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Maternal and placental responses before preterm birth: adaptations to increase fetal thyroid hormone availability?

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Abstract

Background: During pregnancy, maternal thyroid hormone supply is crucial for fetal development. Preterm infants often present with hypothyroxinemia. Preterm birth, either spontaneous or medically indicated, is always the result of a complicated pregnancy. We hypothesized that in preterm birth, the maternal transplacental thyroid hormone supply is influenced by the pregnancy complication and we questioned whether maternal and placental compensatory mechanisms are activated to increase thyroid hormone transfer.

Methods: Observational case-control study in mother-infant-dyads with complicated pregnancies ending in spontaneous preterm birth (n=31) or indicated preterm birth due to vascular complications (n=45) and normal pregnancies (healthy term controls; n=41). At delivery, maternal and cord blood and placenta samples were collected. Cord and maternal plasma concentrations of TSH, total T4, fT4/FTI, total T3 and TBG, and maternal serum concentrations of TPO-antibodies were measured.

Placental maturity was evaluated histologically and mRNA and/or protein levels of thyroid hormone deiodinases (DiO) 1, 2 and 3 and transporters (MCT8, MCT10 and OATP1c1) were quantified.

Results: In indicated and spontaneous preterm births, cord plasma T4 concentrations were lower than in healthy term controls ($P \leq 0.001$), whereas T3 was only decreased in spontaneous preterm birth ($P \leq 0.001$). Compared with spontaneous preterm births and healthy term controls, indicated preterm birth was characterized by higher maternal plasma TSH ($P \leq 0.05$), earlier placental maturation, higher placental DiO2 gene and MCT10 protein levels and lower DiO3 gene levels (all $P \leq 0.01$).

Conclusions: Low T4 was observed in preterm infants irrespective of the cause of preterm birth, while maternal (TSH) and placental (Dio2, Dio3 and MCT10) compensatory responses were only activated in indicated preterm birth due to vascular complications. This may have mediated the normal fetal T3 availability in preterm infants born after indicated preterm birth but not after spontaneous preterm birth.

Introduction

Thyroid hormones are essential for fetal and neonatal maturation and crucial for brain development [1]. Until mid-gestation, the fetus is completely dependent on maternal thyroid hormone supply [2, 3, 4]. The embryogenesis of the fetal structural thyroid system is largely complete at 12 weeks of gestational age, but it is not until mid-gestation that the fetal hypothalamic-pituitary-thyroid-axis starts to function progressively [5]. Nevertheless, maternal thyroid hormone supply remains present and only after birth, the infant's thyroid system functions completely autonomously [6].

Maternal thyroid hormones are transferred through placental thyroid hormone transporters [7]. There are indications of altered expression of monocarboxylate transporters (MCT) 8 and 10 in placentae of pregnancies complicated by intrauterine growth restriction (IUGR) [7]. In rodents, organic anion transporting polypeptide (OATP) 1C, the transporter with the highest affinity for thyroid hormones, plays an essential compensating role when the transplacental passage of maternal thyroid hormones is low, before the onset of fetal thyroid hormone production [8]. Maternal thyroid hormones are activated or inactivated by placental deiodinating enzymes. Iodothyronine deiodinases (DiO) DiO2 and DiO3 are expressed in human placentae [9, 10]. DiO2, an outer ring deiodinase, is the primary activating thyroid hormone enzyme. It catalyzes the conversion of thyroxine (T4) to triiodothyronine (T3) [9]. Dio3, the predominant thyroid hormone inactivating enzyme, catalyzes the monodeiodination of T4 to reverse T3 and of T3 to diiodothyronine [10]. Placental DiO3 activity is 200 times higher than DiO2 activity, suggesting a role for DiO3 in protecting the fetus from maternal thyroid hormone oversupply [11, 12]. Both placental DiO2 and DiO3 activity levels decline as gestation progresses [12]. Preterm birth occurs during the critical period of in-utero transition from maternal to fetal thyroid hormone dependence [13]. Preterm newborns present with lower circulating cord blood T4 levels compared to term newborns [14]. This phenomenon can persist during the first weeks

of life and is known as transient hypothyroxinemia of prematurity (THOP). THOP is defined as transient low T4 levels with thyroid stimulating hormone (TSH) levels in the normal to lower range. The prevalence is approximately 50% in extremely low gestational age neonates (ELGANs, i.e. gestational age <28 weeks) [5]. Consistent with the importance of thyroid hormones in fetal and neonatal development, several studies have demonstrated the association between THOP and low IQ and impaired motor development [15, 16], although other studies failed to show the same results [17, 18], making it still a topic of debate. Sudden loss of maternal thyroid hormone supply is one of the contributing factors to hypothyroxinemia in preterm infants [5, 19]. However, preterm birth has many causes with different manifestations in placental histology and function. In general, they can be classified as spontaneous preterm birth or medically indicated preterm birth [20]. Although the etiology of indicated preterm birth can vary, pregnancy-associated vascular pathology is a particular chronic pregnancy complication, starting early in pregnancy. It is associated with hypoxia, resulting in villous maldevelopment, signs of premature aging of the placenta and subsequently IUGR [21].

To obtain deeper insight in the development of THOP, we hypothesized that in preterm birth, the maternal pregnancy complication influences the transplacental thyroid hormone supply to the fetus and we questioned whether maternal and placental compensatory mechanisms are activated to increase thyroid hormone transfer to the fetus.

Methodology

Patient selection

This study was part of a larger observational case-control study, conducted from September 2011 until March 2014, in a single tertiary care hospital after approval by the local ethical

committee (S53423) of the University Hospitals Leuven and registration at Clinicaltrials.gov (NCT01466023). Patients at risk of preterm delivery and patients with healthy term pregnancies were included in the study after obtaining written informed consent. Pregnancies complicated with fetal congenital malformations were excluded.

For the current study, patients were classified into either spontaneous or indicated preterm birth due to vascular cause (pregnancy-associated hypertension, preeclampsia, HELLP syndrome), based on the clinical diagnosis of the obstetrician, combined with the histological diagnosis of the pathologist [20]. Exclusion criteria were: indicated preterm birth due to non-vascular pathology, maternal antithyroid drug use and multiple pregnancies, since the number of fetuses also influences maternal thyroid function [22].

Relevant clinical data were collected: maternal age, parity, maternal education level, body mass index (BMI), tobacco use, documented and treated thyroid dysfunction, course of betamethasone, mode of delivery, gestational age, birth weight, presence of IUGR, placental weight, placental weight / gestational age and Clinical Risk Index for Babies (CRIB) score, reflecting severity of illness in preterm infants [23].

Sample collection

At delivery, both maternal and fetal cord blood plasma and serum samples were collected, immediately centrifuged and stored at -20°C. Right after birth, the placenta was weighed and full-thickness biopsies were taken from the peri-umbilical region. Placental tissue was dissected off the chorionic plate and washed in iced 0.9% saline solution. One sample was fixed in 4% formaldehyde solution and after 24 hours embedded in paraffin. Six samples were snap-frozen in liquid nitrogen before storage at -80°C [12].

Blood analysis

TSH, total T4, total T3, T4 uptake and T4 binding globulin (TBG) were determined on all the samples. Free T4 index (FTI = total T4 / T4 uptake) and thyroid peroxidase (TPO) antibodies were determined in pregnant women and free T4 (fT4) in neonates. Assays used were as follows: for TSH: sandwich immunoassay with electrochemiluminescence (ECL), Hitachi/Roche-Modular E; for T4, T3 and fT4: competitive immunoassay with ECL, Hitachi/Roche-Modular E; for TBG: RIA-gnost TBG kit, CISBIO; for TPO antibodies: enzyme immunoassay, Genway Biotech. No specific reference ranges for pregnant patients were available. Reference ranges for cord blood were: for TSH: 0.27–4.20 mIU/L; for T4: 5.1–14.1 µg/dL; for T3: 80–200 ng/dL; for fT4: 0.93–1.7 ng/dL; for TBG: 14.0–24.0 mg/L.

Placental mRNA and protein levels

A full-thickness placental biopsy was homogenized and RNA and proteins were isolated with a commercial kit for RNA/protein isolation (Nucleospin NA/protein from Mechery-Nagel). RNA was reverse transcribed and cDNA of Dio1, Dio2, Dio3, MCT8, MCT10 and OATP1C was quantified in real-time as previously described with commercial TaqMan® Assays (Applied Biosystems) [24]. Data were normalized to ribosomal 18S (*Rn18s*) gene expression and expressed as fold change of the mean of the controls. Protein levels of Dio2 (ab77481, Abcam), Dio3 (ab102926, Abcam), MCT8 (PA5-36978, Thermo-Fischer) and MCT10 (PA5-50760, Thermo-Fischer) were quantified as previously described [24]. Concentrations were expressed relative to the means of the controls.

Evaluation of premature aging of the placenta

Hematoxylin and eosin staining was done on 4-µm-thick sections of paraffin-embedded placenta biopsies. Placental images were blinded and independently scored twice for premature aging according to presence of Tenney-Parker changes and increased branching [21]. As a

quality control, in those samples for which a clinical diagnosis was available, scoring was compared to it.

Villi sizes were quantified by the following procedure: Conversion of RGB image to L*a*b and the HSV color spaces to obtain separate color and intensity information in different channels, application of an entropy filter to the intensity channel to obtain a new channel with textural information, use of a logistic regression classifier to assign to each pixel a probability of belonging to villi tissue. The features used corresponded to the pixel values in each of the previously described channels, as well as aggregate values over the entire image obtained from applying Otsu thresholding to each channel [25]. To develop the classifier, a subset of images was manually labeled to indicate the tissue corresponding to villi. After classification, the channel obtained with the probabilities for each pixel was further thresholded to obtain a binary classification of belonging to villi tissue for each pixel. Performance of morphological operations on the selected pixels was performed to generate segments. Only segments of sufficient size and solidity were kept. Henceforth these segments were considered the automatically identified villi. The size of each villus was computed as the area of the corresponding segment, which is equivalent to the number of pixels in the segment. Analyses were performed with the Statistics and Image processing toolboxes in MATLAB 2016b (The MathWorks, Natick, MA).

Statistical analysis

Statistical analyses were done using IBM SPSS Statistics. Shapiro-Wilk was used for normality testing, if $P \leq 0.05$, data were log-transformed and reassessed for normality. Normally distributed data were analyzed using one-way ANOVA tests with post-hoc independent-samples *t*-tests. The nonparametric Kruskal-Wallis and Mann-Whitney U tests were used when

data were not normally distributed. Chi-square test for independence and Fisher's exact tests were used for categorical variables. Correlation analysis was performed with two-tailed Pearson and Spearman Rho coefficient. To correct for potential gestational age-related effects, analyses were repeated in 27 patients with spontaneous preterm birth matched to 27 patients with indicated preterm birth with use of propensity scores obtained by logistic regression for 3 gestational age categories (24–27 weeks, 28–31 weeks, 32–36 weeks). Similarly, placenta analyses were repeated in 20 patients with spontaneous preterm birth matched according to GA to 22 patients with indicated preterm birth. Data are expressed as mean $\pm$ SD or median with interquartile range. Statistical significance was assumed for a two-sided P value ≤ 0.05 .

Results

Patient characteristics

Of the 319 patients who consented to the larger project, 117 patients were found eligible for this analysis (Fig. 1). Table 1 summarizes maternal, neonatal and placental characteristics. Thirty-one patients with spontaneous preterm birth, 45 patients with indicated preterm birth and 41 healthy term controls were compared. Maternal education level was significantly lower in mothers with preterm delivery and they had a higher caesarean section rate compared with healthy term controls. Mothers with indicated preterm birth had significantly higher BMIs and more tobacco use than the other two groups. Also median gestational age was significantly higher in the group with indicated preterm birth than in the spontaneous preterm birth group. In contrast, mean birth weight was not different between the indicated and spontaneous preterm birth groups, but there was significantly more IUGR in the indicated preterm birth group than in the spontaneous preterm birth group. Placental weight in spontaneous and indicated preterm birth was comparable, but placental weight / gestational age ratio was significantly lower in the indicated preterm birth group compared to the spontaneous preterm birth group.

Maternal and neonatal TH plasma concentrations at time of birth

Maternal circulating TSH levels were higher in the indicated preterm birth group compared to the spontaneous preterm birth group and healthy term controls (Fig. 2A). Circulating total T4, FTI and total T3 were comparable in all groups (Fig. 2B-D), as were circulating TBG levels (data not shown, $P=0.4$). TPO antibodies were positive in 5/117 patients: 1/31 in the spontaneous preterm birth group, 2/45 in the indicated preterm birth group and 2/41 in the healthy term controls ($P=1.0$).

Neonatal cord blood TSH levels were comparable in all groups (Fig. 3A). Cord blood T4 and fT4 levels were significantly lower in the spontaneous and indicated preterm birth groups, compared with the healthy term controls (Fig. 3B-C). Cord blood T3 (Fig. 3D) and TBG levels (data not shown, $P<0.001$) were significantly lower in the spontaneous preterm birth group compared to both the indicated preterm birth group and the healthy term controls.

To exclude a potential bias due to the lower median gestational age in the spontaneous preterm birth group, cord blood thyroid hormone levels were also compared in a gestational age matched subset of 27 patients with indicated preterm birth and 27 patients with spontaneous preterm birth. In this subset, neonatal cord blood T3 levels remained significantly lower in the spontaneous preterm birth group compared to the indicated preterm birth group ($P=0.02$), whereas TSH, total and fT4 and TBG were comparable in both groups (data not shown). After adjustment for smoking and obesity ($\text{BMI} \geq 30$ according to the definitions of the World Health Organization [26]), maternal circulating TSH levels were comparable in all groups. Cord blood T4 and fT4 levels remained significantly lower in the spontaneous and indicated preterm birth groups, compared with the healthy term controls ($P<0.001$ and $P<0.005$) (data not shown), whereas cord blood T3 remained significantly lower in the spontaneous preterm birth group

compared to both the indicated preterm birth group and the healthy term controls ($P \leq 0.001$) (data not shown).

Placental expression of TH transporters and deiodinases

Placental tissue was sampled from 20/31 patients with spontaneous preterm birth, 31/45 patients with indicated preterm birth and 35/41 healthy term controls (Patient characteristics: Supplemental Table 1, Maternal and cord blood thyroid hormone levels: Supplemental fig. 1-2). MCT8 mRNA and protein levels were comparable in all groups (Fig. 4A-B). There was overall no difference in MCT10 mRNA levels, but MCT10 protein levels were significantly lower in the spontaneous preterm birth group compared to the indicated preterm birth group and healthy term controls (Fig. 4C-D). OATP1C mRNA levels were below the detection limit and therefore not quantified on protein level.

Dio1 mRNA levels were low and overall not significantly different in the three groups (data not shown, $P=0.2$) and therefore also not quantified on protein level. Dio2 mRNA levels were higher in the indicated preterm birth group, compared to the spontaneous preterm birth group and healthy term controls (Fig. 4E). Dio2 protein levels were only significantly higher in the indicated preterm birth group compared to healthy term controls (fig. 4F). Dio3 mRNA levels were lower in the indicated preterm birth group, compared to the spontaneous preterm birth group and healthy term controls (Fig. 4G), but protein levels were comparable in all groups (Fig. 4H).

In the subset matched for gestational age, MCT8 mRNA, MCT10 mRNA and Dio1 mRNA levels were similar in the indicated and spontaneous preterm birth groups. However, Dio2 mRNA levels remained significantly higher ($P=0.001$) and Dio3 mRNA levels significantly lower ($P = 0.004$) in the indicated preterm birth group compared to the spontaneous preterm

birth group. Protein levels of MCT8, DiO2 and DiO3 were similar, but MCT10 protein levels were significantly higher in the indicated preterm birth group compared to the spontaneous preterm birth group ($P<0.001$) (data not shown). After adjustment for smoking and obesity ($\text{BMI} \geq 30$ according to the definitions of the World Health Organization [26]), DiO2 mRNA levels remained significantly higher ($P<0.001$) and DiO3 mRNA levels significantly lower ($P<0.05$) in the indicated preterm birth group compared to the spontaneous preterm birth group. Also MCT10 protein levels remained significantly lower in the spontaneous preterm birth group compared to the indicated preterm birth group and healthy term controls ($P<0.001$) (data not shown).

Neonatal cord blood T3/T4 ratio and DiO2 mRNA levels correlated significantly in the overall study population ($r = 0.3$, $P = 0.003$) as did MCT10 protein levels with cord blood T3 ($r = 0.2$, $P = 0.03$).

Assessment of premature aging of the placenta

In 38/86 placenta samples, the clinical diagnosis of the pathologist was available and in 34 of these 38 samples, there was agreement in assessment of premature aging of the placenta between the pathologist and the researcher's score system. Early placental maturation correlated significantly with lower median villi area ($r = 0.3$, $P = 0.01$).

In 8/20 placentae of the spontaneous preterm birth group and in 18/31 placentae of the indicated preterm birth group, there were histological signs of early maturation ($P=0.2$). However, in the subgroup matched for gestational age, placentae of the indicated preterm birth group showed significantly more histological signs of early maturation than placentae of the spontaneous preterm birth group ($P=0.04$). In the group of preterm infants (gestational age <37 weeks) as well as in the subgroup matched for gestational age, median villi area was significantly lower

in the indicated preterm birth group than in the spontaneous preterm birth group ($P=0.01$ and $P=0.04$, respectively), indicating early maturation (Fig. 5C, D).

Discussion

This observational study demonstrated that mothers with indicated preterm birth due to chronic pregnancy-associated vascular pathology had signs of thyroid and placental thyroid hormone activating mechanisms, which were not present in mothers with spontaneous preterm birth. This was reflected by increased maternal circulating TSH levels, increased placental Dio2 mRNA levels, decreased placental Dio3 mRNA levels and increased placental MCT10 protein levels together with advanced histological placenta maturation in the indicated preterm birth group but not in the spontaneous preterm birth group. Furthermore, infants of the indicated preterm birth group showed higher circulating T3 levels, compared to gestational age matched peers of the spontaneous preterm birth group. These findings suggest the existence of placental compensating mechanisms to provide the fetus with sufficient thyroid hormones in case of chronic placental pathology.

Cord blood T4 and fT4 levels were significantly lower in both spontaneous and indicated preterm births than in healthy term controls, which is probably due to fetal thyroid hormone system immaturities [5]. However, the expected low cord blood T3 levels, as seen in spontaneous preterm birth, could not be demonstrated in the indicated preterm birth group. They were in the same range as term cord blood T3 levels. This difference remained in the subset matched for gestational age, filtering out a possible maturity-related mechanism. Maternal or placental generated T3 as the source of fetal T3 could be possible explanations for the increased cord blood T3 levels in indicated preterm birth. Similar results were described in neonates with congenital hypothyroidism due to a total iodide organification defect or thyroid agenesis where

substantial amounts of T4 were found in cord blood, indicating maternal transfer of thyroid hormones during late gestation [27]. Remarkably, in these neonates, T4 levels were considerably lower compared to the levels of euthyroid neonates, but T3 concentrations were in the same range [11].

In all groups, cord blood TSH levels were high, but comparable. TSH normally peaks within the first hour after birth and declines progressively over 24–48 hours, partly triggered by cord cutting and cooling. The intrauterine trigger is unknown [28]. The TSH surge has been documented to be limited in preterm newborns, especially in ELGANs [5]. In our study, cord blood TSH levels were similar in preterm and term neonates. This could be partly due to the heterogeneity of gestational age in our groups, with only a minority of ELGANs in both preterm groups. Cord blood TSH levels were shown to be lower in infants born after caesarean section compared to infants born after vaginal delivery [29]. The high number of caesarean sections in this study might have negatively affected median cord blood TSH levels in controls.

Mothers with indicated preterm birth showed an increased health risk profile compared to mothers with spontaneous preterm birth: they had significantly higher BMI and higher incidence of tobacco use. Median maternal circulating TSH levels were slightly increased compared to mothers of the spontaneous preterm birth group and healthy term controls. The median circulating TSH level in the indicated preterm birth group was 2.3 mIU/L, which is still below the recommended upper reference limit in the second and third trimester of pregnancy to define subclinical hypothyroidism [30]. Only 4.3% of mothers were TPO-antibody positive and these were equally distributed among the 3 groups. Thus, thyroid autoimmunity is not an explanation for the increased maternal TSH levels in indicated preterm birth.

Corrected for gestational age, placental weight in indicated preterm birth was significantly lower compared to the other groups. Histologically, there were more signs of early maturation in placentae of the indicated preterm birth group compared with placentae of the spontaneous preterm birth group, reflecting Tenney-Parker changes. These are considered placental adaptation mechanisms in response to persistent hypoxia [31]. The onset of pathophysiology in spontaneous preterm birth is in general more acute and therefore, one would expect less placental compensatory mechanisms.

We observed no differences in MCT8 mRNA and protein levels between all groups. MCT10 mRNA levels were also comparable between all groups, but MCT10 protein levels were significantly higher in the indicated preterm birth group compared to the spontaneous preterm birth group and similar to healthy term controls. Compared to MCT8, MCT10 has a greater ability to transport T3, suggesting that the increase in MCT10 protein levels could indeed contribute to the increased cord blood T3 levels in the indicated preterm birth group [32].

Of further interest was the significant upregulation in Dio2 mRNA levels and downregulation in Dio3 mRNA levels in placentae of indicated preterm birth compared with the other groups. These differences remained statistically significant in placentae matched for gestational age. Increased Dio2 expression would allow more T4 to T3 conversion and decreased Dio3 expression would suggest less thyroid hormone inactivation, together again suggesting a compensatory reaction to increase active T3 in the fetus. Indeed, cord blood T3/T4 ratios correlated well with Dio2 gene mRNA levels. Although possible placental compensation mechanisms on the deiodinase level in infants with IUGR could not be demonstrated by the group of Chan [12], our results are in the same line as other animal and *in vitro* studies. In cultured human placental cells, thyroid hormone deprivation increased Dio2 activity and addition of thyroid hormone to the hormone-depleted medium impaired the rise of Dio2 in a

dose-dependent way [33]. These findings were also demonstrated in several rat tissues *in vitro* and *in vivo*, suggesting an adaptive function for DiO2 [8, 34, 35, 36]. Underlying mechanisms are speculative. Down-regulation of DiO2 mRNA expression mediated by the nuclear T3 receptor, and substrate-induced enzyme inactivation are two known mechanisms of thyroid hormone mediated DiO2 inactivation in rodent brain [37, 38, 39]. DiO3 expression in brain is positively controlled by thyroid hormones, probably at the pretranslational level [37, 40]. However, it is hypothetical to extrapolate these findings of DiO2 and DiO3 regulation to the human placenta. It is also debatable whether a local increase of placental DiO2 acts to increase local placental T3 bio-availability rather than to supply the fetus with T3, since T3 also plays a role in placentation [41].

The following limitations have to be highlighted. Due to the clinical context, heterogeneity in patients, reflected in the study groups, was inevitable. Although mothers with use of antithyroid drugs were excluded, we did not exclude mothers on levothyroxine treatment. Yet, post-hoc statistical analysis, after exclusion of those patients, confirmed our findings. Also, the classification into either spontaneous or indicated preterm birth was based on the clinical diagnosis of the obstetrician, combined with the histological diagnosis of the pathologist, which may contain misdiagnoses. A recent cross-sectional study indeed showed an incorrect classification of preterm birth into spontaneous or indicated preterm birth subtypes in 5%–15% of the cases, even with experienced researchers [42]. Also in the assessment of premature aging of the placenta, imperfections may be present, since there was no complete agreement between the diagnosis of the pathologist and the researcher's score system. However, the assessment was combined with objective measurements of the median villi area and placentae with histological signs of early maturation correlated well with smaller median villi area, making the assessment more robust. We did not collect data on first trimester maternal circulating thyroid

hormone levels, which would have given more information on the evolution to the pre-delivery levels. We also observed a discrepancy between gene and protein expression for MCT10, Dio2 and Dio3. Posttranscriptional or posttranslational upregulation might be hypothetical explanations for this discrepancy between gene and protein expression [38] or be related to lack of sensitivity of Western blot quantifications. Finally, measurement of direct actions of T3 on the placenta, for example thyroid hormone receptor-regulated inflammation-related molecules [43] would have strengthened our findings, but were beyond the scope of the current study.

Conclusion

Hypothyroxinemia was observed in preterm infants irrespective of the cause of preterm birth, but maternal (TSH) and placental (Dio2, Dio3 and MCT10) compensatory responses were only activated in indicated preterm birth, which may have mediated the normal fetal T3 availability in infants born after indicated preterm birth but not after spontaneous preterm birth. More research is necessary to reveal the underlying regulating mechanisms.

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Author Disclosure Statement

No competing financial interests exist for any of the authors.

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- Mothers were sent back to referring hospital (n=31), logistical reasons (n=75), insufficient blood available in preterm infants (n=15)

Figure 1. Patient selection flow chart.

Figure 2. Maternal TH values around delivery in spontaneous preterm, indicated preterm and healthy term birth. Panel (a) Maternal circulating TSH levels (Kruskal–Wallis test). Panel (b) Maternal circulating total T4 levels (ANOVA). Panel (c) Maternal circulating fT4 levels (ANOVA after logarithmic transformation). Panel (d) Maternal circulating total T3 levels (ANOVA). Data are presented as medians and interquartile ranges. Spontaneous preterm birth (SPB) (n=31), indicated preterm birth (IPB) (n=45), and healthy term birth (controls) (n=41).

Figure 3. Neonatal TH values in umbilical cord blood in spontaneous preterm, indicated preterm and healthy term birth. Panel (a) Neonatal circulating TSH levels (Kruskal–Wallis). Panel (b) Neonatal circulating T4 levels (ANOVA). Panel (c) Neonatal fT4 levels (ANOVA). Panel (d) Neonatal circulating T3 levels (ANOVA after logarithmic transformation). Data are presented as medians and interquartile ranges. Spontaneous preterm birth (SPB) (n=31), indicated preterm birth (IPB) (n=45) and healthy term birth (controls) (n=41).

Figure 4. Placental mRNA and protein levels of thyroid hormone transporters MCT8 and MCT10 and deiodases DiO2 and DiO3 in spontaneous preterm, indicated preterm and healthy term birth. Panel (a) MCT8 mRNA levels (Kruskal–Wallis). Panel (b) MCT 8 protein levels (Kruskal–Wallis). Panel (c) MCT10 mRNA levels (Kruskal–Wallis). Panel (d) MCT 10 protein levels (ANOVA after logarithmic transformation). Panel (e) DiO2 mRNA levels (ANOVA after logarithmic transformation). Panel (f) DiO2 protein levels (ANOVA after logarithmic transformation). Panel (g) DiO3 mRNA levels (Kruskal–Wallis). Panel (h) DiO3 protein levels (Kruskal–Wallis). Data are presented as medians and interquartile ranges. Spontaneous preterm birth (SPB) (n=20), indicated preterm birth (IPB) (n=31), and healthy term birth (controls) (n=35).

Figure 5. Assessment of placenta maturation. Panel (a) Placental section, hematoxylin, and eosin staining, pictures taken with Leica microscope, magnification 10x. Panel (b) Same placental section after computer processing. Panel (c) Median villi area in spontaneous preterm birth (n=20) and indicated preterm birth (n=22) (Mann–Whitney U test). Panel (d) Median villi area in spontaneous preterm birth (n=18) and indicated preterm birth (n=19) in placenta matched for gestational age (Mann–Whitney U test). Data are presented as medians and interquartile ranges. SPB: Spontaneous preterm birth; IPB: indicated preterm birth.

Table 1. Patient characteristics.

Characteristic	Spontaneous preterm birth (SPB) (n = 31)	Indicated preterm birth (IPB) (n = 45)	Controls (n = 41)	p Value
Maternal characteristics				
Median age (years) (±IQR)	30.0 (±8.0)	29.0 (±7.0)	31.0 (±8.0)	.2
Primipara – no. (%)	16 (51.6)	29 (64.4)	22 (53.7)	.4
Abortions – no. (%)	11 (35.5)	18 (40.0)	10 (24.4)	.3
Recurrent abortion (>2) – no. (%)	2 (6.5)	2 (4.4)	2 (4.9)	.9
Maternal higher education – no. (%)	21 (67.7) (n = 31)	22 (52.4) (42)	38 (92.7) (n = 41)	<.001
Mean BMI (±SD)	23.4 (4.0) (n = 9)	26.2 (5.6) (11 = 44)	23.6 (4.2) (n = 41)	.01
Tobacco use – no. (%)	1 (3.2)	10 (22.2)	1 (2.4)	.003
Spontaneous conception – no. (%)	29 (93.5)	42 (93.3)	36 (87.8)	.6
Documented and treated thyroid dysfunction – no. (%)	1 (3.2)	4 (8.9)	4 (9.8)	.5
Complete course of betamethasone ≤34 w – no. (%) (n)	25 (86.2) (n = 29)	20 (87.0) (n = 23)	Not applicable	1.0
Median gestational age (weeks) (IQR)	31.0 (±6.0)	34.0 (±6.0)	39.0 (±2.0)	<.001
Median gestational age, subgroup ≤34 w (weeks) (IQR) (n)	30.0 (±6.0) (n = 29)	31.0 (±4.0) (n = 23)	Not applicable	.9
Vaginal delivery – no. (%)	16 (51.6)	14 (31.1)	27 (65.9)	.005
Neonatal characteristics				
Male sex – no. (%)	21 (67.7)	22 (48.9)	26 (63.4)	.8
Mean birth weight (g) (±SD)	1714.0 (±647.0)	1878.0 (±884.0)	3243.0 (±474.0)	<.001
IUGR (birth weight < P10) – no. (%)	0 (0.0)	16 (35.5)	4 (9.8)	<.001
Mean CRIB score (birth weight ≤1500 g and/or gestational age <31) (±SD) (n)	5.6 (±5.6) (n = 15)	4.6 (±4.5) (n = 19)	Not applicable	.6
Placental characteristics				
Mean weight placenta (g) (±SD)	430.8 (±122.4)	383.1 (±152.5)	580.0 (±141.1)	<.001
Weight placenta/GA (±SD)	14.1 (±3.3)	11.2 (±3.8)	14.7 (±2.9)	<.001

IQR: interquartile range; SD: standard deviation; BMI: body mass index; IUGR: intrauterine growth restriction; CRIB score: clinical risk index for babies (birth weight ≤ 1500 g and/or gestational age < 31 weeks), range 0–23; GA: gestational age.