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Improvement of fetal pulmonary hypertension and maturity after reversal of ductal constriction: a prospective cohort study

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This study shows for the first time that fetal estimated mean pulmonary artery pressure decreases and pulmonary vascular maturation increases after reversal of ductus arteriosus constriction by suspension of the causal agent, being these effects strongly correlated and independent of evolution of gestational age.

What are the clinical implications of this work?

Demonstration that fetal pulmonary vascular maturity and pulmonary hypertension improve when ductus arteriosus constriction is resolved by restriction of prostaglandin inhibitors stresses the utmost importance of adequate maternal guidance in late gestation, in order to treat and prevent this potentially severe functional fetal disorder.

ABSTRACT

Objectives: To test the hypothesis that estimated mean pulmonary artery pressure (MPAP) decreases and pulmonary vascular maturation assessed by the ratio of pulmonary artery flow acceleration time and ejection time (AT/ET) increases after reversal of fetal ductus arteriosus constriction, by suspension of the causal agent (polyphenol-rich foods or nonsteroidal anti-inflammatory drugs), and that these effects are independent of evolution of gestational age, inferences not yet demonstrated in the clinical setting. **Methods:** This is a prospective cohort study, comparing Doppler echocardiographic ductal flow dynamics parameters, MPAP and AT/ET in 70 third trimester fetuses at the moment of ductal constriction diagnosis and after 2 weeks of discontinuation of prostaglandin inhibitors. MPAP was estimated by Dabestani equation [PMAP = 90 - (0.62 x AT)] and vascular maturity by AT/ET ratio, according to reported validations. Statistical analysis utilized t test for comparison of the variables at diagnosis and after reversal of ductal constriction. Variations of MPAP and AT/ET at these 2 moments were also compared to the expected variations at the same gestational period in normal fetuses according to control reference curves of both variables constructed in 305 normal fetuses from normal pregnant women encompassing the third trimester. **Results:** Normalization of mean systolic and diastolic ductal velocities (1.86 ± -0.34 to 1.38 ± 0.41 m/s, $p<0.0001$ and (0.41 ± 0.11 to 0.21 ± 0.065 m/s, $p<0.0001$, respectively) and of pulsatility index (1.99 ± 0.20 to 2.55 ± 0.42, $p<0.0001$) was demonstrated after 2 weeks. In this period, mean MPAP decreased (66.7 ± 6.90 to 54.5 ± 6.70 mmHg, $p< 0.0001$), and AT/ET ratio at the pulmonary artery increased (0.20 ± 0.06 to 0.33 ± 0.07, $p< 0.0001$). Variation of mean MPAP was -12.3 ± -0.30 mmHg, $p<0.001$ (variation in normal fetuses in 2 weeks in the same gestational period = -2.3 ± -

0.19 mmHg [5.3 times more]), and variation of pulmonary AT/ET was 0.14 ± 0.08, p<0.001 (variation of AT/ET in normal fetuses in the same gestational period = + 0.015 ± 0.08, [9.3 times more]). **Conclusion:** Resolution of fetal ductal constriction is followed by fall in the estimated mean pulmonary artery pressure and by increase in pulmonary vascular maturity, in a significant higher degree than the observed in normal fetuses in the same gestational age evolution period.

INTRODUCTION

Constriction of fetal ductus arteriosus is a prevalent functional disorder, with potential for several neonatal complications such as pulmonary hypertension, cardiac failure and even death. Maternal utilization of anti-inflammatory pharmacological or dietary substances, such as nonsteroidal anti-inflammatory drugs (NSAD) and polyphenol-rich foods, respectively, which inhibits prostaglandin synthesis, has been widely shown to be responsible for the majority of cases[1-13]. There is habitual reversal after suspension of the causal agent and it has been already demonstrated that, in polyphenol-induced fetal ductal constriction, increase of maternal prostaglandin levels occurs after this reversal[11,12].

Fetal lung maturity is delayed when there is an acute decrease in pulmonary vascular area[14,15], such as in acute pulmonary hypertension due to pulmonary vasoconstriction. This event may be the result of constriction of fetal ductus arteriosus and reversible following its reversal.

Time events related to fetal pulmonary artery flow, such as acceleration and ejection times, have been shown to be of paramount importance in estimating pulmonary artery pressure and pulmonary vascular maturity by flow Doppler analysis, being variation of pulmonary artery fetal acceleration time the main parameter involved[16-20]. It seems logical to assume that fetal pulmonary hypertension should improve after the constricted ductus arteriosus reopens as a result of suspending prostaglandin inhibitors, while the delayed vascular maturation secondary to pulmonary vasoconstriction during ductal constriction should improve at the same time, but these are inferences that have not yet been demonstrated in the clinical setting.

This study was designed to test the hypothesis that estimated mean pulmonary artery pressure decreases and pulmonary vascular maturation increases after reversal of fetal constriction and that these effects are independent of evolution of gestational age.

PATIENTS AND METHODS

Study design and participants

This was a prospective cohort study, according to the *STROBE* statement[21], of women with singleton pregnancy at ≥ 28 weeks undergoing fetal echocardiography for any indication.

Two previous studies have already shown that maternal suspension of pharmacological or dietary inhibitors of prostaglandin synthesis (NSAD or polyphenol-rich foods, respectively) was followed after 2 weeks by reversal of ductal constriction[11,12]. Thus, as the same inclusion and exclusion criteria herein described were used and the methodology did not change, the analysis of this intervention was not the purpose of the present study. The subjects were pregnant women submitted to fetal echocardiography at the Fetal Cardiology Unit of the Institute of Cardiology of Rio Grande do Sul, in Porto Alegre, Brazil. Participants were examined between March 2017 and March 2019. The study group included cases with a diagnosis of fetal ductal constriction, as evidenced by echocardiographic findings of ductus arteriosus (DA) peak systolic velocity ≥ 1.40 m/s, DA maximum diastolic velocity ≥ 0.30 m/s and DA pulsatility index (determined as [peak systolic velocity − peak diastolic velocity]/mean velocity) ≤ 2.2, caused by maternal use of known prostaglandin inhibitors (anti-inflammatory pharmacological agents or polyphenol-rich foods)[13]. In addition to these parameters, Doppler analysis of mean pulmonary artery pressure (MPAP), estimated by the Dabestani equation (MPAP= [90 – 0.62 x AT]) and pulmonary acceleration time/ejection time ratio (AT/ET) to assess pulmonary vascular maturity, were also performed at the same time, as already described[18,22]. All measurements were obtained at the moment of diagnosis of ductal constriction and two weeks after discontinuation of

the causal agents, when its resolution was expected, according to previous studies[11,12]. These findings were also compared to a control group of fetuses with normal echocardiographic diagnosis encompassing the same gestational age range (28-38 weeks), in which normal reference curves for estimated MPAP and AT/ET were constructed, in order to compare them to the findings of the study group, to exclude the potential confounder effect of gestational age. Exclusion criteria for fetal characteristics in both groups were: cardiac malformations, or any other malformations; intrauterine growth restriction; increased nuchal translucency (> 95th percentile); chromosomal disorders suspected on fetal echocardiography or obstetric ultrasound morphological assessment; signs of any type of hydrops fetalis, or heart arrhythmia. Since the study group was made up exclusively by fetuses with ductal constriction, this was the only accepted cardiac alteration. Maternal exclusion criteria, both in the study group and in the normal group used for the construction of reference curves, included: hypertension, diabetes mellitus, structural or functional cardiac abnormality, current infection and positive serology for toxoplasmosis, HIV, cytomegalovirus, hepatitis C; current use of illicit drugs, alcohol or smoking; multiple pregnancy; ongoing labor or premature rupture of membranes; unsuitable echocardiographic window; having received previous nutritional guidance in relation to restricted intake of polyphenol-rich foods; and refusal to participate in the study.

The predictor variable of this study was reversal of ductal constriction after suspension of maternal consumption of pharmacological or dietary prostaglandin inhibitors, utilizing a group of fetuses with this disorder who were subjects of another parallel study. Fetal Doppler echocardiographic criteria both for diagnosis and reversal of ductal constriction have been reported and were here detailed with the sole purpose of

categorization as before and after resolution of this condition. The outcome variable of the present study was the modification of MPAP and AT/ET ratio (pulmonary hypertension and pulmonary vascular maturity) before and after reversal of ductal constriction.

The study was approved by the Research Ethics Committee of the Institute of Cardiology of Rio Grande do Sul (CAAE: 20606619.1.0000.5333). All participants provided signed informed consent after being fully informed of the purpose of the study. The study was conducted according to guidelines of Resolution 466/12 of the National Council of Health for research with human beings, including anonymity and privacy of participants.

Logistics

In cases with fetal ductal constriction, the mothers received orientation to suspend the usage of any pharmacological agent with potential for prostaglandin inhibition, mainly nonsteroidal anti-inflammatory drugs and nutritional guidance to restrict consumption of polyphenol-rich foods, according to protocols already published[13,23]. All women were directed to return after 2 weeks for follow-up Doppler echocardiographic assessment.

Fetal echocardiography was performed with commercially available ultrasound equipment capable of producing high-resolution images in M-mode, two-dimensional, continuous and pulsed-wave Doppler, and color-flow mapping (GE Vivid E9, GE Healthcare Ultrasound, Milwaukee, WI, USA), and devoted exclusively to the project. Electronic transducers were used with frequencies of 1.7–5MHz. Echocardiographic examinations assessed, by sequential analysis, the atrial situs, heart position, systemic and pulmonary venous drainage, atrioventricular and ventricular – arterial connections,

foramen ovale, aortic arch, ductal arch, and possible malformations. The DA was evaluated by color-flow Doppler in a longitudinal plane of the ductal arch or a transverse plane at the level of three vessels and trachea. The presence of flow turbulence was registered. The 2 mm pulsed-wave Doppler sample was positioned at the most distal, descending aortic end of the DA, with a maximum angle of 30°, without angle correction; 100 – 200-Hz filters, 50 cm/s scanning and five spectrum curves were applied. Clear Doppler signals and uniform waveforms were recorded. Fetal heart rate was recorded. In one of the cycles, the peak systolic, peak diastolic and end-diastolic velocities (m/s) were determined. The DA pulsatility index was calculated automatically by the echocardiography system after manual tracing of the spectral curve, using the equation: (peak systolic velocity − peak diastolic velocity)/mean velocity. Fetal ductal constriction was diagnosed by the following criteria: ductal peak systolic velocity ≥ 1.40 m/s, maximum ductal diastolic velocity ≥ 0.30 m/s and ductal pulsatility index ≤ 2.2[13]. Additional parameters indicative of functional effects of ductal constriction were also evaluated, but were not essential to the diagnosis; these included ductal flow turbulence on color flow mapping, right-to-left ventricle diameter ratio ≥ 1.3, pulmonary artery to aorta diameter ratio ≥ 1.3, bulging of the interventricular septum into the left ventricle during the cardiac cycle and tricuspid valve regurgitation. To assess main pulmonary artery flow, the Doppler sample-volume was positioned at the main pulmonary artery, as close to the pulmonary valve as possible, with a maximal angle of 20º, without angle correction. Acceleration time, ejection time, AT/ET ratio and MPAP were also obtained at the same time. Acceleration time was considered the time interval between the start of the pulmonary flow ejection and the peak of the ejection curve. The ejection time was measured between the start and the end of the

pulmonary flow ejection curve (**Figure 1**). Women were included in the study group after agreement between at least two researchers with respect to the diagnostic criteria of fetal ductal constriction. Two weeks after the first visit, a second Doppler echocardiographic examination was performed, by an investigator blinded to the study, group allocation and results of the first examination. Normal values of transductal flow velocities and pulsatility index, as well as absence of any other associated abnormalities or complications, were considered as a favorable outcome, and were confirmed by a second examiner. On this occasion, participants underwent echocardiographic reevaluation and answered again a questionnaire about usage of pharmacological or dietary prostaglandin inhibitors. On reassessment, the participants of the study group were guided to maintain polyphenol-rich food restriction and suppression of anti-inflammatory drugs until the end of pregnancy, and to consume alternative foods to replace the essential micronutrients present in polyphenol-rich foods.

For construction of MPAP and AT/ET ratio reference curves (control group), only normal fetuses from normal mothers with gestational age range from 20 to 37 weeks were included in the study, as already exposed.

Precautions were taken to control for main biases: selection biases, by following rigorously the inclusion and exclusion criteria for the selection of study participants; assessment biases, by ensuring that all measurements were performed by observers experienced in fetal echocardiography using strict predetermined parameters; and confounding factors, by including in the interventional group only fetuses within the third trimester and by constructing local reference curves of MPAP and AT/AT ratio plotted against gestational age, in order to compare the behavior of these variables excluding the effect of gestational age. Only echocardiographic images of good quality

were considered. Doppler angle < 30° was used in measurements. Data were collected during periods of fetal apnea and with no movement.

Sample size

The estimation of the sample size for an expected difference of the mean and SD of at least 15% in MPAP decrease and AT/ET ratio increase before and after resolution of fetal constriction, based on a previous pilot study of 18 patients (26% of the total sample), with a power of 90% and a significance level of 5% for AT/ET ratio and 1% for MPAP, was of 61 pregnant women.

For construction of the local nomograms of fetal MPAP and AT/ET ratio, from 19 to 37 weeks, also based on previous studies, and using as the main outcome the reduction of MPAP and the increase of AT/ET according to gestational age and considering an alpha error of 5% and a statistical power of 90%, a sample size of 286 mothers was estimated[22].

Statistical analysis

Results were expressed as follows: Student's *t*-test for independent samples was used for comparison of continuous variables with normal distribution between groups. Pre and post-treatment diagnostic parameters of fetal ductal constriction, pulmonary hypertension (MPAP) and pulmonary vascular maturity (AT/ET) were analyzed by Student´s *t*-test for paired samples. Variations of MPAP and AT/ET at these 2 moments were also compared to the expected variations at the same gestational period in normal fetuses using the Student's *t-test*. Correlation between MPAP and AT/ET ratio was tested by Pearson correlation test. For the construction of the reference curves of MPAP and AT/ET ratio, a simple linear regression was used to evaluate the relationship between these variables and gestational age, with a polynomial regression model adjusted with the coefficient of correlation[24]. A significance level of 5% was considered statistically significant. SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA) was used for data analysis.

RESULTS

Seventy women were recruited for the study group and there were no losses during the study period. Therefore, all the 70 pregnancies with fetal DA constriction (mean gestational age 27.75 ± 6.98 and mean maternal age 28.17 ± 8.12) were analyzed at the moment of diagnosis and reassessed after 2 weeks, upon guided suspension of maternal consumption of dietary or pharmacological prostaglandin inhibitors (polyphenol-rich foods or nonsteroidal anti-inflammatory agents). All patients were in the third trimester of pregnancy. Three hundred and five normal pregnant women, with normal fetuses, were examined from 19 to 37 weeks, to construct local reference curves of acceleration time/ejection time ratio and mean pulmonary artery pressure plotted against gestational age, which are expressed at **Figure 2**. The time range used for comparison with the study group was the period of 26-37 weeks, representing the period when ductal constriction is detected. MPAP decreased significantly with the evolution of the gestation (r = 0.51, $P < 0.001$), while AT/ET ratio increased with the gestational age (r = 0.53, $P < 0.001$). There was a strong inverse correlation between these variables (r = 0.89, $P = 0.001$), which is depicted at **Figure 3.**

Echocardiographic data before and after intervention in the study group are presented in **Figure 4**, which demonstrates the documentation and magnitude of ductal constriction reversal in the intervention group. The group showed in the same period a significant decrease in the mean of MPAP (66.7 ± 6.90 mmHg *vs* 54.5 ± 6.70 mmHg, $P < 0.001$) and an increase in mean AT/ET ratio (0.20 ± 0.06 *vs* 0.33 ± 0.07, $P < 0.001$) (**Figure 5**). The inverse correlation between MPAP and AT/ET ratio already observed in the normal reference group was also present in the study group, both at the moment of the

diagnosis of ductal constriction (r = 0.67, P <0.001) and after reversal of this condition (r = 0.57, P <0.001) (**Figure 3**).

The variation observed in the decrease in MPAP, compared to the expected physiological difference obtained from the reference curve in the same gestational range was -12.3 mmHg ± -0.30 mmHg, being the expected variation -2.3 ± -0.19 mmHg (P < 0.001, 5.3 times more). As for the increase in AT/ET ratio, this variation was 0.14 ± 0.08, with an expected variation from the nomogram 0.015 ± 0.08 (P < 0.001, 9.3 times more).

DISCUSSION

This study shows for the first time in humans that resolution of fetal ductal constriction is followed by fall in the estimated mean pulmonary artery pressure and by increase in pulmonary vascular maturity, in a significant higher degree than the observed in normal fetuses in the same gestational age range period.

Constriction of fetal ductus arteriosus is a potentially severe functional disorder, which has been widely shown to be one of the most frequent causes of neonatal pulmonary hypertension[25-32].

With vascular neoformations and gradual increase in pulmonary maturity, mean pulmonary artery pressure, high in early gestation, have a physiological tendency to progressively decrease toward late pregnancy, but this impact in pulmonary vascular reactivity may not occur or be lower in the presence of fetal ductal constriction[26,28,29,31,33]. Persistent neonatal pulmonary hypertension may occur in this situation, as a result of pulmonary vascular hyperreactivity due to the abrupt changes in postnatal circulation[25,29,31].

At the second and third trimester, alveolization start in terminal bronchioles and differentiation in pneumocytes I and II occurs with formation of surfactant, the lipoproteic combination which regulates alveolar superficial tension, and this process increases gradually, improving pulmonary maturation, especially at the third semester[34]. At the same time, increase in pulmonary vascularization occurs, with development of the intrapulmonary arteries and their transversal arteries, as well as an increase in arterial wall elasticity[35,36]. This phenomenon reflects the intimate relationship between pulmonary artery pressure and pulmonary maturity, while providing the rationale to understand why when one is affected, the other shows inversely proportional response.

The literature has consistently shown that fetal pulmonary maturity, traditionally assessed by amniotic fluid tests, such as surfactant/albumin ratio and others, could be predicted by the Doppler analysis of pulmonary artery flow, utilizing the AT/ET ratio, with high sensibility and specificity, being the cutoff from 0.32 to 0.35, depending of the methodology utilized, due to progressive increase of acceleration time toward the end of gestation[36-40]. Since the Doppler assessment of MPAP was proposed and validated in adults, utilizing as the dependent variable the AT, the Dabestani formula was used to estimate fetal pulmonary arterial pressure, whose behavior during gestation is consistent with the known pattern of decrease toward the end of gestational age and inversely correlated with variation of AT/ET ratio.

Transient pulmonary hypertension, such as occurs in ductal constriction, has been already shown to increase lung arteriolar resistance and reactivity[41,42], with lower pulmonary flow. Since flow in pulmonary trunk is related at the same time to systemic (ductal) and peripheral (pulmonary vascular bed) circuits, which are in parallel, its impedance reflects simultaneously this rheological effect at both sites. Decreased acceleration time is the Doppler marker of this phenomenon, and reversal of this effect after reopening of the ductus expresses resolution of both pulmonary hypertension and delayed lung maturity. These events occur at the third trimester, in a 2-3 weeks span, and the rate of variation of acceleration time (thus in AT/ET ratio and estimated MPAP) was much higher than the physiological differences observed in the reference curves – decrease of more than 5 times in MPAP and increase of almost 9 times in AT/ET. These original findings stress the role of ductal constriction upon delayed pulmonary maturity, being the mean AT/ET during this condition far below the accepted cutoff of around 34%, with a significant increase toward normal values after its reversal. Since both

variables depend on the AT, the expected inverse correlations between MPAP and AT/ET ratio both in normal fetuses and in the study group at the moment of diagnosis of ductal constriction and after its resolution were confirmed.

As for the limitations of this study, the potential bias of obtaining data at a single center is intrinsic, even though present in the mind of researchers and thus avoiding delay in generating evidence, especially since safety of the patients was not at risk. Another limitation could be that reproducibility of the main variables was not calculated in the present project, but they have been already tested in other studies and are available in the literature. The intrinsic limitation of using surrogate Doppler measurements for fetal estimated pulmonary maturity and pulmonary arterial pressure is acknowledged, but they have been previously validated and allow noninvasive assessment of parameters otherwise inaccessible in everyday clinical settings and in the search of new knowledge. To control potential confounder and assessment biases, inclusion criteria were strictly followed and only echocardiographic images of good quality were considered.

There are practical implications of the present study, that along with its original nature, represent its external validity. Many echocardiographic and Doppler parameters have already been used to assess severity of fetal ductal constriction, such as those related to the ductal flow itself (flow velocities and pulsatility index) and to the cardiac repercussion of this functional disorder[13]. Evaluation of mean pulmonary artery pressure and of pulmonary maturity, based mainly in Doppler analysis of the acceleration time and ejection time in pulmonary arterial flow, brings to bedside physiological parameters of great value. Demonstration that pulmonary vascular maturity may be influenced by the presence of acute pulmonary hypertension secondary to ductus arteriosus constriction, and that these alterations may be reversible after its resolution, are

important to clinical practice, corroborating the need to prevent its occurrence. Adequate maternal guidance to avoid consumption of both pharmacological and dietary substances with known prostaglandin inhibition effects in the third trimester of pregnancy is essential (Recommendation Class I, Level of Evidence A)[13,43,44]. Adding to the assessment of ductal constriction informations on the degree of pulmonary hypertension and how pulmonary maturity is expected to improve after resolution of this disorder could be of benefit from the prognostic viewpoint.

In conclusion, and in accordance with the conceptual hypothesis of this study, improvement of fetal pulmonary hypertension after reversal of ductal constriction is accompanied by amelioration of pulmonary vascular maturity, reflected respectively by decrease in Doppler assessed mean pulmonary artery pressure and increase in acceleration time/ejection time ratio in pulmonary artery flow.

Conflict of interest: The authors have no conflict of interest to disclose.

Abbreviations:

(NSAD) - Nonsteroidal anti-inflammatory drugs

(DA) - Ductus arteriosus

(MPAP) - mean pulmonary artery pressure

(AT/ET) - Acceleration time/Ejection time ratio

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FIGURES TITLES

Figure 1. ACQUISITION OF DOPPLER PULMONARY ARTERY FLOW.

Figure 1. *A*: Acquisition of Doppler pulmonary artery flow. Anatomic specimen of a fetal thorax (sagittal plane). RV: right ventricle; PA: pulmonary artery; DA: ductus arteriosus; AO: aorta. *B:* Diagram of a fetal echocardiogram in a short axis plane. RA: right atrium. *C*: Fetal Doppler tracing of the pulmonary artery ejection flow guided by a 2D image of RV and PA. Acceleration time (AT): time interval between the start of the pulmonary flow and the peak of the ejection curve; ejection time (ET): time between the start and the end of the pulmonary flow ejection curve.

Figure 2. REFERENCE CURVES OF MEAN PULMONARY ARTERY PRESSURE AND ACCELERATION TIME / EJECTION TIME RATIO IN NORMAL FETUSES ACCORDING TO GESTATIONAL AGE.

Fig. 2. *A*: Reference curve of estimated mean pulmonary artery pressure (MPAP) according to gestational age (GA). *B:* Reference curve of acceleration time / ejection time ratio (AT/ET) according to gestational age.

Figure 3. CORRELATION OF MEAN PULMONARY ARTERY PRESSURE AND PULMONARY ARTERY ACCELERATION TIME / EJECTION TIME RATIO IN CONTROL GROUP AND INTERVENTION GROUP, BEFORE AND AFTER REVERSAL OF DUCTAL CONSTRICTION.

Fig. 3. Correlation of acceleration time/ejection time ratio and estimated mean pulmonary artery pressure (MPAP) and in the control group (A) and in the intervention group, before (B) and after (C) reversal of ductal constriction.

Figure 4. DUCTUS FLOW BEHAVIOUR IN THE INTERVENTION GROUP AFTER 2 WEEKS OF RESTRICTION OF PROSTAGLANDIN INHIBITORS SUBSTANCES.

Fig. 4. The figure demonstrates the documentation and magnitude of ductal constriction reversal in the intervention group. A: Mean systolic velocity; B: Mean diastolic velocity; C: Mean pulsatility index.

Figure 5. BEHAVIOUR OF PULMONARY ARTERY PRESSURE AND ACCELERATION TIME / EJECTION TIME RATIO BEFORE AND AFTER REVERSAL OF DUCTAL CONSTRICTION.

Fig. 5. Mean of mean pulmonary artery pressure (MPAP) (A) and mean acceleration time/ejection time (AT/ET) (B) in the intervention group, before and after reversal of ductal constriction.