Diagnostic procedures for assessing the severity of alloimmune fetal anemia

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Fetal cardiac contractility before and after intrauterine transfusion

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Abstract

Objective: To evaluate the effect of fetal anemia and intrauterine transfusion on ventricular shortening fraction.
Methods: Intrauterine transfusion was performed at a median gestational age of 31 weeks (range 19-35). Median hemoglobin concentration before and after intrauterine transfusion was 7.9 g/dl (range 2.7-13.7) and 14.3 g/dl (range 12.7-16.1) respectively. The end-diastolic and end-systolic transverse dimensions of the left and right ventricles were obtained using M-mode ultrasound. The shortening fractions of both ventricles were calculated at three time points: before, immediately after and one day after intrauterine transfusion. The blood volume given at intrauterine transfusion was expressed as a percentage of estimated fetoplacental blood volume.
Results: Complete measurements were obtained from 49 transfusions in 23 fetuses. Both, left and right, ventricular shortening fractions differed significantly between the three time points. Left ventricular shortening fraction decreased immediately after transfusion in 43 (88 %) of the 49 fetuses. Right ventricular shortening fraction decreased immediately after transfusion in 42 (86 %) of the 49 fetuses. At the first intrauterine transfusion, there was only a weak correlation between the decrease in shortening fraction of both ventricles and the transfused volume (left: $R^2 = 0.15$; $p = 0.20$ / right: $R^2 = 0.005$; $p = 0.81$).
Conclusion: Transfusion significantly decreases the shortening fraction of both ventricles of the fetal heart. There is, however, little correlation between the decrease in shortening fraction and the volume of red cells given at intra-uterine transfusion.

Introduction

A variety of sonographic measurements can be used to describe fetal cardiac function. These include morphological measurements, such as the cardio thoracic ratio[1], dynamic measurements, such as ventricular shortening fraction measured in the four-chamber view as transverse ventricular diameter[2-4] ventricular length[5], or area[6], Doppler measurements, such as isovolumetric contraction time [7], and combined measurements such as stroke volume or cardiac output.[2] Fetal anemia, as well as intrauterine transfusion (IUT), is supposed to have an effect on fetal cardiac contractility.

Previous studies have focused on functional[8-11], morphological[12], Doppler[13-17] or combined measurements[9; 10; 18; 19] In this study, we have chosen to evaluate ventricular shortening fraction in severely anemic fetuses before and after IUT. M-mode measured four-chamber view transverse shortening fraction probably corresponds best with the visual impression of cardiac contractility on a B-mode image. A further advantage of ventricular shortening fraction is that it has been shown to be fairly constant throughout normal pregnancy.[2-4]

We wanted to investigate the possible use of ventricular shortening fraction as a diagnostic tool in the diagnosis of fetal anemia and in the assessment of cardiac overload during IUT. Our hypothesis for this study was that fetal anemia increases and that intra-uterine transusion decreases the fetal cardiac contractility. To test this hypothesis, we measured the left and right ventricular shortening fractionsin a group of alloimmune anemic fetuses before intra-uterine transfuion, immediately after (within 30 minutes) and a day after IUT.

Methods

Setting and Patients

Leiden University Medical Center is the national referral center for the treatment of fetal anemia in the Netherlands. Our methods for diagnosis and treatment of severe fetal alloimmune anemia have been described previously.[20] Between March 2001 and May 2002, M-mode ultrasound of the fetal heart was performed before, immediately after, and one day after all IUTs. The institutional review board gave approval for this prospective study, and all women gave oral informed consent. Severe fetal anemia was defined as hemoglobin-deficit ≥ 5 SD, moderate fetal anemia as 2 SD ≤ hemoglobin-deficit < 5 SD, and absence of anemia as hemoglobin-deficit < 2 SD of the normal mean.[21] Hydrops was classified as mild when a distinct rim of ascites was present with or without pericardial effusion. [22] Hydrops was classified as severe when ascites was abundant (free-floating intra-abdominal organs) with or without pericardial effusion, skin edema, or pleural effusion.[22]

Procedures

Fetal blood sampling was performed, and packed red cells with a hematocrit of 76-84% were given intravenously. The volume given at IUT depended on gestational age and on the severity of anemia. Hemoglobin was measured in the initial fetal blood sample of the transfusion. The hemoglobin concentration target after IUT was 14 g/dl. The transfused volume was expressed as a percentage of the estimated fetoplacental blood volume (FBV): (transfused volume at IUT / estimated FBV) *100. To calculate estimated FBV, we used estimated fetal weight determined by ultrasound, together with the assumption of a FBV of 100 ml/kg.[23] Before the procedure, meperidine (75 mg), promethazine (25 mg) and indomethacin (50 mg) were given to the mother. Atracurium (0.4 mg/kg) was given to the fetus immediately after the initial blood sample was taken.

Measurements

M-mode measurements were performed before IUT (0-6 hours), immediately after (within ½ hour), and one day after (12-24 hours). In the

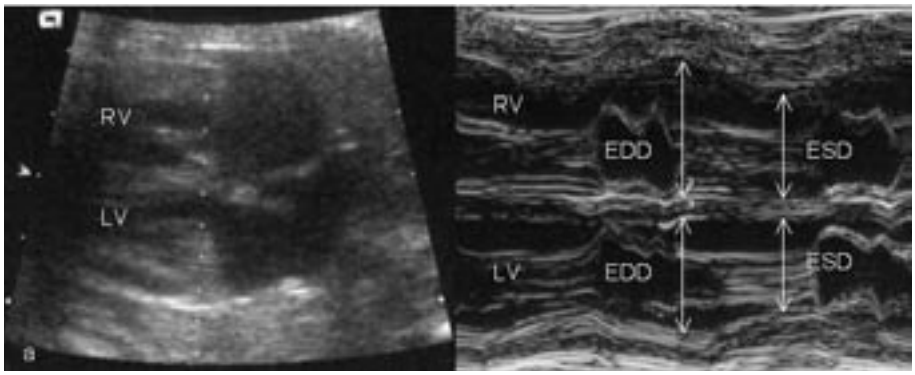

Figure 1 - Fetal heart (a) and the orientation of the M-mode cursor, placed perpendicular to the interventricular septum, just below the tips of the atrioventricular valves.
M-Mode measurement (b) of the systolic and diastolic transverse ventricular diameters.
LV = left ventricle, RV = right ventricle, EDD = end-diastolic dimension, ESD = end-systolic dimension

four-chamber view of the fetal heart, the cursor was placed perpendicular to the interventricular septum, just below the tips of the atrioventricular valves. The end-diastolic dimension (EDD) and the end-systolic dimension (ESD) of the left and the right ventricles were then obtained (Figure 1). M-mode measurements were obtained in the absence of fetal breathing and body movements. M-mode studies were done by one of two experienced operators (ES, KT) using an Acuson Sequoia (Acuson, Mountain View, CA) ultrasound machine with a 6.0 MHz probe. The left ventricular shortening fraction (LVSF) and the right ventricular shortening fraction (RVSF) were calculated using the following formula: shortening fraction = (EDD – ESD) / EDD. The percentage decrease between shortening fraction before and that after transfusion was calculated as (1 – (shortening fraction $_{\text{after IUT}}$ / shortening fraction $_{\text{before IUT}}$)) * 100. The percentage decrease in shortening fraction was correlated with the transfused volume as a percentage of estimated FBV.

Normal values

LVSF and RVSF were measured in 13 uncomplicated pregnancies with normal outcome. Each patient was measured five times with an interval of 4 weeks, between 18 - 36 weeks gestation. Inter-observer limits of agreement (95 % confidence interval of differences) for LVSF and RVSF were -0.014 to +0.024 and -0.023 to +0.023 respectively.[24]

Statistics

To compare the changes in shortening fraction between the three time points in patients with the first IUT and in patients with the following IUT, a linear mixed model with random effect was used.[25] This model was also used to compare means between controls and patients, to correct for the fact that several measurements per person were performed. Pearson R^2 values were calculated between percentage decrease in shortening fraction at first IUT and transfused volume as a percentage of estimated FBV. Statistical analysis was performed using SPSS 10.0 (SPSS, Chicago, IL) and SAS proc mixed (SAS, Cary, NC). A value of $p < 0.05$ was considered significant.

Results

During the study period, 85 IUTs were performed in 30 pregnancies. Thirty-six procedures were excluded from analysis, because of incomplete measurements due to fetal breathing, body movements or fetal position in utero (n=23), lack of time to perform the measurements (n=8), maternal obesity (n=4), or because intravascular access was not obtained and intraperitoneal transfusion was performed (n=1). Complete measurements were obtained from 49 IUTs in 23 fetuses in 23 women. Study population characteristics are shown in Table 1. There were no major complications following IUT in our study population. Minor complications occurred in 4 IUTs: fetal bradycardia for less than 1 minute (n=2), bleeding from the puncture site for less than 3 minutes (n=2).

Figure 2 shows mean (± 2 SEM) LVSF and RVSF in normal (control) fetuses (these measurements were longitudinally obtained) as well as in anemic fetuses before the first IUT, immediately after the first IUT, and one day after the first IUT. Ventricular shortening fraction was higher in anemic (first IUT) than in normal fetuses but his difference was not statistically significant (LVSF p=0.280, RVSF p=0.075). Mean (± 2 SEM) LVSF was 0.27 (0.24-0.29) in normal (control) fetuses. For the 13 fetuses that received their first transfusion, mean LVSF was 0.30 (0.24-0.35) before IUT, 0.19 (0.16-0.23) immediately after IUT, and 0.30 (0.26-0.34) one day after IUT

Table 1 - Characteristics of the 49 IUTs.

Maternal age (completed years), median (range) 32 (19-43)	
Gestational age (completed weeks), median (range) 31 (19-35)	
Type of alloimmunization	
D	41
Kell	3
c	5
Hydrops	
none	43
mild	3 (3 D alloimmunizations)
severe	3 (1 D and 2 Kell alloimmunizations)
Order of IUTs	
first	13
second	15
third	11
fourth	5
fifth	4
sixth	1
Infused volume	
infused volume (ml), median (range)	71 (14-114)
infused volume/estimated FBV (%), median (range) 44 (14-85)	
Hemoglobin	
hemoglobin before IUT (g/dl), median (range)	7.9 (2.7 – 13.7)
hemoglobin after IUT (g/dl), median (range), (n = 47) 14.3 (12.7 – 16.1)	
Degree of anemia	
none (Hb > -2 SD)	1
moderate (-2 SD≥ Hb > -5 SD)	11
severe (Hb≤ - 5 SD)	37

IUT = intra-uterine transfusion, FBV = fetoplacental blood volume, Hb = Hemoglobin, SD = standard deviation

(Figure 2). Mean (± 2 SEM) RVSF was 0.21 (0.18-0.25) in normal (control) fetuses. For the 13 fetuses that received their first transfusion, mean RVSF was 0.27 (0.23-0.32) before IUT, 0.16 (0.11-0.22) immediately after IUT, and 0.23 (0.18-0.27) one day after IUT (Figure 2). In previously transfused fetuses, similar changes were observed: LVSF was 0.28 (0.26-0.31) before IUT, 0.22 (0.18-0.25) immediately after IUT, and 0.33 (0.30-0.35) one day after IUT. RVSF was 0.31 (0.28-0.35) before IUT, 0.18 (0.14-0.23) immediately after IUT, and 0.23 (0.20-0.26) one day after IUT. Mean fetal heart rate (± 2 SEM) was 138 (136-140) in normal fetuses, 139 (133-144)

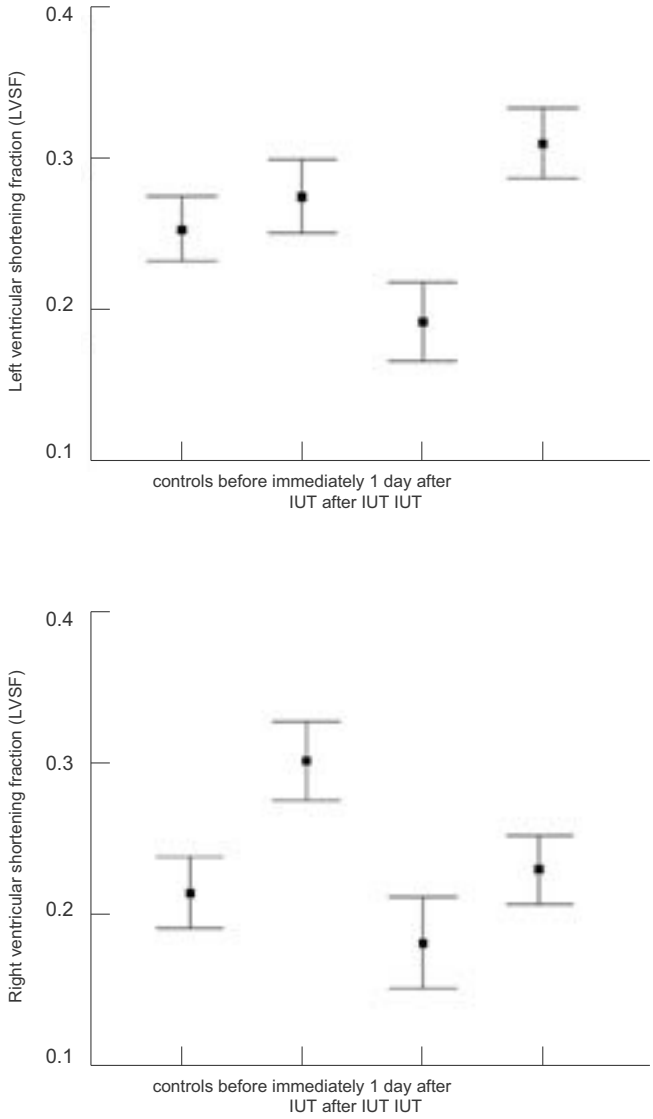

Figure 2 - Shortening fraction (mean ± 2 SEM) of the left (LVSF) and the right (RVSF) ventricle in 13 normal controls (taken in account that the measurements are longitudinally obtained with according change in SEM), and in 13 anemic fetuses before, immediately after, and one day after the first IUT. Ventricular shortening fraction was higher in anemic than in normal fetuses but this difference was not statistically significant. In anemic fetuses, LVSF and RVSF differed significantly between the three time points ($p<0.001$).

in anemic fetuses before the first IUT, 133 (126-139) immediately after the first IUT, and 141 (138-145) one day after the first IUT. In anemic fetuses LVSF and RVSF, as well as fetal heart rate, differed significantly between the three time points. This applied to fetuses receiving the first transfusion as well as for previously transfused fetuses ($p<0.004$).

Figure 3 shows the relation between ventricular shortening fraction before and immediately after IUT. The 45-degree line divides the fetuses with a decrease in shortening fraction from the fetuses with an increase. LVSF decreased in 43/49 (88 %) of IUTs, RVSF decreased in 42/49 (86 %) of IUTs. Hydropic fetuses showed a similar pattern as non-hydropic fetuses, although there was a tendency towards higher shortening fractions in hydropic fetuses.

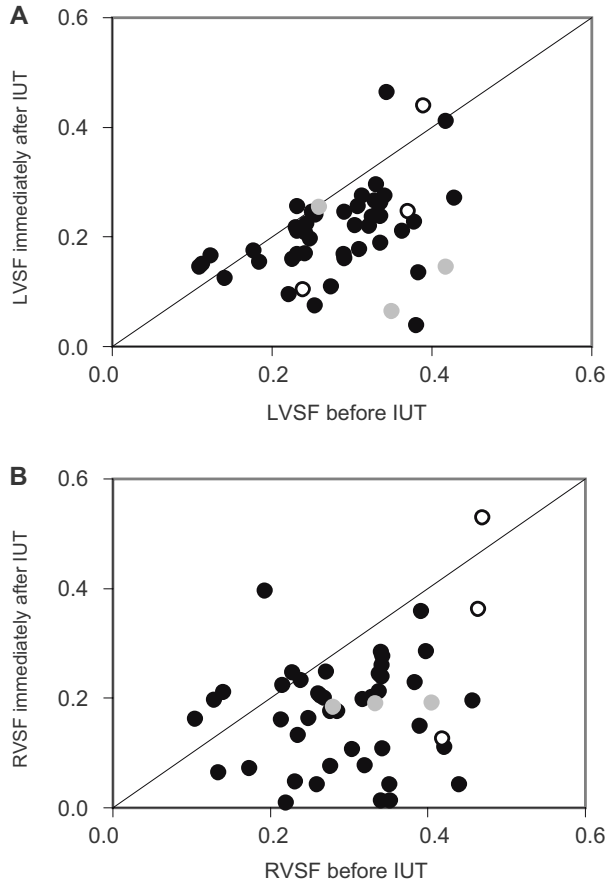

Figure 3 - Relation between ventricular shortening fractions before and immediately after IUT (49 IUTs in 23 fetuses). Each dot represents the two ventricular shortening fractions concerning one IUT, the value before IUT is on the x-axis and the post-transfusion value is on the y-axis. (A) Diagram for the left ventricular shortening fraction (LVSF) and (B) for right ventricular shortening fraction (RVSF).
The 45º line divides the fetuses with a decrease in shortening fraction (right of the line) from the fetuses with an increase in shortening fraction (left of the line). Black circles represent non-hydropic fetuses, grey circles represent mildly hydropic fetuses, and open circles represent severely hydropic fetuses.

Figure 4 shows the relation between percentage decrease in shortening fraction during transfusion and the transfused volume as a percentage of estimated FBV in 49 IUTs. We found only weak correlations for this relation at the first IUT (left: $R^2 = 0.15$; p= 0.20 / right: $R^2 = 0.005$; P = 0.1.

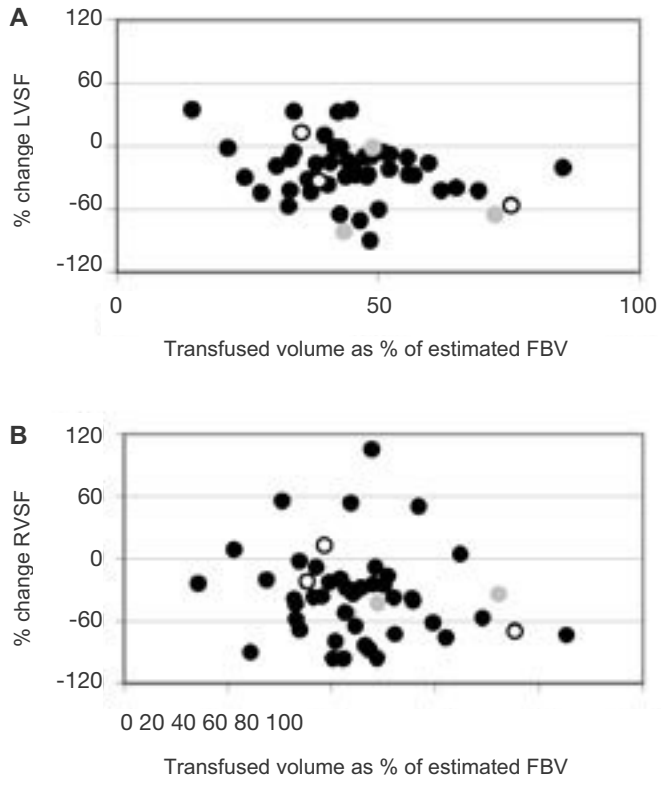

Figure 4 - Relation between change in shortening fraction and transfused volume in 49 IUTs. The change in shortening fractions is expressed as a percentage of the pre-transfusion value (minus value for decrease and plus value for increase). The percentage decrease in shortening fraction during transfusion was calculated as (1 – (shortening fraction after IUT / shortening fraction before IUT)) * 100. The transfused volume is expressed as a percentage of estimated fetoplacental blood volume (FBV). Transfused volume was calculated as: (transfused volume at IUT / estimated FBV) *100. Black circles represent non-hydropic fetuses, grey circles represent mildly hydropic fetuses, and open circles represent severely hydropic fetuses.
(A) Diagram for effect on left ventricular shortening fraction (LVSF) and (B) for right ventricular shortening fraction (RVSF).

Discussion

We measured LVSF and RVSF in normal fetuses and in anemic fetuses before and after IUT. We found that both LVSF and RVSF were higher in anemic than in normal fetuses, though this was not statistically significant. Fetal heart rate significantly decreased immediately after IUT. Furthermore, we found a substantial and statistically significant decrease in both LVSF and RVSF immediately after IUT. One would expect shortening fraction to decrease more after the administration of relatively large volumes of packed red cells. However, we found only a very weak correlation between the decrease in shortening fraction and the transfused volume (as a percentage of estimated FBV).

A review of normal LVSF and RVSF values as measured by different researchers is presented in Table 2.[2-4; 26; 27] Some researchers found a small decrease in ventricular shortening fraction with advancing gestational age; most researchers, however, found no significant effect of gestational age. As shown in Table 2, mean LVSF and RVSF are, in different research papers, close to 33%, ranging between 0.26 to 0.48 for the left ventricle and 0.21 to 0.42 for the right ventricle. We do not have an explanation

Table 2 - Literature review of reference values for ventricular shortening fraction measured by M-mode in normal fetuses.

First author, year	*Range of gestational age (weeks)*	*Number of fetuses*	*Number of measurements*	*Mean LVSF (SD)*	*Mean RVSF (SD)*
Wladimiroff et al,[27] (1981)	27-33	27	27	0.29 (0.07)	0.29 (0.06)
	34-40	26	26	0.26 (0.06)	0.26 (0.06)
De Vore et al,[3] (1984)	18-41	82	82	0.33 (0.04)	0.32 (0.04)
Koyanagi et al,[26] (1990)	18-41	104	104	0.26 (0.02)	0.29 (0.02)
Agata et al,[2] (1991)	37-40	34	34	0.34 (0.06)	---
Hsieh et al,[4] (2000)	10-40	42	241	0.48 (0.13)	0.42 (0.11)
Present study	18-36	13	65	0.27 (0.08)	0.21 (0.10)
Weighted mean of above mentioned studies	---	328	---	0.32 (0.06)	0.31 (0.06)

LVSF = left ventricular shortening fraction, RVSF = right ventricular shortening fraction, SD = standard deviation

for the differing range of normal values in previous studies, other than the difference between imaging techniques and the position within the ventricle from where the measurements are taken.[3] In our study mean LVSF was 0.27 and RVSF 0.21. These normal values are rather low in comparison to most of those in the studies listed in Table 2. Therefore, we suggest that sonographers should create their own reference ranges for ventricular shortening fraction.

We think that the strength of our study lies in the fact that we measured the shortening fraction on both sides of the heart in the same fetus before and after changing its FBV. This change was substantial in most cases. Further, we measured a relatively large number of fetuses with severe anemia. There are also some weaknesses in our study. First, the repeatability of M-mode measurements in the fetus has been described as poor.[28] However, in 1990, Veille et al. assessed the error of the cursor placement and found that there was no statistical difference between measurements of the right and the left ventricle at two different levels (at the tips of the atrioventricular valves and at the insertions of the valves) either during diastole or during systole.[29] Therefore the placement of the M-mode cursor at the atrioventricular valves in ventricular shortening fraction measurements is less critical than would be expected. Further, inter-observer variability in M-mode ventricular shortening fraction was measured by two groups of researchers.[27;29] They found a measuring error of 5%. Inter-observer limits of agreement in our study were –0.014 to +0.024 for LVSF and –0.023 to +0.023 for RVSF. Second, our standard medication before IUT included indomethacin. In some fetuses indomethacin causes transient constriction of the ductus arteriosus, even after short-term use.[30] This may have influenced LVSF and RVSF after IUT. Indeed, in 1997, Harada et al. showed that administration of indomethacin decreased the right ventricular area shortening fraction, but not the left ventricular area shortening fraction.[31]

Other authors have reported on cardiac function before and after intrauterine transfusion. Their findings are summarized in Table 3. In short, cardiac output decreased immediately after IUT whereas measures of afterload increased immediately after IUT.[8-11; 14; 16; 17; 19]Twelve hours

Table 3 - Summary of results of previous studies on cardiac function before and after intrauterine transfusion.

Authors, year	*No. of fetuses*	*No. of IUTs*	*Measure of cardiac function*	*Immediate effect of IUT*	*Effect of IUT after > 12 hours*
Copel et al,[13] 1988	24	64	Umbilical artery, pulsatility index		no change in the pulsatility index for any of the vessels
			Descending aorta, pulsatility index		
Copel et al,[18] 1989	11	11	Left ventricular output		no significant changes
			Right ventricular output		no significant changes
Weiner et al,[11] 1989	8	20	Umbilical venous pressure	increase with 4.2 mmHg	
Mari et al,[15] 1990	16	16	Middle cerebral artery, pulsatile index		no significant difference in the pulsatility index for any of the vessels
			Internal carotid artery, pulsatility index		
			Anterior cerebral artery, pulsatility index		
			Thoracic aorta, pulsatility index		
			Abdominal aorta, pulsatility index		
			Renal artery, pulsatility index		
			Femoral artery, pulsatility index		
			Umbilical artery, pulsatility index		
			Heartrate		no change in heartrate
Mari et al,[16] 1990	13	13	Middle cerebral artery, pulsatilel index	decrease with 0.70	decrease with 0.01
			Internal carotid artery,	decrease with 0.57	decrease with 0.10
			Anterior cerebral artery, pulsatility index	decrease with 0.69	decrease with 0.08
			Umbilical artery, pulsatility index	decrease with 0.29	decrease with 0.05
			Heartrate	significant increase in heartrate	no significant changes
Moise et al,[10] 1990	21	38	Umbilical venous pressure	increase with 1.7 mm Hg	
			Left ventricular output	decrease with 19%	
			Right ventricular output	decrease with 22%	
			Heartrate	no change in heartrate	
Rizzo et al,[19] 1990	12	12	Left ventricular output	decrease with 63.84 ml/min/kg	
			Right ventricular output	decrease with 52.35 ml/min/kg	
			Heartrate	no significant changes	
Oepkes et al,[17] 1993	21	21	Ductus venosus peak velocity	increase with 0.26 m/s	decrease with 0.09 m/s
			no significant changes	decrease with 0.09 m/s	
d' Ancona et al,[14] 1997	14	14	Portal vein	Increase with 0.7 of the H/L ratio	
Goodrum et al,[8] 1997	21	27	Mean umbilical arterial pressure	increase with 4.6 mm Hg	
			Heartrate	bradycardia in 5/16 IUTs	
Kilby et al,[9] 1998	6 fetal lambs	6	Left ventricular afterload	increase with 7.2 mm Hg/ml	
			End-diastolic pressure	increase with 6.5 mm Hg	
			End-diastolic vollume	increase with 0.9 ml/kg	
			Left ventricular output	decrease with 28 ml/kg/min	
			Heartrate	no significant changes	
This study	23	49	Left ventricular shortening fraction	decrease from 0.30 to 0.19	no significant decrease
			Right ventricular shortening fraction	decrease from 0.27 to 0.16	no significant decrease
			Heartrate	significant decrease	no significant decreas E

H/L ratio, ratio between high (peak) and low (nadir) velocities in the portal vein

after IUT, however, these changes were no longer demonstrable.[13; 15;18] Our findings, that VSF decreased immediately after IUT and normalized within 12 hours are, thus, in accordance with the findings of these previous studies. Further, we have related the change in VSF with the relative increase in blood volume during IUT. However, we found only a weak correlation.

In 1999 Ulm et al. described two pregnancies in the same woman where they performed 11 and 13 transfusions respectively.[32] In their case report, they suggest that continuation of intravascular therapy until term may represent a reasonable alternative to selective premature delivery even in cases with highly aggressive maternal rhesus alloimmunization. Our study suggests that a smaller number of IUTs but with a larger volume do not endanger the condition of the fetal heart. Massive IUT (a mean of 45% of FPV in 15 minutes) was well tolerated in our study. Although we perform IUT from 16 weeks' gestation onwards and aim at term delivery, the maximum number of IUT per pregnancy in our clinic has been 7 with low overall mortality and morbidity.[22]

In anemic fetuses middle cerebral artery peak velocity is increased. [33] After IUT this peak velocity of the middle cerebral artery decreases.[34] It has been suggested that fetal hematocrit as well as left ventricular contractility are the main determining factors for these changes in middle cerebral artery peak velocity.[35; 36] Our finding of a decreased LVSF after IUT supports the suggestion that decreased cardiac contractility contributes to the decrease in middle cerebral artery peak velocity after IUT.

In conclusion, fetal anemia had only a minor effect on M-mode measured shortening fraction of the left and right ventricles of the heart. Intrauterine transfusion, on the other hand, had a clear effect, with both LVSF and RVSF decreasing significantly. This corresponds well with the visual impression of decreased contractility on B-mode ultrasound during and immediately after IUT. This effect on contractility showed, however, little correlation with the transfused volume given at IUT.

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