Fetal Pain

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Chapter 4

Fetal stress hormone changes during intrauterine transfusions

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Abstract

Objective To document fetal stress hormone and Doppler changes after intrauterine transfusions (IUTs) in either the intrahepatic portion of the umbilical vein (IHV) or the placental cord insertion (PCI).

Method Pregnant women scheduled for IUT for fetal anaemia (N=25) were included prospectively. Cortisol, β-endorphin and noradrenalin concentrations in fetal plasma and middle cerebral artery pulsatility index before and after transfusion were compared. Transfusions were performed through the IHV, thus puncturing the fetus, or at the PCI.

Results There were no measurable differences between the transfusion sites.

Conclusion In anaemic fetuses undergoing transfusion, Doppler changes and fetal stress hormone changes were unrelated to the site of needle insertion.

Introduction

Owing to the expanding numbers of invasive fetal procedures, there is an increasing awareness for the concept of fetal pain. There is, however, much debate as to whether the fetus is mature enough to be able to perceive pain at all [1-7]. Giannakoulopoulos et al found that intrauterine needling in the fetal intrahepatic vein (IHV) resulted in alterations in the fetal stress hormones noradrenalin, beta-endorphin and cortisol [8, 9] and in a decrease in pulsatility index of the middle cerebral artery [10]. These changes were ablated by administration of analgesics to the fetus and were not observed if the IUT was performed through the umbilical cord root at placental cord insertion (PCI, which is not innervated)[11]. The above suggests that the fetus is able to generate a response to a noxious stimulus, which may be interpreted as the capability to perceive a sensation of pain. Even more, this response seems to be alterable by analgesics in a way similar to infants [8, 9]. Interpreting these responses however has to be done cautiously, as a stress response not necessarily implies pain. But as the occurrence of pain seems unlikely without an accompanying stress response, the fetal stress response has been used as a surrogate measure for pain [12, 13]. Until now, reports on the fetal stress response came from only one research group. Herein, we aimed to duplicate their observations by documenting any eventual response to a potential painful stimulus. We performed a prospective study comparing the fetal stress response between women undergoing intrauterine transfusions (IUTs) for alloimmune fetal anaemia in the intrahepatic portion of the umbilical vein, thereby subjecting the fetus to a potential painful stimulus, or through the umbilical cord root, which is not innervated and as such, no noxious stimulus was present.

Materials and Methods

The Leiden University Medical Centre (LUMC) is the national referral centre for the treatment of fetal anaemia. Our methods for case selection, surveillance and treatment of red cell alloimmunisation have been described previously [14].
Women with singleton pregnancies scheduled for IUTs for fetal red cell alloimmunisation between 20 and 35 weeks were offered study participation. Women were enrolled from September 2004 until December 2007. Exclusion criteria were morbid obesity, fetal structural anomalies or fetal hydrops. Fetuses could be part of the study several times.

All women received indomethacin 50 mg rectally half an hour before the start of the procedure. We did not stratify for site of needle insertion (IHV or PCI) as this was decided on just prior to commencing the procedure depending on the position of the fetus, placenta and cord or other technical factors.
The main outcome measure was the influence of site of needle insertion on fetal stress hormone and Doppler changes during IUT.
Initially, the study was designed as a randomised controlled trial comparing remifentanil with placebo in IUTs in either IHV or PCI (the study was registered with ClinicalTrials.gov, number NCT01013558). However, unfortunately only after completion of the trial we discovered that the dosage of remifentanil was accidently set at 0.08 µg/kg/min instead of the planned 0.15 µg/kg/min. This dosage was so low, that we expect it will not have any analgesic effect on the fetus. Therefore we will restrict us to a detailed description of the results in the placebo group.

Immediately before and after the procedure, Doppler flow velocity waveforms from the middle cerebral artery were obtained. All measurements were performed by an experienced sonographer, with an Acuson Sequoia (Mountain View, California, US) ultrasound machine with a 6MHz probe. All measurements were achieved in periods of fetal rest and apnoea. An angle <20◦ between the vessel and Doppler beam was used, and angle correction was applied. Doppler waveforms were obtained from three consecutive waveforms with similar appearance and a narrow band of frequencies and one of these was analysed.
Following ultrasound funipuncture, all fetuses received atracurium (0.4 mg/kg) intravenously. In addition to the collection of clinical blood samples (before commencing and after completing the IUT), 4 mL of fetal blood was drawn for the hormone assays of noradrenalin, beta-endorphin and cortisol. The preprocedural sample was collected immediately after access to the fetal circulation was established. The postprocedural sample was collected 2 minutes after the total amount of transfusion blood was given to the fetus. In procedures performed at gestational ages of 24 weeks or less we limited the amount of blood to be sampled to a maximum of 2 mL, to avoid adverse effects. All samples were kept on ice until centrifugation (1200 rpm), which was done within 30 minutes. Plasma samples were stored at -80˚C until analysis. Cortisol and beta-endorphin levels were assayed using a standardised radioimmunoassay and noradrenalin levels were assayed using high-pressure liquid chromotography. Analysis was performed in one batch.

Power analysis showed that a sample size of 10 in each group (two-tailed $\alpha = 0.05$, $\beta = 0.80$, standard deviation of differences 50%) would be sufficient to detect a 50% rise in plasma concentration of cortisol, beta-endorphin and noradrenalin after IUT based on previous studies (Giannakoulopoulos et al. 1994; Radunovic et al. 1992).

Data were analysed using SPSS version 16.0. Statistical analysis to compare the groups was performed using the paired and unpaired t-test, as appropriate. A linear mixed model analysis showed that a correlation within fetuses was unlikely as the p value of the random patient effect was far from significant. We therefore considered each observation independent and used a non-parametric test (in particular Kruskal-Wallis) to assess differences in medians. Univariate analyses showed that site of puncture, gestational age at puncture, initial haemoglobin, final haemoglobin, amount of blood transfused and number of intrauterine transfusions did not correlate with stress hormone changes. There was a tendency of increase in cortisol concentration with increasing gestational age, however since gestation was not significantly different between the groups we did not consider this as a candidate for confounding.

As pain is a learned response this could mean that the results could have been influenced by the fact that we did not only include first procedures. However, excluding the repetitive transfusions did not change the results.

Data are given as mean (SD). A P-value of <0.05 was considered statistically significant.

Ethics approval of the study protocol was granted by the Leiden University Medical Centre Ethics Board.

Results

Data of 25 IUTs, performed in 18 different women, were included. Fourteen transfusions were performed in the IHV and 11 in the PCI. In one case the samples could not be analysed due to technical difficulties related to storage of the blood samples. One patient asked for maternal pain relief and was therefore excluded from the study. As such, we will discuss 23 cases: 12 IUTs in the IHV and 11 in the PCI. Five cases were included two times. In 3 cases both transfusions were performed in the PCI, in one case both transfusions were performed in the IHV and in one case the first transfusion was performed in the IHV and the second one in the PCI.

The characteristics of the study population are given in table 1. Pretransfusion haemoglobin levels were slightly lower in the PCI group, this difference was statistically significant.

Table 1. Characteristics of the study population.

	IHV (N=12)	PCI (N=11)	P-value
Gestational age at IUT (weeks)	29 (5·2)	27 (4·4)	0·51
Haemoglobin before IUT (g/dl)	8·2 (1·0)	5·8 (1·0)	0·001
EFW at IUT (grams)	1500 (850)	1317 (716)	0·58

Numbers are given as mean (SD). IHV: intrahepatic vein, PCI: placental cord insertion, IUT: intrauterine transfusion, EFW: estimated fetal weight.

Transfusion data are given in table 2. There were no significant differences between both groups except for a slightly higher amount of transfused blood (as percentage of total fetoplacental blood volume) in the PCI group.

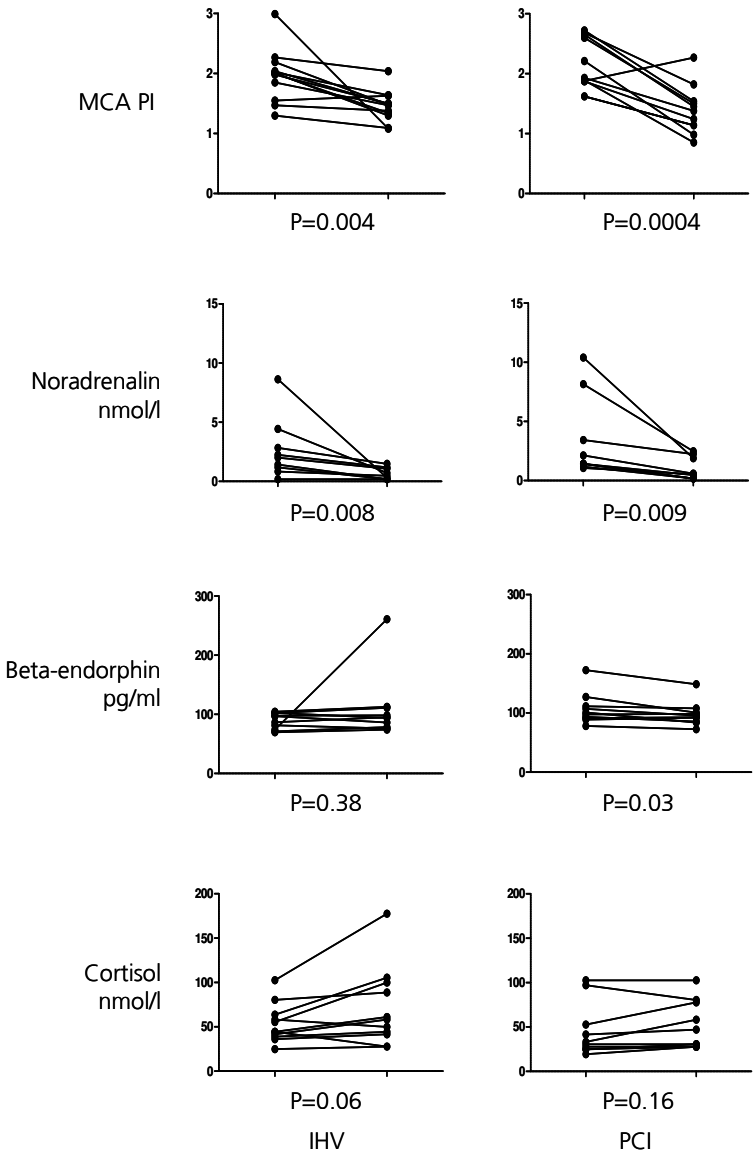
Table 2. Transfusion data.

	IHV (N=12)	PCI (N=11)	P-value
Haemoglobin after IUT (g/dl)	15·0 (0·7)	15·1 (0·9)	0·78
pH before IUT	7·42 (0·07)	7·41 (0·03)	0·79
pH after IUT	7·34 (0·07)	7·33 (0·05)	0·65
Transfusion volume (ml)	50 (20·6)	56 (21·3)	0·44
Transfusion volume (% FPV)	31 (8·1)	40 (12·6)	0·04
Duration of IUT (min)	25 (9·2)	25 (7·6)	0·98

Numbers are given as mean (SD). IHV: intrahepatic vein, PCI: placental cord insertion, IUT: intrauterine transfusion, FPV: fetoplacental blood volume.

Changes in Doppler flow measurements as well as stress hormone changes are displayed in figure 1 and table 3. There were no statistical differences in Doppler changes and fetal stress hormone changes between both groups. The pulsatility index of the MCA decreased significantly, irrespective of the site of transfusion. Noradrenalin concentrations decreased significantly in both groups. Beta endorphin concentration decreased slightly in the PCI group (P=0.03), but did not change in the IHV group (P=0.38). Cortisol concentrations did not change significantly. There were no significant differences in delta pulsatility index or delta stress hormones between both groups.

Figure 1. Changes in fetal Doppler flow profiles and stress hormones noradrenalin, beta-endorphin and cortisol during IUT



IHV: intrahepatic vein, PCI: placental cord insertion

Table 3. Changes in MCA PI and fetal stress hormones noradrenaline, beta-endorphin and cortisol during intrauterine transfusions.

		MCA PI	Noradrenalin (nmol/l)	Beta-endorphin (pg/ml)	Cortisol (nmol/l)
IHV	N	12	9	10	11
	Before	1·97 (0·43)	2·41 (2·53)	88·6 (13·8)	53·7 (22·2)
	After	1·45 (0·26)	0·59 (0·49)	108·7 (55·2)	71·1 (44·4)
	P value	0·004	0·008	0·38	0·06
	Delta (Δ)	0·52	0·34	-20·0	-17·4
PCI	N	11	9	10	10
	Before	2·15 (0·43)	3·40 (3·45)	106·8 (26·7)	45·5 (30·2)
	After	1·39 (0·40)	0·97 (0·95)	97·8 (20·3)	51·0 (27·4)
	P value	0·0004	0·009	0·03	0·16
	Delta (Δ)	0·76	0·41	9·0	-5·5
	95%CI between Δ changes	-0.69 to 0.19	-0·25 to 0·38	-69·0 to 10·8	-31·1 to 7·3

Numbers are given as mean (SD). MCA PI = pulsatility index of the middle cerebral artery, IHV = intrahepatic vein, PCI = placental cord insertion

There was 1 distinct outlier. This patient had a very high beta-endorphin concentration after transfusion in the IHV. She had two IUTs as part of the study (both in IHV) and only at the second transfusion such a rise was measured (GA 32 weeks, haemoglobin level transfused 8.7-15.0 g/dL, duration of IUT 20 minutes, procedure uncomplicated and without any obvious details). Significance levels did not change following exclusion of this outlier.
There was a significant correlation between cortisol and gestational age and between cortisol level prior to IUT and haemoglobin level, with a tendency towards a higher cortisol level with increasing gestational age and higher pretransfusion haemoglobin levels. However, there was no significant correlation between delta cortisol and gestational age or delta cortisol and pretransfusion haemoglobin level. There was no significant correlation between pretransfusion stresshormone levels or delta stress hormone levels and volume of blood transfused.
There were no serious fetal or maternal adverse events during the study period.

Discussion

In this study we could not confirm earlier observations on a difference in changes in Doppler measurements and fetal stress hormones in women undergoing intrauterine transfusion either by cord root or intrahepatic vein needle insertion. This was certainly an unanticipated finding.
There are several limitations to our study. First, posttransfusion fetal blood samples were taken after a significant amount of blood had been added to the total fetal blood volume. This may dilute stress hormone levels which may have led to an underestimation of the concentration of the stress hormones. However, this is not avoidable and identical in other studies that have measured stress hormone concentrations in intrauterine transfusions. Second, we routinely used atracurium for fetal neuromuscular blockade and indomethacin to prevent uterine contractions. We consider it unlikely that these medications influenced our results. Atracurium has been shown not to alter fetal heart rate and heart rate variability and is unlikely to influence a stress response [15]. Maternally administered indomethacin reaches a maximal fetal concentration only several hours after administration and we gave indomethacin 30 minutes prior to the procedure [16].
To our knowledge, only one other research group studied the effect of a potentially painful stimulus on human fetal stress hormones. Giannakoulopoulos et al. reported a

rise in fetal stress hormone concentrations and changes in fetal brain bloodflow profiles during IUTs in nine women through the IHV, and absence of these changes when needling the placental root of the umbilical cord [8-10]. We found, in a comparable group of women, no differences between intrahepatic and placental cord insertion transfusions. The most striking difference is that we measured a decrease in noradrenalin levels whereas Giannakoulopoulos et al observed a rise. One potential explanation might be that the fetuses in their study were less anaemic than those in our study. Another possibility is that fetal blood was transfused at a different speed, which might influence the stress reaction by the end of the IUT.

The pretransfusion concentrations of all three stresshormones were at the upper end of what is described as normal fetal values [17-19]. Since these higher concentrations were present in all fetuses and in the PCI group no potential painful stimulus was present, this would suggest that all fetuses experienced stress at the start of the transfusion, irrespective of the site of needling. We speculate that these pre-procedural elevated stress hormone concentrations reflect a status of pre-existing stress rather than procedure related stress, for instance due to the haemodynamic consequences of anaemia. This is supported by the fact that we found no increase in concentrations of beta-endorphin or cortisol after the start of the procedure, whereas the decrease in noradrenalin concentration could reflect a reduced stress level after IUT. It is also conceivable that these changes are a sign of the cardiovascular adaptation to volume expansion during intrauterine transfusions. The decrease in pulsatility index of the middle cerebral artery in all groups supports this assumption, as we have described before [20].

Previous research has suggested that some presumably painful fetal conditions can lead to long-term changes in infant sensitivity [21]. Therefore, despite the fact that we were unable to show a significant rise in fetal stress hormones with needling the fetal liver, we feel it remains imperative to further investigate the potential harm from inflicting fetal stress, and the possible role of fetal analgesia. If another attempt should be made to measure fetal stress hormone changes, we suggest this should be done in procedures not involving anaemic fetuses, and without applying a volume load. The focus should lie on finding both safe and effective analgesia, for both fetus and mother.

Conclusion

In anaemic fetuses undergoing transfusion, Doppler changes and fetal stress hormone changes were unrelated to the site of needle insertion. This suggests that needle transgression of the fetus is not the cause of fetal stress hormone changes during intrauterine transfusions.

Additional information on remifentanil groups:

Table 1: Characteristics of the study population

Table 2: Transfusion data.

Table 3: Changes in MCA PI and fetal stress hormones noradrenalin, beta-endorphin and cortisol during intrauterine transfusions.

Figure 1: Changes in fetal Doppler flow profiles and stress hormones noradrenalin, beta-endorphin and cortisol during IUT with either remifentanil or placebo.

Table 1. Characteristics of the study population.

	Remifentanil (N=23)			Placebo (N=23)		
	IHV (N=13)	PCI (N=10)	P-value	IHV (N=12)	PCI (N=11)	P-value
Gestational age at IUT (weeks)	29 (4·4)	27 (3·0)	<0·0001	29 (5·2)	27 (4·4)	0·51
Haemoglobin before IUT (g/dl)	7·4 (1·5)	6·8 (0·6)	<0·0001	8·2 (1·0)	5·8 (1·0)	0·001
EFW at IUT (grams)	1507 (620)	1164 (427)	<0·0001	1500 (850)	1317 (716)	0·58

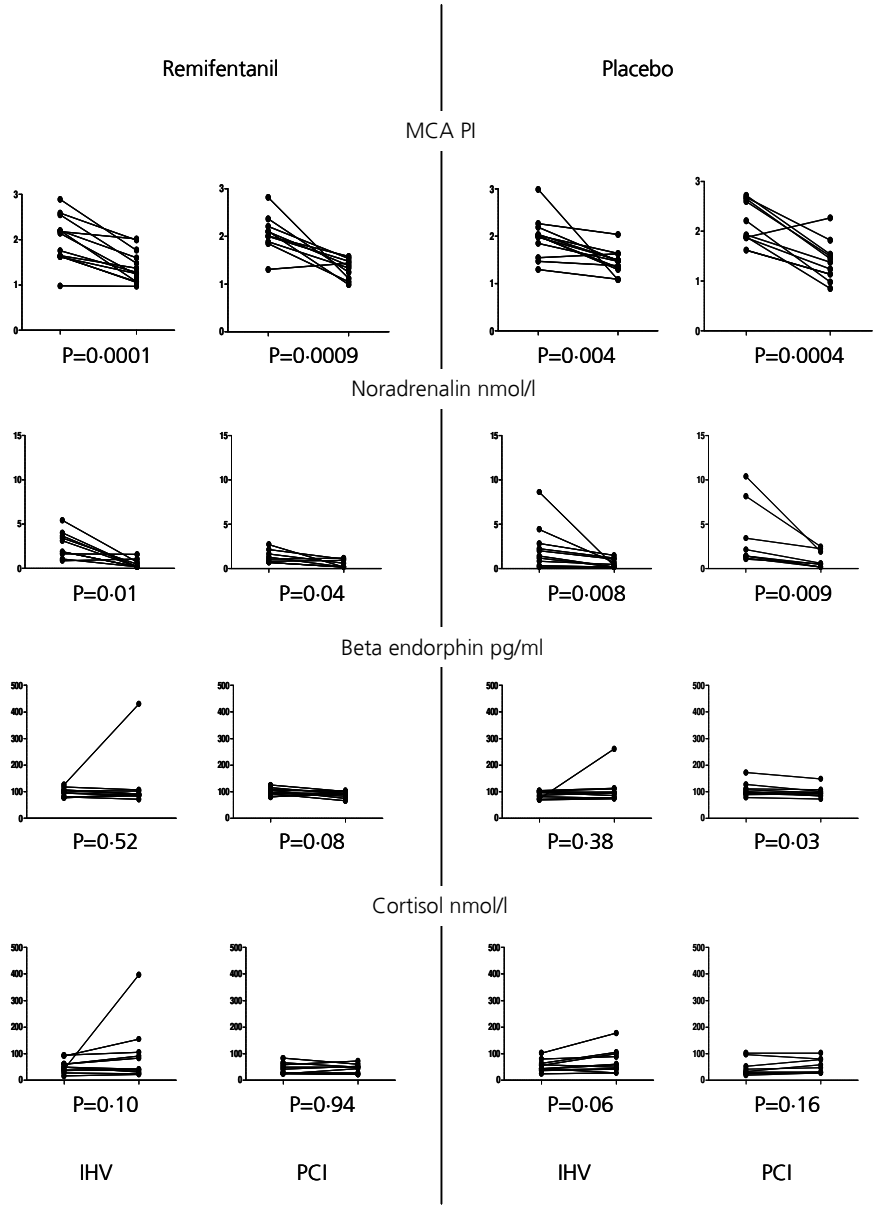
Numbers are given as mean (SD). IHV: intrahepatic vein, PCI: placental cord insertion, IUT: intrauterine transfusion, EFW: estimated fetal weight.

Table 2. Transfusion data.

	Remifentanil (N=23)			Placebo (N=23)		
	IHV (N=13)	PCI (N=10)	P-value	IHV (N=12)	PCI (N=11)	P-value
Haemoglobin after IUT (g/dl)	14·7 (0·8)	15·8 (0·7)	0·04	15·0 (0·7)	15·1 (0·9)	0·78
pH before IUT	7·43 (0·05)	7·41 (0·03)	0·25	7·42 (0·07)	7·41 (0·03)	0·79
pH after IUT	7·37 (0·05)	7·38 (0·04)	0·58	7·34 (0·07)	7·33 (0·05)	0·65
Transfusion volume (ml)	57 (22·2)	52 (15·7)	0·54	50 (20·6)	56 (21·3)	0·44
Transfusion volume (% FPV)	34 (11·3)	38 (5·3)	0·35	31 (8·1)	40 (12·6)	0·04
Duration of IUT (min)	26 (9·5)	23 (6·3)	0·47	25 (9·2)	25 (7·6)	0·98

Numbers are given as mean (SD). IHV: intrahepatic vein, PCI: placental cord insertion, IUT: intrauterine transfusion, FPV: fetoplacental blood volume.

Figure 1. Changes in fetal Doppler flow profiles and stress hormones noradrenaline, beta-endorphin and cortisol during IUT with either remifentanil or placebo.



IHV: intrahepatic vein, PCI: placental cord insertion

Table 3. Changes in MCA PI and fetal stress hormones noradrenaline, beta-endorphin and cortisol during intrauterine transfusions.

		MCA PI	Nor-adrenalin (nmol/l)	Beta-endorphin (pg/ml)	Cortisol (nmol/l)
IHV placebo	N	12	9	10	11
	Before	1·97 (0·43)	2·41 (2·53)	88·6 (13·8)	53·7 (22·2)
	After	1·45 (0·26)	0·59 (0·49)	108·7 (55·2)	71·1 (44·4)
	P value	0·004	0·008	0·38	0·06
	Delta (Δ)	0·52	0·34	-20·0	-17·4
PCI placebo	N	11	9	10	10
	Before	2·15 (0·43)	3·40 (3·45)	106·8 (26·7)	45·5 (30·2)
	After	1·39 (0·40)	0·97 (0·95)	97·8 (20·3)	51·0 (27·4)
	P value	0·0004	0·009	0·03	0·16
	Delta (Δ)	0·76	0·41	9·0	-5·5
IHV remifentanil	N	13	10	11	11
	Before	2·07 (0·53)	2·71 (1·46)	97·1 (16·9)	51·7 (24·3)
	After	1·44 (0·36)	0·49 (0·49)	120·8 (103·2)	93·8 (108·7)
	P value	0·002	0·002	0·42	0·22
	Delta (Δ)	0·64	0·38	-23·0	-42·0
PCI remifentanil	N	10	9	10	9
	Before	2·07 (0·39)	3·40 (3·45)	100·5 (14·1)	45·0 (21·0)
	After	1·32 (0·21)	0·97 (0·95)	90·6 (12·5)	44·7 (17·5)
	P value	0·0009	0·009	0·09	0·95
	Delta (Δ)	0·75	0·41	9·9	0·31

Values are given as mean (SD). MCA PI = pulsatility index of the middle cerebral artery, IHV = intrahepatic vein, PCI = placental cord insertion

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