The placenta in fetal congenital heart disease

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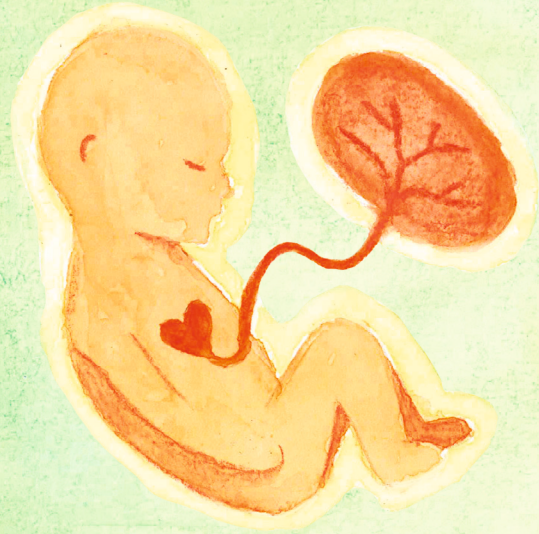

CHAPTER 3

Placenta histology related to flow and oxygenation in fetal congenital heart disease

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ABSTRACT

Background

Fetuses with congenital heart defects (CHD) show delayed neurodevelopment, fetal growth restriction (FGR) and placenta related complications. The neurodevelopmental delay may be, partly, attributed to placental factors.

Aim

As both placental development and fetal aortic flow/oxygenation influence neurodevelopment, placentas were compared within fetal CHD groups based on aortic oxygenation and flow, aiming to unravel the true effects in the developmental processes.

Study design

Placental tissues of pregnancies with fetal CHD and healthy controls were selected from biobanks of two Dutch academic hospitals (LUMC, UMCU). Additionally, biometry and Dopplers were assessed.

Subjects

CHD cases with reduced oxygenation (RO) towards the fetal brain were compared to cases with reduced flow (RF) in the aortic arch and healthy controls. Genetic abnormalities, termination of pregnancy, fetal demise and/or multiple pregnancies were excluded.

Outcome measures

Histological outcomes were related to fetal Dopplers and biometry. A placenta severity score was used to assess the severity of placental abnormalities per case.

Results

In CHD, significantly more delayed maturation, maternal vascular malperfusion, fetal hypoxia and higher placenta severity scores (median 14 in RO, 14 in RF, 5 in controls, $p<0.001$) were observed. Doppler abnormalities (PI UA >p90, PI MCA <p10, CPR <p10) and FGR were more often found in CHD. There were no differences in placental abnormalities, fetal growth and fetal Dopplers between cases with RO and RF.

Conclusion

Fetal hemodynamics in the ascending aorta could not be related to placenta characteristics. We hypothesize that placental development influences neurodevelopment in excess of hemodynamics in CHD cases.

INTRODUCTION

Congenital heart defects (CHD) are the most common congenital anomalies, with an incidence of five to eight per thousand newborns [1]. Advancements in medical care over the past decades have greatly improved the survival of neonates with CHD and the focus of research has shifted to optimizing long-term outcomes and quality of life for these children. [1-3] Neurodevelopmental delay is frequently observed in children and adolescents with CHD and it is now an vital part of the prenatal counseling for parents. [4-11]

Altered neurodevelopment can manifest as early as the fetal period, as shown by multiple fetal imaging studies [4, 5, 12-15]. Although impaired fetal brain growth has primarily been attributed to diminished cerebral blood flow or decreased oxygenation in the ascending aorta in fetal life, caused by the heart defect, the impact of other intrauterine factors should not be underestimated. Analysis of a large set of consecutively studied fetuses revealed that decreased fetal growth and altered placental development, rather than aortic flow and oxygenation, were significant determinants influencing brain growth in fetuses with CHD. [6-9]

Both fetal growth restriction (FGR) and pre-eclampsia are mediated via the placenta, and occur more frequently in pregnancies with fetal CHD [16-23]. In addition, more placental abnormalities are found in pregnancies with fetal CHD [23-25]. There is a knowledge gap on the interaction between placenta, heart defects, and intrauterine development, given that altered placental development and decreased fetal growth could potentially contribute to reduced brain development in fetal CHD and placental diseases occur more frequently in these pregnancies. We hypothesize that altered placental development in CHD affects neurodevelopment comparable to growth restricted fetuses without CHD.

In this study histopathological examination of the placenta of CHD cases was performed and compared to placentas of healthy controls. To assess the hemodynamic effect of the heart defect on the fetal brain in interaction with the placenta, CHD cases were classified into two groups: (1) CHD causing reduced oxygenation in ascending aorta and normal flow towards the brain (simple transposition of the great arteries (TGA); and (2) CHD causing reduced flow towards the brain with either mixed or normal oxygenation in the ascending aorta (hypoplastic left heart syndrome (HLHS), aortic valve stenosis (AoS) or aortic arch hypoplasia (AAH)). Additional, fetal biometry and Doppler indices were related to placenta histology, including a placenta severity score, to evaluate the impact of placental abnormalities on fetal development in these cases.

MATERIALS AND METHODS

Patient selection

In this multicenter prospective case-control study, 18 CHD cases with reduced oxygenation (RO) (simple TGA) and 19 CHD cases with reduced flow (RF) towards the brain

(7 HLHS, 2 AoS, 10 AAH) were identified in the biobanks of two academic hospitals in The Netherlands; the Leiden University Medical Centre (LUMC) and University Medical Centre Utrecht (UMCU). In these biobanks, clinical data and placental tissue of pregnancies with fetal severe CHD were collected from January 2016 to May 2022 (UMCU) and from April 2020 to June 2022 (LUMC). Placental tissue of 23 healthy controls (both mother and fetus) were collected from the biobank of the LUMC. Multiple pregnancies, pregnancies with a confirmed or suspected genetic syndrome, cases with termination of pregnancy and cases with intra-uterine fetal demise were excluded from this study.

Clustering of CHDs into the different groups is previously described and based on theoretical hemodynamics.[7, 26] Cases of two different groups were included in this study. One group consisting of cases with normal flow and reduced oxygenation (RO) to the brain, this group comprises of cases with simple TGA. The other group consists of cases with reduced flow (RF) towards the brain. This group consists of fetuses that all underwent a sternotomy to correct their arch anatomy after birth, indicating these cases had arch hypoplasia over a significant length, as all coarctation are operated by a lateral thoracotomy in our center. All had an aortic valve diameter of ‹4.5mm at 30-32 weeks GA. Furthermore they had fetal reversed or obstructed flow in the aortic arch 30-32 weeks GA.

Data collection

The LUMC biobank consists of consecutive included cases with severe CHD (defined as CHD that requires surgery within the first year of life). Included cases followed a standard clinical pathway, including ultrasound assessments of fetal biometry and Dopplers every four weeks. Clinical data and outcomes were registered in the PRECOR-registry, including all CHD cases of the CAHAL (Centre for Congenital Heart Diseases Amsterdam Leiden).

In the UMCU, a prospective, observational cohort study was conducted, enrolling cases with severe CHD. Cases followed a standardized clinical protocol that included histological evaluation of the placenta. Fetal biometry and Doppler imaging were not conducted routinely and were performed based on clinical necessity.

After identification of cases, data on maternal characteristics (age, parity, medical history, smoking, obstetric history), fetal characteristics (gender, fetal biometry, fetal Doppler indices, fetal head circumference measured by ultrasound) and data on the course of pregnancy (gestational age and mode of birth, pregnancy complications) were collected from the biobanks and electronic patient files. Maternal morbidity was defined as pre-existent maternal illness, including diabetes, hypertension, cardiac disease, renal disease, thyroid disease and auto-immune disease.

The fetal Doppler indices studied included the pulsatility index (PI) of the middle cerebral artery (MCA) and the umbilical artery (UA), as well as the derived cerebroplacental ratio (CPR). The measurements were performed by trained ultrasonographers every four weeks,

at a gestational age between 20-36 weeks. At least two measurements were performed per assessment to ensure consistency in the measurements. A PI of the MCA ‹p10, PI of de UA ›p90 and CPR ‹p10 are signs of abnormal umbilical cord flow and/or fetal brainsparing, most probably caused by placenta insufficiency, and were considered abnormal.

Placental weight was measured without the umbilical cord and membranes (Figure 1), and assessed as described by Pinar et al [27]. Umbilical cord insertion was categorized as normal (central and paracentral) or abnormal (marginal, defined as ‹ 1.5 cm from the placental disk border or velamentous, defined as insertion into the fetal membranes). Coiling was defined as hypocoiling with an index of ‹ 0.1, normal 0.1-0.3 and hypercoiling with an index › 0.3 [28].

Placenta histology was assessed following the Amsterdam criteria [28]. The placenta severity score, previously described by Nijman et al., was used to define the extent and severity of placental abnormalities in each case [29].

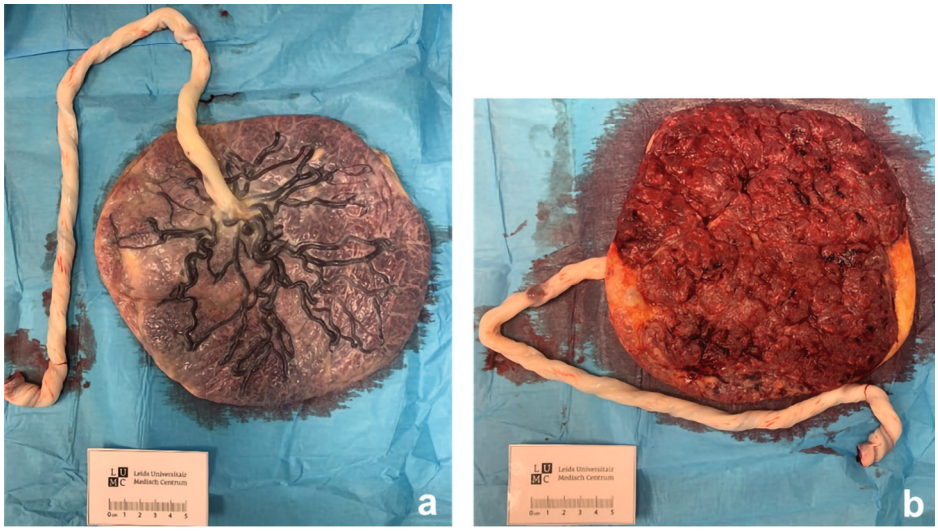

Figure 1: Macroscopic placenta images
1a: fetal side with umbilical cord and membranes attached
1b: maternal side with umbilical cord and membranes attached

Statistical analysis

Continuous data with normal distributions are presented as mean ± standard deviation (SD) and continuous non-normal distributed data are presented as median ± interquartile range (IQR). Categorial data are presented as numbers and percentages (n, %). Independent sample t-tests and Chi-square tests are used where appropriate. Additional multivariate logistic regression analyses are performed to correct for possible confounders (gestational age of delivery and birth mode). A p-value of ‹0.05 was considered significant.

Table 1: Clinical characteristics of included cases

	CHD with reduced oxygenation* n = 18	CHD with reduced flow † n = 19	p-value**	Controls n = 23	p-value***
Maternal age (median, IQR)	32 (7)	33 (3)	N.S.	33 (6)	N.S.
Parity (n,%)			N.S.		N.S.
0	5 (27.8)	9 (47.4)		11 (47.8)	
1	11 (61.1)	6 (31.6)		9 (39.1)	
2	0	4 (21.1)		3 (13.0)	
3	1 (5.6)	0		0	
› 3	1 (5.6)	0		0	
Gestational age at time of delivery (n, %)			N.S.		0.047
‹ 37 weeks	2 (11.1)	2 (10.1)		0	
37-41 weeks	16 (88.9)	16 (84.2)		17 (73.9)	
› 41 weeks	0	1 (5.3)		6 (26.1)	
Maternal morbidity (n, %)	3 (16.7)	1 (5.3)	N.S.	1 (4.3)	N.S.
Maternal medication (n, %)	3 (16.7)	3 (15.8)	N.S.	1 (4.3)	N.S.
Maternal smoking (n, %)	2 (11.1)	0	N.S.	0	N.S.
Pregnancy complication in previous pregnancy (n, %)					
Pregnancy induced hypertension	0	0	N.A.	0	N.A.
Pre-eclampsia	0	0	N.A.	0	N.A.
Fetal growth restriction	0	1 (5.3)	N.S.	0	N.S.
Macrosomia	0	0	N.A.	0	N.A.
Stillbirth	0	1 (5.3)	N.S.	0	N.S.
Gestational Diabetes	2 (11.1)	1 (5.3)	N.S.	0	N.S.
Pregnancy complication in current pregnancy (n, %)					
Pregnancy induced hypertension	1 (5.6)	0	N.S.	0	N.S.
Pre-eclampsia	0	1 (5.3)	N.S.	0	N.S.
Gestational Diabetes	2 (11.1)	2 (10.5)	N.S.	2 (8.7)	N.S.
Birth mode (n, %)			N.S.		0.015
Vaginal	14 (77.8)	10 (52.6)		21 (91.3)	
Cesarean section	4 (22.2)	9 (47.4)		2 (8.7)	
Birthweight percentile (n, %)					
‹ 2.3th percentile	1 (5.6)	3 (15.8)	N.S.	0	N.S.
› 97.7th percentile	1 (5.6)	2 (10.5)	N.S.	0	N.S.
Genetic testing performed (n, %)	9 (50.0)	16 (84.2)	0.006	0	‹0.001
Number of fetal ultrasound assessments (median, IQR)	3 (2.3)	4 (4)	N.S.	4 (1.0)	N.S.

* simple transposition of the great arteries
† hypoplastic left heart syndrome, aortic valve stenosis and/or aortic arch hypoplasia
** p-value CHD with reduced flow vs CHD with reduced oxygenation
*** p-value CHD vs controls

RESULTS

Characteristics of study subjects

Patient characteristics are shown in Table 1. A significant difference in gestational age at time of delivery was observed between CHD cases and controls, as 6/23 controls (26.1%) delivered after 41 weeks of gestation compared to only 1/37 case (2.7%) in the CHD group. Cesarean section (CS) rates differed significantly, with only 2/23 controls (8.7%) as compared to 4/18 CHD cases (22.2%) with RO, and 9/10 CHD cases (47.4%) with RF (p=0.015 between cases and controls). All CS performed in the control group and the group of CHD with RO were elective, whilst 4 of the CS performed in the group with RF were emergency CS due to fetal distress.

FGR was more common in CHD cases (4/37, 10.8% CHD cases, 0 controls), though this did not reach statistical significance. There was no difference in prevalence of FGR between the two types of CHD. No significant differences in characteristics on maternal health or pregnancy complications between the groups were found.

Table 2: Placenta characteristics

	CHD with reduced oxygenation* n = 18	CHD with reduced flow † n = 19	p-value**	Controls n = 23	p-value***
Placenta weight percentile (n, %)					
‹ 10th percentile	6 (33.3)	5 (26.3)	N.S.	4 (17.4)	N.S.
› 90th percentile	2 (11.1)	4 (21.1)	N.S.	4 (17.4)	N.S.
Abnormal umbilical cord insertion (n, %)	0	5 (26.3)	0.027	0	0.005
Single umbilical artery (n, %)	0	1 (5.3)	N.S.	0	N.S.
Coiling (n, %)					
Hypocoiling	3 (16.7)	3 (15.8)	N.S.	0	0.020
Hypercoiling	4 (22.2)	1 (5.3)	N.S.	3 (13.0)	N.S.
Delayed maturation (n, %)	12 (66.7)	9 (47.4)	N.S.	5 (21.7)	0.008
Maternal vascular malperfusion (n, %)	11 (61.1)	14 (73.7)	N.S.	5 (21.7)	‹0.001
Fetal vascular malperfusion (n, %)	2 (11.1)	6 (31.6)	N.S.	3 (13.0)	N.S.
Fetal hypoxia (n, %)	14 (77.8)	14 (73.7)	N.S.	2 (8.7)	‹0.001
Inflammatory lesions (n, %)	6 (33.3)	7 (36.8)	N.S.	13 (56.5)	N.S.
Placenta severity score (median, IQR)	14 (11)	14 (11)	N.S.	5 (8)	‹0.001

* simple transposition of the great arteries
† hypoplastic left heart syndrome, aortic valve stenosis and/or aortic arch hypoplasia
** p-value CHD with reduced flow vs CHD with reduced oxygenation
*** p-value CHD vs controls

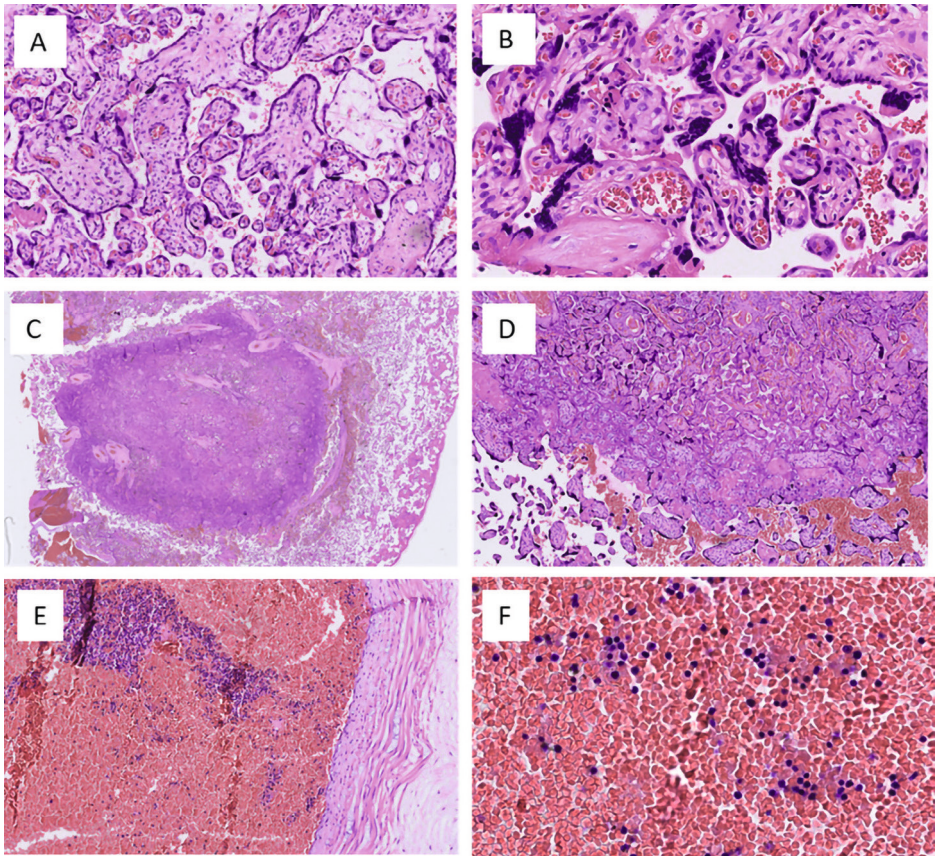

Figure 2: Histology of placental abnormalities found in our cohort with placentas of fetal congenital heart defects
A: Delayed maturation in a placenta of 38 weeks with large immature villi (HE, 100x)
B: Ischemia with increased syncytial knotting of trophoblast (HE, 200x)
C-D: infarction of placental parenchyma (HE, 5x) with close-up in D (HE, 50x)
E-F: example of increased amount of nucleated fetal erythrocytes in fetal circulation (umbilical cord at 39 weeks, HE 100x) with a close-up of nucleated fetal erythrocytes (HE, 400x)

Placenta characteristics

There was no significant difference in placenta weight percentiles between the groups (Table 2). Of CHD cases with RF 5/19 (26.3%) had an abnormal umbilical cord insertion (i.e., marginal or velamentous) as compared to none of the CHD cases with RO (0) and no controls (0) (p=0.005 between CHD cases and controls, p=0.027 between RF and RO). Furthermore, hypocoiling of the umbilical cord was exclusively observed in the CHD groups (p=0.020), with an occurrence of 3/18 (16.7%) in cases with RO and 3/19 (15.8%) in RF. There was no difference in the frequency of hypercoiling between CHD cases and controls, and there was no difference between hypocoiling and hypercoiling among the different groups of CHD.

More than half of the CHD cases had delayed villous maturation, with 12/18 (66.7%) in cases with RO and 9/19 (47.4%) in RF, compared to 5/23 (21.7%) of the controls (p=0.008) (Figure 2). In addition, maternal vascular malperfusion lesions were significantly more frequent in CHD cases (11/18, 61.1% in RO, 14/19, 73.7% in RF and in only 5/23, 21.7% of the controls, p<0.001 between CHD cases and controls). Notably, there was no significant difference in maternal vascular malperfusion lesions between the different types of CHD. Only 2/23 controls (8.7%) had signs of fetal hypoxia in the placenta, whereas 14/28 (77.8%) of CHD cases with RO and 14/19 (73.7%) of the CHD cases with RF had those placental characteristics (p<0.001 between CHD cases and controls). There was no significant difference in the frequency of single umbilical artery, fetal vascular malperfusion lesions or inflammatory lesions between the groups. Placenta severity score was significantly higher in CHD cases as compared to controls (p<0.001) although there was no significant difference between the two types of CHD.

Given the variations in gestational age at time of delivery and birth mode between the groups (Table 1), additional multivariate analyses were performed to correct for gestational age and birth mode. These subanalyses did not yield additional results.

Table 3: Fetal ultrasound results

	CHD with reduced oxygenation* n = 18	CHD with reduced flow † n = 19	p-value**	Controls n = 23	p-value***
Abnormal fetal Doppler § (n, %)					
PI UA >p90	5/15 (33.3)	3/13 (23.1)	N.S.	2 (8.7)	0.010
PI MCA <p10	7/15 (46.7)	9/13 (69.2)	N.S.	8 (34.8)	0.007
CPR <p10	2/15 (13.3)	7/13 (53.8)	N.S.	2 (8.7)	<0.001
Fetal head circumference ¶ (n, %)					
<p3	0	2 (10.5)	N.S.	0	N.S.
p3-p10	1 (5.6)	1 (5.3)	N.S.	0	N.S.

PI = pulsality index, MCA = middle cerebral artery, UA = umbilical artery, CPR = cerebroplacental ratio
* simple transposition of the great arteries
† hypoplastic left heart syndrome, aortic valve stenosis and/or aortic arch hypoplasia
§ during one or more assessment(s)
¶ during the latest assessment
** p-value CHD with reduced flow vs CHD with reduced oxygenation
*** p-value CHD vs controls

Table 4: Fetal growth and Dopplers and placental abnormalities - maturation

	Delayed maturation n = 26	Normal maturation n = 34	p-value
Abnormal fetal Doppler* (n, %)			
PI UA ›p90	5/17 (29.4)	5/34 (14.7)	0.021
PI MCA ‹p10	9/17 (52.9)	15/34 (44.1)	0.036
CPR ‹p10	5/17 (29.4)	6/34 (17.6)	0.031
Fetal head circumference † (n, %)			
‹p3	2 (7.7)	0	N.S.
p3-p10	1 (3.8)	1 (2.9)	N.S.
Birthweight percentile (n, %)			
‹ 2.3th percentile	3 (11.5)	1 (2.9)	N.S.
› 97.7th percentile	2 (7.7)	1 (2.9)	N.S.
Placenta weight percentile (n, %)			
‹ 10th percentile	9 (34.6)	6 (17.6)	N.S.
› 90th percentile	6 (23.1)	4 (11.8)	N.S.

PI = pulsality index, MCA = middle cerebral artery, UA = umbilical artery, CPR = cerebroplacental ratio
* during one or more assessment(s)
† during the latest assessment

Table 5: Fetal growth and Dopplers and placental abnormalities – Placenta severity score

	Placenta severity score (median, IQR)	p-value (compared to normal)
Fetal Doppler*		
PI UA ›p90 (n = 10, 16.7%)	12 (12)	N.S.
Normal PI UA (n = 41, 78.8%)	8 (10)	
PI MCA ‹p10 (n = 24, 40.0%)	10 (12)	N.S.
Normal PI UA (n = 27, 51.9%)	8 (8)	
CPR ‹p10 (n = 11, 18.3%)	12 (10)	0.028
Normal CPR (n = 38, 76.0%)	7 (10)	
Fetal head circumference † (n, %)		
‹p3 (n = 2, 3.3%)	23 (IQR N.A.)	N.S.
p3-p10 (n = 2, 3.3%)	13 (IQR N.A.)	N.S.
HC ›p10 § (n = 55, 91.7%)	9 (11)	
Birthweight percentile (n, %)		
‹ 2.3th percentile (n = 4, 6.7%)	9 (11)	0.032
2.3th – 97.7th percentile § (n = 53, 88.3%)	10 (11)	
› 97.7th percentile (n = 3, 5.0%)	9 (11)	N.S.
Placenta weight percentile (n, %)		
‹ 10th percentile (n = 15, 25.0%)	18 (11)	‹0.001
10th – 90th percentile § (n = 35, 58.3%)	8 (9)	
› 90th percentile (n = 10, 16.7%)	8 (11)	N.S.

PI = pulsality index, MCA = middle cerebral artery, UA = umbilical artery, CPR = cerebroplacental ratio
* during one or more assessment(s)
† during the latest assessment
§ considered normal

Fetal growth and Dopplers

In the CHD group, 5/15 cases (33.3%) with RO and 3/13 cases (23.1%) with RF had abnormal flow in the UA (PI ›p90) during one or more ultrasound assessments as opposed to 2/23 controls (8.7%) (p=0.010 between CHD cases and controls) (Table 3). More than half of the CHD cases (7/15, 46.7% in cases with RO and 9/13, 69.2% in cases with RF) had abnormal flow in the MCA (PI ‹p10) during one or more ultrasound assessments in contrast to only 8/23 controls (34.8%) (p=0.007). CHD cases exhibited an abnormal CPR (‹p10) more often (2/15, 13.3% in RO, 7/13, 53.8% in RF), contrary to only 2/23 (8.7%) in healthy controls (p‹0.001)). Although there was a marked difference in abnormal CPR between the two groups of CHD, this did not reach statistical significance. Fetal head circumferences ‹p3 and ‹p10 were not significantly different between the groups.

Fetal growth and Dopplers in relation to placental abnormalities

Additional analyses were performed, relating placental abnormalities to fetal growth and Doppler indices (Table 4, Appendix C1-2). Cases with delayed villous maturation had significantly more abnormal MCA and UA flows and significantly more abnormal CPR values (Table 4). Small fetal head circumference (‹p3 and p3-p10), low birthweight and low placenta weight were more frequent in cases with delayed villous maturation; however, this did not reach statistical significance. No significant relations were identified in fetal growth parameters and Doppler indices in cases with maternal vascular malperfusion or fetal hypoxia (Appendix A1-2).

Placenta severity scores are significantly higher in cases with abnormal fetal Dopplers, low birthweight percentile and low placental weight (Table 5). Placenta severity scores were higher in cases with a small head circumference, however, this did not reach statistical significance.

DISCUSSION

This multicenter prospective study validates prior observations indicating a higher prevalence of abnormal umbilical cord insertion, delayed villous maturation, maternal vascular malperfusion lesions and signs of fetal hypoxia in placentas of pregnancies with fetal CHD [23-25]. This supports the hypothesis that vascular development is altered in placentas of pregnancies with fetal CHD. Our study, however, did not reveal differences in placental abnormalities, fetal growth parameters and results of Doppler examinations between CHD subtypes causing reduced flow towards the fetal brain (with either mixed or normal oxygenation in the systemic circulation) and CHD subtypes with normal flow and decreased oxygenation in the ascending aorta.

Placental signs of fetal hypoxia (defined as increased nucleated erythrocytes in fetal circulation) are often a compensation of decreased oxygen transport across the placenta. This placental abnormality was more frequently observed in the placentas of CHD cases,

consistent with previous literature [23]. The fact that we did not find differences between the two CHD groups, supports the theory of the placenta-heart axis, in which the parallel development of the placenta and the fetal heart is highlighted [30, 31]. Numerous genes have been identified that influence both the development of the placenta and the fetal heart simultaneously [23, 31]. According to this placenta-heart axis theory, abnormal early placentation and fetal hypoxia in the first trimester contribute to the development of fetal CHD. In addition, circulatory changes and hypoxia induced by the CHD may lead to an abnormal development of the placenta. This may result in placenta insufficiency, in which the transfer of oxygen and nutrients between the mother and fetus is reduced. An adaptive response to chronic fetal hypoxia may become apparent, in which blood flow is redistributed to the brain in order to provide sufficient resources and oxygen for the brain to develop and function. Ongoing placenta insufficiency further contributes to the FGR, delayed (neuro)development and adverse pregnancy outcomes [30]. Our study result support this hypothesis, as we found more abnormal Doppler indices related to placenta insufficiency and brainsparing in cases with fetal CHD. In addition, we found more abnormal Dopplers in cases that had delayed villous maturation of the placenta, which is related to congenital abnormalities and adverse pregnancy outcomes, rather than FGR [33, 34]. The placenta abnormalities found in our CHD cohort could either be cause or consequence, as proposed in the theory of the placenta-heart axis. Similar to cases with FGR, we found more maternal vascular malperfusion lesions in our CHD cohort. [35] Nonetheless, we also found more delayed villous maturation in CHD cases, which is related to circulatory abnormalities, rather than FGR [33, 34]. Therefore, the placental abnormalities in CHD cannot solely be attributed to the higher incidence of FGR in these cases. The underlying etiology of both types of placental abnormalities and the relation to cardiac development remains unclear.

In previous studies, it has been hypothesized that both diminished flow towards the fetal brain and, to a lesser extent, decreased oxygenation in the ascending aorta, cause hypoxia in the fetal brain and thereby contribute to neurodevelopmental delay in fetuses with CHD. [4, 5, 12, 13, 36, 37] Nonetheless, other studies suggest that decreased fetal growth and placenta insufficiency had more influence on brain growth in fetuses with CHD than aortic flow and oxygenation in itself. [6-9] Our results point towards the same direction, as we observed no difference in placental abnormalities, fetal growth and fetal Dopplers, between CHD causing diminished flow towards the fetal brain and CHD causing decreased oxygenation in the ascending aorta. This implies that hemodynamic alterations caused by the CHD cannot solely explain the altered fetal and placental developmental outcomes found in these cases. This supports the hypothesis that altered placental function in fetal CHD contributes to the neurodevelopmental delays observed in fetuses and infants with CHD, rather than exclusively from fetal hemodynamic alterations caused by the CHD. However, since our study did not include fetal brain volumes, additional research is needed to disentangle the respective contributions of altered placenta function and fetal cardiac anatomy to impaired brain growth in CHD.

In our CHD cases with reduced ascending aortic flow, abnormal umbilical cord insertion (marginal, velamentous) was frequently found, finding its etiology in early embryogenesis [38]. This is comparable to the results of a previous study by Albalawi *et al.*, as they found significantly more abnormal cord insertions in cases with fetal CHD as compared to controls [39]. In their study, abnormal cord insertion was more frequent in both left heart disease and conotruncal defects, whereas in our study there were no cases with abnormal cord insertion in the CHD group with simple TGA, which is a conotruncal defect. This difference might be attributed to the variation of included subtypes, as Albalawi included all conotruncal defects, whilst in our study only cases with simple TGA were included.

This study again supports the complexity of the underlying multifactorial mechanism through which various heart defects, each with its distinct hemodynamic effects, affect placental development. To gain a more comprehensive understanding of the relationship between CHD and altered placental development, research with a focus on vascular development of the maternal uteroplacental surface and genetic alternations in the embryo, related to placental hypoxia, is essential. Future studies aimed at improving the (neuro)developmental outcomes in fetal CHD, should take placenta morphology, fetal biometry and placenta related complications like PE into account. In addition, larger sets of pooled data are needed to truly understand if there are minute differences in placental pathology for these relatively rarer conditions, together with functional imaging data to better understand in vivo changes in flow and oxygenation in both the aortic arch, the brain and the placenta.

Strengths

The main strength of this study is the prospective collection of materials for biobanking in two hospitals included in this study and the inclusion of a control group. Previous studies on this topic are mostly retrospective and therefore prone to selection bias. Our cohort is with that reliable, as our findings are comparable to findings in previous studies.

In addition, in this study we related placental outcomes to ascending aortic flow and oxygenation, and we differentiated between different types of placental abnormalities and with that, identified the location of the abnormalities in the placenta. With this information, the hypothesis of abnormal placentation in pregnancies with fetal CHD is confirmed, and we showed that the abnormal placental development is unlikely related to aortic flow and oxygenation.

Limitations

The first limitation of this study is the small sample size, especially regarding the Doppler indices and growth outcomes. Therefore, the effect of the CHD and placental characteristics on developmental outcomes could not have been established.

Another limitation is the fact that gestational age and birth mode were significantly different between the groups. Difference in gestational age is inevitable, as in cases with fetal CHD labor is often induced so the patient can deliver in a hospital where neonatal cardiac care can be provided. In addition, as placental related pregnancy complications are more common in pregnancies with fetal CHD, labor is often induced before the term date. To assess the effect on the results, additional analyses were performed to correct for these factors. There were no additional differences in maternal health characteristics and pregnancy complications between the groups.

Cases with a genetic diagnosis, either pre- or postnatally tested, were excluded from this study. However, not all included cases underwent genetic testing. In the group of CHD cases with low oxygenation, 50% of the cases had genetic testing without an abnormal finding. This group comprises of cases with a simple TGA, a diagnosis that is seldomly related to an underlying genetic disorder [40]. In the group of CHD with reduced aortic flow, which is known for more frequent underlying genetic disorders, 5 cases were not tested (14%). Whole Exome Sequencing (WES) was performed in almost half of 11 CHD cases with reduced flow (47.8%). As we excluded all cases suspect for a genetic diagnosis after birth and all cases with additional congenital abnormalities or dysmorphias, we expect that the genetic profile of the vast majority of the included cases is normal.

Conclusions

Delayed villous maturation, maternal vascular malperfusion lesions and fetal hypoxia are more common in placentas of pregnancies with fetal CHD. Our study did not establish a clear correlation between placenta abnormalities and subtypes of CHD, categorized based on aortic flow and oxygenation. This corresponds with the hypothesis that the influence of fetal CHD on brain growth and (neuro)development may be more significantly impacted by factors such as decreased fetal growth and abnormal placental development, rather than solely alterations in aortic flow or oxygenation caused by the CHD. Larger sets of pooled data are needed to further elaborate on this hypothesis.

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APPENDIX A1: Fetal growth and Dopplers and placental abnormalities – maternal vascular malperfusion

	Maternal vascular malperfusion n = 30	No maternal vascular malperfusion n = 30	p-value
Abnormal fetal Doppler* (n, %)			
PI UA ›p90	3/24 (12.5)	7/27 (25.9)	N.S.
PI MCA ‹p10	9/24 (37.5)	15/27 (55.6)	N.S.
CPR ‹p10	6/24 (25.0)	5/27 (18.5)	N.S.
Fetal head circumference † (n, %)			
‹p3	1 (3.3)	1 (3.3)	N.S.
p3-p10	1 (3.3)	1 (3.3)	N.S.
Birthweight percentile (n, %)			
‹ 2.3th percentile	2 (6.7)	2 (6.7)	N.S.
› 97.7th percentile	1 (3.3)	2 (3.3)	N.S.
Placenta weight percentile (n, %)			
‹ 10th percentile	7 (23.3)	8 (26.7)	N.S
› 90th percentile	5 (16.7)	5 (16.7)	N.S.

PI = pulsality index, MCA = middle cerebral artery, UA = umbilical artery, CPR = cerebroplacental ratio
* during one or more assessment(s)
† during the latest assessment

APPENDIX A2: Fetal growth and Dopplers and placental abnormalities – fetal hypoxia

	Fetal hypoxia n = 30	No fetal hypoxia n = 30	p-value
Abnormal fetal Doppler* (n, %)			
PI UA ›p90 with normal PI MCA	4/23 (17.4)	6/28 (21.4)	N.S.
PI MCA ‹p10 with normal PI UA	12/23 (52.2)	11/28 (39.3)	N.S.
CPR ‹p10	6/23 (26.1)	5/28 (17.9)	N.S.
Fetal head circumference † (n, %)			
‹p3	0	2 (6.7)	N.S.
p3-p10	1 (3.3)	1 (3.3)	N.S.
Birthweight percentile (n, %)			
‹ 2.3th percentile	1 (3.3)	3 (10.0)	N.S.
› 97.7th percentile	2 (6.7)	1 (3.3)	N.S.
Placenta weight percentile (n, %)			
‹ 10th percentile	8 (26.7)	7 (23.3)	N.S.
› 90th percentile	6 (20.0)	4 (13.3)	N.S.

PI = pulsality index, MCA = middle cerebral artery, UA = umbilical artery, CPR = cerebroplacental ratio
*during one or more assessment(s)
† during one or more assessment(s)