05**
Elements				
As (ng/g)	2.29 (1.47–4.97)	2.71 (1.50–8.42)	2.14 (1.39–3.96)	0.455
Cd (ng/g)	4.45 (3.09–5.48)	4.71 (3.96–5.00)	3.35 (2.43–4.48)	**<0.05**
Cr (μg/g)	4.87 (2.59–14.8)	7.03 (3.42–29.8)	4.28 (2.50–11.2)	0.363
Hg (ng/g)	6.75 (2.64–14.2)	8.09 (2.91–11.8)	3.23 (1.76–14.3)	0.505
Pb (ng/g)	7.67 (5.49–12.6)	7.67 (5.84–12.8)	8.17 (5.11–12.3)	0.812
Se (μg/g)	0.16 (0.15–0.17)	0.16 (0.15–0.18)	0.16 (0.15–0.18)	0.747
Zn (μg/g)	8.80 (8.31–9.45)	8.80 (8.39–9.39)	8.33 (7.95–8.96)	**<0.05**
Mn (μg/g)	0.18 (0.09–0.41)	0.18 (0.10–0.87)	0.11 (0.09–0.17)	**<0.05**
Cu (μg/g)	0.94 (0.81–1.03)	1.00 (0.88–1.22)	0.89 (0.80–0.97)	**<0.05**
Fe (mg/g)	0.19 (0.14–0.27)	0.18 (0.14–0.34)	0.20 (0.14–0.23)	0.501
Ca (mg/g)	0.52 (0.24–1.06)	0.45 (0.21–1.14)	0.72 (0.41–0.89)	0.563
K (mg/g)	1.97 (1.86–2.11)	1.97 (1.86–2.09)	1.92 (1.78–2.09)	0.313
Mg (μg/g)	81.1 (75.5–89.8)	79.3 (75.9–89.4)	75.9 (71.6–87.9)	0.162
Na (mg/g)	1.83 (1.71–2.00)	1.92 (1.74–2.01)	1.85 (1.63–2.01)	0.522
Al (μg/g)	0.39 (0.26–0.67)	0.41 (0.26–0.51)	0.36 (0.27–0.48)	0.765
Σ heavy metals (μg/g)[c]	4.80 (2.39–14.8)	7.05 (3.44–29.8)	3.99 (1.23–11.1)	0.237
Σ elements (mg/g)[d]	4.89 (4.47–5.53)	4.77 (4.31–6.17)	4.80 (4.55–5.18)	0.520
VE				
α-tocopherol (μg/g)	15.1 (12.6–18.0)	13.6 (12.8–16.5)	14.3 (12.2–16.6)	0.602
β-tocopherol (ng/g)	93.6 (84.1–110)	82.7 (75.9–93.3)	87.3 (80.0–111)	0.145
γ-tocopherol (ng/g)	311 (234–369)	288 (237–344)	274 (229–430)	0.798
β-tocotrienol (ng/g)	5.79 (3.15–8.00)	5.42 (5.10–6.40)	5.67 (4.63–9.80)	0.668
α-tocopherylquinone (ng/g)	407 (291–601)	412 (244–598)	425 (344–644)	0.614
Σ VE (μg/g)	16.0 (13.3–18.9)	14.6 (13.5–17.2)	15.1 (12.9–17.7)	0.585

Abbreviations: VE, vitamin E. [a]Median (25th–75th percentiles). [b]The difference of TH and element levels between peri-insertion, mid-disc, and periphery regions were compared using Kruskal-Wallis test. The different regions were divided according to the distance from the spot to the center of the placenta, < 2 cm for peri-insertion, 2–4 cm for mid-disc, and >4 cm for periphery. [c]The sum of As, Cd, Cr, Hg, and Pb. [d]The sum of all the elements measured.

4 Discussion

4.1 Chemical analysis in placentome

The TH levels in human basal plate of placenta reported here were similar to previous findings using the entire human placenta [31]. Previous studies reported that the average levels of As, Cd, Hg, Pb, Se, Zn, Mn, Cu, Ca, K, Mg, Fe, and Al in human placenta were 0.006, 0.004, 0.008, 0.034, 0.2, 10, 0.08, 0.9, 770, 1685, 100, 69, and 0.25 μg/g fw, respectively [18,35,36]. These values were in line with our results although the authors did not indicate the sampling positions. The placental levels of BFRs measured here were also close to previous reports [37]. However, higher levels of Cr, Fe, and PCBs were observed in this study [35–37], probably due to the differences in exposure level, blood content, potential contamination during sampling, and sample size. NE in cord blood measured here was about 10 times lower compared with previous study while DA was higher [38]. Monoamines were < LOD in all placenta samples, probably because of the high monoamine oxidase activity in placenta [39]. VEs are fat-soluble nutrients that are important during early stage of life. We quantified several VEs in placentome, in which α-tocopherol is the dominating congener.

4.2 Distribution of biomarkers in placenta and umbilical cord

In placenta, the fetal blood vessels locate close to the surface of the expanding trophoblastic villi to approximate with maternal vessels [2]. This interface allows the exchange of chemicals between maternal and fetal circulations. The following mechanisms are potentially involved: (1) Passive diffusion [3]; (2) Transporting proteins, i.e., transthyretin (TTR) [40], metallothionein [41], and transferrin [42]; (3) Local metabolism, i.e., deiodination [43], oxidation [44,45], and sulfotransferation [46]; (4) Lipid content [10] and blood content [16]. Environmental exposure [40,46–48] and maternal physiological conditions [18,22,49] can influence this process by interfering with the expression of transporters and enzymes.

Only a few previous studies examined the transfer of certain elements in human placentome [16,18]. Our results show that THs and elements behave differently in human and porcine placentome. This is probably due to the structural differences between human and porcine placentas. Human placenta is hemochorial in which the placental trophoblast is in direct contact with maternal blood, while pig placenta is epitheliochorial in which the trophoblast is apposed to the epithelium of the uterus [50]. The ample fetal villi section is rich in umbilical arteries and veins. The chemical distribution variability in this section of placenta mainly results from the differences in blood content [16].

Trans~~Trans~~-placental delivery of THs is regulated by plasma membrane transporters, iodothyronine deiodinases and proteins within trophoblast cells [1]. While T_4 level was higher in the basal plate of human placentome, T_4, rT_3 and 3,3′-T_2 showed higher levels in umbilical cord of porcine tissues. This is probably due to the differences in the expression of TTR and deiodinases. Besides, the THs in term placenta may also originate from fetal circulation. The placenta is known to block the transfer of Cu and Cd, but is a weak barrier for Pb and Mn [35,51,52], which explains the higher Cu and Cd levels but lower Pb and Mn levels in human placenta here. This has potential clinical implications because Cd exposure could reduce the essential Ca and Zn transfer to the fetus by competitively binding with metallothionein [36], while perinatal Pb exposure may contribute to the etiologies of autism spectrum disorder [4]. Previous studies observed higher levels of Ca and Zn in maternal compartment than in fetal portion [16,18], which agrees well with our study. However, de Moraes *et* al. found lower Ca level in placenta of adult mother [18], suggesting an age-dependent expression of Ca^{2+} transporter/channel. Human placenta could partially block the As transfer [36]. However, we did not find significant difference of As level between human placenta and cord. Fe from maternal circulation is transferred to the fetus by binding with

transferrin along a unidirectional pathway [53], leading to lower Fe level in human umbilical cord. An active transfer of Hg in placenta was suggested previously [54], which was not found in this study, probably due to the high variation of Hg concentration in our data. Some other studies assessed the distribution of elements by comparing their levels in maternal plasma and cord blood, which revealed higher levels of Cd, Cu, Zn, and Se in maternal plasma [52,53]. These results are in line with our study. Previous study observed lower α-tocopherol level in cord blood than in maternal circulation [55], which is in line with our results.

Although 9 monoamines were targeted for analysis, only NE and DA were quantified in human cord blood while the other congeners were <LOD in all samples. These results suggest that the NE and DA in cord blood might be of fetal origin.

4.3 Distribution variability of chemicals in placental basal plate

The poor reliability of certain THs and elements in placentome observed here is consistent with previous reports [16,17]. rT_3, Cd, Zn, Mn, Cu in human basal plate, and As, Cd, Cr, Al, and Σ heavy metals in porcine basal plate showed significant differences among the regions. These results corresponded well with their low ICC values, although we did not observe regional difference for some other chemicals with low ICC such as Pb in human basal plate. Besides, the significant differences of biological variation of certain THs compared with measurement variation also confirmed their low ICCs. To our knowledge, only one study assessed the regional distributions of chemicals in placenta, in which higher Fe level was found in the central region of human placenta. The authors attributed to the regional differences in blood flow [16]. In this study Fe concentration was similar among these regions (p = 0.501), indicating similar blood content among the subsamples. Manci *et* al. also observed higher Ca concentration in the periphery region [16]. In our study, Ca level in the periphery was higher but not significant. Besides, we observed higher concentrations of As, Cd, Cr, Al, and Σ heavy metals in the periphery region of porcine placenta. These results indicate that the regional differences might be due to the regional expressions of transporting proteins and metabolic enzymes. VEs in basal plate generally showed good reliability in basal plate, probably due to their high fat solubility.

Exposures to the most congeners of PCBs and PBDEs had a high degree of reliability in placenta in our study, with ICCs of 0.40–0.98 except PCB-81, -126, and BDE-208. However, all the PCDD/Fs and their WHO-TEQ values showed low reliability. These findings suggest different transfer mechanisms of PCDD/Fs with PCBs and PBDEs. For example, PCDD/Fs may induce the expression of cytochrome P450 enzymes which may interfere with their metabolism and clearance [56], leading to larger variances. Besides, the poor linearity of the ICCs of PCDD/Fs with their levels indicates that the low reliability might be the results of low concentrations and high measurement variation. The low reliability of certain THs and elements, however, might be due to biological variances.

Taken together, our results indicate that substantial exposure misclassification may happen in epidemiological studies when placental TH and element exposures are classified using a single spot placenta or umbilical cord samples. To avoid potential sampling bias, previous studies advised to collect the entire placenta or clearly define the location of the sampling sites [11]. However, due to the differences in placental distribution characteristics, we propose to consider different sampling procedures. However, collecting the entire placenta or pooled samples from different regions seems unavoidable for most THs, elements and PCDD/Fs. Some other chemicals such as monoamines in cord blood, however, may originate from the fetus. Placenta is not an appropriate source for such biomarkers to assess maternal situations.

Placental sampling is critical due to the size and potential heterogeneity of biomarkers. A proper sampling protocol is essential for generating subsamples to evaluate placental distribution. Burton *et* al. summarized the factors surrounding placenta collection that may influence data obtained, and proposed that placenta should be sampled from at least 4 sites [57]. Our method stepped further and proved the regional variation of biomarkers in placenta.

4.4 Strengths and limitations

This study has several strengths: (1) >100 chemicals (THs, elements, POPs, VEs, and monoamines) were analyzed in this study, which can represent the most prominent constituents in intrauterine environment; (2) We comprehensively assessed the distribution properties of the chemicals. This could help to optimize the sampling strategy, reduce sampling bias, and better indicate the disease etiology and progression; (3) The cryo-sampling procedure could be used to assess the distribution of other biomarkers and larger sets as well as retrospective studies on persistent biomarkers. Pooling of these subsamples could reduce sample size and sampling bias; (4) Fine-powder samples were generated using this protocol, which are very suitable for storage, transportation, as well as chemical or biological analysis; (5) This protocol can be conducted off delivery room, and thus, the degradation/transformation of the biomarkers can be avoided. Our study also has certain limitations. For example, the number of tissues is limited, which may reduce the statistical power. Previous studies observed differences of the element levels between maternal and fetal placenta [18,21]. TH and elemental analyses in this study were performed in basal plate although the levels here were similar to previous reports with entire placenta. Besides, our protocol may not be suitable for biomarkers that are not stable during freeze-thaw cycles. Finally, metal tools were used in this protocol, which may raise contamination for elemental analysis.

To conclude, a novel cryo-based collection of placentome off delivery room, its storage, sampling, and subsampling obtaining homogenous powders were developed to enable studies of large sets of biomarkers and repetitive and retrospective analysis of persisting markers. We investigated the placental distribution of various biomarkers using the subsamples. We found heterogeneous distributions for certain THs, elements, and PCDD/Fs, while PCBs, PBDEs, and VEs showed high reliability. Our results suggest different modes of placental distribution, and highlight the challenges of assessing intrauterine exposures because of the sampling bias. Different sampling strategies should be applied according to their distribution variation. Our protocol enables future studies to reduce sampling bias and increase reproducibility. Further studies regarding the regional differences of transporting proteins and enzymes are warranted. This protocol can serve as a starting point for future studies to improve placenta sampling technique and reproducibility.

Author contribution

Z.-M. L was involved in the study design, data-acquisition, measurement performance, data-analysis and manuscript writing. B. B., Q. B., B. H., C.C., and B. M. were involved in measurement performance. J. P.-F. and K. F. were involved in sample collection. M. D. A. and K.-W. S were involved in the study design, sample collection, data interpretation, and manuscript review. All authors have read and approved the final version of this manuscript.

Declaration of competing interest

There are no conflicts of interest.

Acknowledgements

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Appendix A Supplementary data[Instruction: Please replace the Supplementary data with the attached file.]

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2020.08.009.

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The corrections made in this section will be reviewed and approved by a journal production editor. The newly added/removed references and its citations will be reordered and rearranged by the production team.

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Highlights

- A protocol was developed for placenta sampling to reduce sample size and bias.
- The distribution of a wide variety of biomarkers was evaluated.
- Both human and porcine placentome were employed.
- Different chemicals showed different modes of distribution.

Appendix A Supplementary data

The following is the Supplementary data to this article:

Multimedia Component 1

Multimedia component 1

alt-text: Multimedia component 1

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