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FETAL VENTRICULAR INTERACTIONS AND WALL MECHANICS DURING DUCTUS ARTERIOSUS OCCLUSION IN A SHEEP MODEL

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Abstract

We investigated the effect of fetal sheep ductus arteriosus occlusion (DO) on the distribution of cardiac output, and left (LV) and right (RV) ventricular function by tissue and pulsed Doppler at baseline, after 15 and 60 minutes of DO induced by a vascular occluder, and 15 minutes after DO was released. Ductal occlusion decreased fetal pO₂. The mean LV output increased ($p < 0.001$) from 725 to 1013 ml/min, and RV (1185 vs. 552 ml/min) and systemic (1757 vs. 1013 ml/min) cardiac outputs fell ($p < 0.001$) after 15 minutes of DO, when compared to baseline. Pulmonary vascular impedance decreased and volume blood flow (Q_p) increased over 3-fold during DO, while foramen ovale volume blood flow (Q_{FO}) remained unchanged. LV systolic function was unaffected, while isovolumic relaxation velocity deceleration decreased. RV functional indices remained unchanged. We conclude that DO increased Q_p, not Q_{FO}. LV output increased, however not as much as RV output fell, resulting in decreased systemic cardiac output. During DO, LV exhibited diminished relaxation.

Keywords

echocardiography; hemodynamics; imaging; pregnancy; regional blood flow

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Introduction

In fetal circulation, ductus arteriosus connects the main pulmonary artery to the descending thoracic aorta. In a sheep fetus, almost 90% of right ventricular (RV) output is directed through ductus arteriosus into the descending aorta thus bypassing the lung circulation that is under acquired vasoconstriction at near term gestation (Lewis et al. 1976; Morin et al. 1988). In an acute sheep experiment with exteriorized fetus, a mechanically induced ductal occlusion (DO) for 30 seconds led to an increase in the pulmonary arterial pressure, a significant decrease in the RV output, a moderate increase in the left ventricular (LV) output, and a 34% fall in combined cardiac output (Tulzer et al. 1991a). Furthermore, RV systolic diameter increased and fractional shortening decreased. Tricuspid regurgitation was seen immediately after DO and it resolved when DO was released. This study raises important questions, first what is the mechanism leading to increased LV output and how the acute changes in ventricular loading conditions affect LV and RV function.

In humans, fetal ventricular loading conditions can be affected by a number of pregnancy complications. Ductal constriction is a well-known complication of maternal use of nonsteroidal anti-inflammatory agents (Moise et al. 1988). In addition, ductal constriction can develop without any predisposing factors. Furthermore, spontaneous closure of ductus arteriosus may occur during the last trimester of pregnancy (Hofstadler et al. 1996; Wei et al. 2011). In severe placental insufficiency, a shift in fetal cardiac output from RV to LV has been demonstrated (Makikallio et al. 2002). The foramen ovale (FO) that is an opening between the right and left atrium can play a critical role in the maintenance of fetal well-being in placental insufficiency. It seems that a fetus with a small FO size is unable to tolerate placental insufficiency as well as a fetus with a larger FO (Kiserud et al. 2004).

Tissue Doppler imaging is an ultrasound modality based on velocity of movement of the myocardium. Parameters derived from the myocardial movement during isovolumic contraction period are shown to be load-independent indices of cardiac contractility (Vogel et al. 2002; Vogel et al. 2003). Tissue Doppler technique is effective in evaluating long axis function and can detect earlier and/or milder forms of myocardial dysfunction in adults compared to standard echocardiography (Nestaas et al. 2011). Furthermore, studies on fetal sheep have shown that during acute acidemia, myocardial velocities become abnormal while cardiac output is still maintained (Acharya et al. 2008).

Immediately following DO, RV afterload increases and the entire RV output must circulate through the lungs into the left atrium. Furthermore, the RV output is sensitive to afterload (Reller et al. 1992; Thornburg et al. 1983). In order to maintain adequate fetal systemic cardiac output, LV blood return and thus preload must increase. An increase in the blood flow across FO would seem to be the primary mechanism in the process to increase LV preload. In addition, increased preload and ventricular end-diastolic pressure should improve ventricular systolic performance.

Previous reports on human fetuses with DO have proposed that the blood flow redistribution between RV and LV is characterized by increased right-to-left shunting across the dilated FO (Hofstadler et al. 1996; Wei et al. 2011). Furthermore, from the physiologic standpoint it

is crucial to understand the mechanisms and limits of the fetal heart to respond alterations in the cardiac loading conditions. The present fetal sheep study was designed to test the hypothesis that prolonged DO would lead to significantly increased FO volume blood flow and thus LV blood return. A rise in the LV preload would improve LV systolic function and output, while the increase in the RV afterload would have a negative impact on RV function. The specific aims of this study were to investigate the effects of DO on 1) ventricular and systemic cardiac outputs, FO and pulmonary volume blood flows, 2) RV and LV free wall longitudinal movement by tissue Doppler imaging to understand the ventricular wall mechanics and function during DO, and 3) fetal peripheral arterial and venous blood flow velocity waveforms, and fetal arterial and venous blood pressures.

Materials and Methods

Data obtained from nine singleton ovine pregnancies were included in this experiment. The Oregon Health and Science University Animal Care and Use Committee approved all experiments reported in this paper.

Surgical preparation and instrumentation

Surgery was performed at 120–126 gestational days (term 145 days). General anesthesia was induced with a diazepam (10mg) ketamine (400mg) mixture and the ewe was intubated. Anesthesia was maintained with 1.5–2.5% isoflurane and 25% nitrous oxide with the balance oxygen. A midline abdominal incision was made to access the uterus. The fetal head and upper body were delivered. Polyvinyl catheters were inserted into the carotid artery and internal jugular vein placing the catheter tips in the ascending aorta and superior vena cava. A left lateral thoracotomy was performed and ductus arteriosus was isolated, allowing for the placement of a 6 mm vascular occluder (In Vivo Metric, Healdsburg, CA). A 3 lead 28 gauge silver coated copper electrocardiogram wire (New England Wire Tech., Lisbon, NH) was placed subcutaneously on the fetal chest. The fetal chest was then closed in anatomic layers. A separate polyvinyl catheter was placed in the amniotic cavity to monitor intra-amniotic pressure. All incisions were closed and the fetus received an intra-amniotic injection of Penicillin G (1 million Units). All catheters and wires were then tunneled to a pouch on the ewe's flank. Post-operative pain was controlled with Buprenex (0.3 mg) twice a day for 3 days.

Invasive data acquisition

After a 5-day recovery period at 125–131 gestational days, fetal ultrasonographic data was acquired. Ewes were studied under general anesthesia. Prior to induction of anesthesia fetal blood gases were evaluated. Each ewe was prehydrated with 1 liter of lactated Ringer to prevent general anesthesia associated hypotension. Thereafter, Ringer's solution was infused at a fixed rate of 200 mL/h. Each ewe was given midazolam (0.2 mg/kg) to assist with minimizing general anesthesia. After induction of anesthesia with propofol (4–7 mg/kg) the ewe was intubated. Anesthesia was maintained with 1–2 % isoflurane mixed with oxygen enriched air. Anesthesia was titrated to keep the ewe's heart rate and blood pressure normal and allow for ultrasound imaging without discomfort while minimizing the physiologic alterations associated with its use. Propofol was occasionally given as a bolus as needed to

maintain anesthesia. A maternal peripheral arterial line was placed in the femoral artery to allow for blood gas and blood pressure monitoring. The sheep were positioned supine with a left lateral tilt. The animals were allowed to stabilize for 30 minutes prior to collecting baseline ultrasound measurements.

During the study, fetal electrocardiogram leads were connected to the ultrasound equipment. Fetal and maternal blood pressures were monitored continuously with pressure transducers calibrated with a mercury manometer and digitally stored using commercial hardware and software (Powerlab, ADI, Castle Hill, Australia). The recordings were analyzed in 1-minute periods and the median value per variable was chosen to represent a particular minute. The means of the last 5 minutes of each phase were used in the analyses. Fetal blood pressures were referenced to intra-amniotic pressure. Heart rate was determined from the pressure waveform. Fetal blood gases and serum samples were collected just before beginning the ultrasonographic data collection at each study point.

Ultrasonographic data acquisition

Ultrasonographic examinations were performed by a single investigator (J.R.). After baseline ultrasonographic data were collected, the ductal occluder was inflated with saline until resistance was met. Complete occlusion was confirmed by color Doppler ultrasonography, with no blood flow across the ductus arteriosus. Ultrasonographic data, identical to baseline study, were obtained 15 and 60 minutes after DO. After the 60-minute DO data was collected, the ductal occluder was completely deflated to restore ductal patency. The last set of ultrasonographic measurements was taken 15 minutes after the DO was released.

Ultrasonographic examination was done using a Vivid 7 Dimension ultrasound system (GE Vingmed Ultrasound, Horten, Norway) with a 10 MHz phased-array transducer. Pulsed Doppler was used to obtain ventricular outflow blood velocity waveforms. The angle of insonation was maintained at <15 degrees. From aortic and pulmonary valve blood flow velocity waveforms, time-velocity integral was obtained by planimetry of the area underneath the Doppler spectrum (Bernard et al. 2012). Pulmonary and aortic valve diameters were measured during systole using the leading edge method to calculate their cross sectional areas. Volumetric blood flows (Q) across the pulmonary and aortic valves were calculated ($Q = \text{cross sectional area of the valve} \times \text{time-velocity integral} \times \text{fetal heart rate}$). RV output equals the volume blood flow across the pulmonary valve and LV output equals the volume blood flow across the aortic valve (Bernard et al. 2012). Previous fetal sheep studies have shown that the proportion of lung volume blood flow (Q_p) of the combined cardiac output is 8 % at near term gestation (Rudolph and Heymann 1970). This result was used to estimate fetal Q_p at baseline and DO release phases. During DO, RV output equals Q_p . Foramen ovale volume blood flow (Q_{FO}) was estimated by subtracting Q_p from LV output. At baseline and at the post DO release phase, systemic cardiac output was estimated by subtracting Q_p from combined cardiac output. During DO, systemic cardiac output equals LV output. Right and LV fractional shortenings were calculated from M-mode recordings using the following formula: ventricular fractional shortening [%] = [(inner

diastolic diameter-inner systolic diameter)/inner diastolic diameter] $\times$ 100 (Bernard et al. 2012).

The longitudinal velocities of the RV and LV free wall during the cardiac cycle were assessed using pulsed-wave tissue Doppler imaging, with the sample volume (1–1.5 mm) placed at the level of the atrioventricular valve annuli and aligned as parallel as possible to the myocardial wall ($<15^\circ$ angle of insonation). Myocardial velocities were recorded during three to six cardiac cycles at a sweep speed of 100 mm/s. The frame rate was maximized. Peak myocardial velocities were measured during isovolumic relaxation (IVRV), early ventricular filling (E'), atrial contraction (A'), isovolumic contraction (IVCV) and ventricular systole (S') (Figure 1). The isovolumic myocardial acceleration and deceleration were calculated by dividing the peak IVCV and IVRV by the time intervals from the onset to the peak of these velocity waveforms (Acharya et al. 2008). In addition, the isovolumic contraction (IVCT) and relaxation times (IVRT) were measured and their proportions (%) of the total cardiac cycle were calculated (Acharya et al. 2008). Global ventricular function was evaluated by the index of myocardial performance (MPI = [IVRT + IVCT]/ejection time), which describes the combined systolic and diastolic function of the ventricle (Tei et al. 1997).

Blood velocity waveforms for the ductus arteriosus, umbilical artery, right pulmonary artery, pulmonary vein, ductus venosus, and inferior vena cava were obtained to calculate their pulsatility index (PI = [peak systolic velocity – end diastolic velocity]/time-averaged maximum velocity over the cardiac cycle) values. In the presence of tricuspid valve regurgitation, right ventricular contractility was assessed by calculating dP/dT (Tulzer et al. 1991b).

Statistics

The summary measurements are presented as means and standard deviation (SD) unless otherwise stated. Repeatedly measured variables were analyzed using linear mixed model. Pairwise comparisons between different time points were performed only if the overall change over time according to linear mixed model was significant ($P < 0.05$). Two-tailed P-values are presented, and all analyzes were performed using SAS for windows (version 9.1.3, SAS Institute Inc., Cary, NC, USA).

Results

Maternal blood gas values and arterial blood pressure remained stable during the experiment (data not shown). After 15 minutes of DO, fetal arterial oxygen saturation was lower than at baseline. Other blood gas parameters did not differ from the baseline values (Table 1). Fetal pO₂ and arterial oxygen saturation were lower after 60 minutes of DO than at baseline. Fetal pCO₂, mean arterial blood pressure and central venous pressure were not affected (Table 1).

Ductal occlusion increased LV stroke volume and output, while RV stroke volume and output decreased. Despite increased LV output, systemic cardiac output fell (Table 2). During DO, Q_P was greater than at baseline. However, Q_{FO} did not change during DO (Table 2, Figure 2).

During DO, tissue Doppler-derived LV A' velocity increased (Table 3). The LV IVCV and its acceleration were not affected. Furthermore, the LV IVRV remained unchanged. However, LV IVRV deceleration was lower at 60-minute DO phase than at baseline. Left ventricular MPI and IVRT% remained unchanged during the experiment. After the release of DO, LV IVCT% was greater than at baseline conditions (Table 3). In the RV, A' velocity was greater at 15-minute DO phase than at baseline (Table 4). The RV IVCV, IVCV acceleration, IVRV and its deceleration did not change significantly when compared to their baseline values. Right ventricular IVCT%, IVRT% and MPI were not affected by DO (Table 4).

Right pulmonary artery PI values decreased during DO (Table 5, Figure 3). After DO was released, right pulmonary artery PI values returned back to the baseline level. Pulmonary vein PIV values increased after DO was released. Both inferior vena cava and ductus venosus PIV values were greater during DO than at baseline and after the release of DO. Umbilical artery PI values were not affected. At baseline and after the release of DO, ductus arteriosus PI values were comparable (Table 5).

Left ventricular inner systolic diameter decreased and LV fractional shortening increased during DO. In the RV, DO increased inner systolic diameter and decreased fractional shortening. Right ventricular contractility, assessed by calculating dp/dT from the tricuspid valve regurgitation jet, was not affected by DO (Table 6).

Discussion

As anticipated, prolonged DO decreased RV output and increased LV output. The following major findings were observed in the present study. Contrary to our expectations, Q_{FO} did not change during DO. A significant rise in Q_P was responsible for an increase in LV output. Despite this shift in fetal cardiac output from the RV to LV, systemic cardiac output fell significantly. Increased LV preload was associated with signs of diastolic dysfunction, i.e. decreased rate of change of relaxation velocity, with no evidence of improved LV systolic function, i.e. no change in the acceleration of contraction velocity. On the other hand, the RV was able to maintain its systolic and diastolic functions despite a significant increase in afterload.

An increase in the LV output could not fully compensate for the simultaneous drop in the RV output following a complete DO, thus leading to diminished systemic cardiac output. Our finding is in agreement with an acute fetal sheep experiment with DO (Tulzer et al. 1991a). Despite this drop, fetal arterial blood pressure was maintained most likely by peripheral vasoconstriction. Immediately after DO, RV afterload increases with a rise in the main pulmonary arterial pressure. In normal circumstances, about 8 % of combined cardiac output is directed to the fetal sheep lung circulation and thus to the left atrium (Rudolph and Heymann 1970). At near term gestation fetal pulmonary arterial circulation is regulated mainly by fetal pO_2 (Lewis et al. 1976; Morin et al. 1988). A drop in fetal oxygenation leads to vasoconstriction and the effect of an increase in fetal oxygenation is opposite. In the present study fetal pO_2 decreased in the ascending aorta during DO. The most likely explanation is that the proportion of Q_P of the LV output increased. In the pulmonary artery,

the oxygen content of the blood is lower than in the ascending aorta in normal circumstances (Rudolph 1985). Other explanation could be a significant reduction in the placental volume blood flow and perfusion. However, fetal $p\text{CO}_2$ did not change significantly during DO suggesting that adequate placental perfusion was maintained. Pulmonary volume blood flow increased and vascular impedance, as indicated by right pulmonary artery PI, decreased significantly during DO. Most likely a rise in the main pulmonary arterial pressure increases the shear stress in the pulmonary arterial bed and this could stimulate inducible nitric oxide synthase in smooth muscle cells leading to increased Q_P (Rairigh et al. 1999). In fetal sheep, constriction of ductus arteriosus initially increases Q_P and decreases pulmonary vascular resistance that is followed by active vasoconstriction. It seems that nitric oxide - endothelin-1 interactions play a significant role in the regulation of pulmonary vascular tone following ductal constriction (Ovadia et al. 2002). Furthermore, pulmonary arteriovenous shunts that are present in fetal pulmonary circulation may be important in DO associated increase in Q_P (McMullan et al. 2004). We cannot precisely determine the percent increase in Q_P that is due to increased pulmonary artery pressure (passive pressure /flow relationship). Importantly, we found no significant change in Q_{FO} during DO. A rise in Q_P and thus in LV preload could impair right-to-left shunting across foramen ovale. However, studies on sheep have shown that of the two forces that determine foramen ovale flow in the fetus, pressure difference and kinetic energy, the latter is far larger than the former (Anderson et al. 1985). In human fetuses it has been shown that the size of the foramen ovale can be an important factor in the maintenance of fetal well-being in placental insufficiency (Kiserud et al. 2004). In addition, human fetuses who are unable to increase Q_{FO} in placental insufficiency, have more often retrograde net blood flow in the aortic isthmus thus predisposing the fetal brain to less oxygenated blood (Makikallio et al. 2003). The results of the present study suggest that at near term gestation, Q_{FO} is already near or at its maximum capacity. This could also explain the normal physiologic development of RV dominance during the last trimester of pregnancy, when pulmonary arterial circulation is subject to hypoxemic vasoconstriction. Altogether, our findings show that pulmonary circulation has a critical role in the maintenance of fetal systemic volume blood flow when ductus arteriosus is acutely occluded.

We found that LV IVCV and its acceleration that is a load-independent index of myocardial contractility (Vogel et al. 2002) did not change significantly during DO. Isovolumic contraction time represents the time interval when the ventricular pressure increases from the atrial to systemic level. Ductal occlusion increases blood return to the left atrium and thus LV end-diastolic volume and preload. Left ventricular systolic function did not improve, but cardiac output increased significantly. There was a modest increase in the fetal heart rate that could in part be responsible for increased cardiac output. During DO, RV pressure is elevated thus changing interventricular septal dynamics during the cardiac cycle and during systole pushing the septum towards the left ventricular lateral wall. In fact, during DO LV inner systolic diameter was significantly less than at baseline and after the release of occlusion. This could lead to decreased end-systolic volume and improved emptying of the LV. This is supported by increased LV fractional shortening and stroke volume during DO. In addition, A' velocity was increased during DO demonstrating that a rise in left atrial filling improved its performance. During DO, LV IVRV deceleration

decreased significantly when compared with the baseline values. Isovolumic relaxation period represents the time interval when the pressure in the ventricle decreases from the systemic to atrial pressure level. Myocardial movement during isovolumic relaxation phase of the cardiac cycle is less affected by ventricular loading conditions than during the ventricular filling (Vogel et al. 2002; Vogel et al. 2003). Our finding suggests that acutely increased LV preload following DO leads to LV diastolic dysfunction. Altogether, our results show that an acute increase in the LV preload is not associated with improved systolic function. It has been shown that fetal sheep LV operates at the breakpoint of the function curve and the output cannot be augmented much above the resting cardiac output by volume loading alone (Thornburg and Morton 1986). Furthermore, it appears that LV diastolic function is disturbed by a severe and prolonged increase in the preload.

In the RV, tissue Doppler-derived indices of cardiac systolic and diastolic function were not affected by DO, demonstrating that RV is capable to tolerate acutely increased afterload. In addition, RV dP/dT, calculated from the tricuspid valve regurgitation jet, did not change during DO, further supporting the concept that RV was able to maintain its contractility. Furthermore, A' velocity increased immediately after the occlusion suggesting an augmented atrial contraction. While RV inner diastolic diameter was not affected, DO increased inner systolic diameter, thus significantly decreasing RV fractional shortening, as shown in a previous study (Tulzer et al. 1991a). Right ventricular fractional shortening is sensitive to changes in afterload and thus not necessarily reflect the ventricular function (Tulzer et al. 1991a). In human fetuses with spontaneous DO, as a response to increased afterload, RV enlargement and hypertrophy progresses, its fractional shortening decreases and its pressure becomes very high, indicating good RV contractility (Hofstadler et al. 1996). These findings are similar to our observations. However, if DO persists, RV contractility may deteriorate, its pressure may fall, and echocardiographic evidence of RV endocardial scarring may appear indicating RV failure (Hofstadler et al. 1996). Altogether, our results show that fetal RV can tolerate acutely and severely increased afterload, at least in short term.

Ductal occlusion significantly increased pulsatility of ductus venosus and inferior vena cava blood flow velocity waveforms. Augmented atrial contraction could increase the pulsatility in the systemic veins by reducing forward blood flow in the ductus venosus during atrial contraction and similarly, in the inferior vena cava blood velocity waveform profile by reducing forward flow or augmenting reverse flow during atrial contraction. In addition, an increase in ductus venosus and inferior vena cava PIV values combined with a rise, although statistically nonsignificant, in the central venous pressure strengthen the concept that foramen ovale is unable to increase its volume blood flow during DO.

There are several limitations to the applicability of our results to humans. The surgical procedures may constitute a significant stress. However, the recovery period after surgery should be long enough for the recovery of fetal myocardial function (De Muylder et al. 1983). Data acquisition was performed while the ewe was under general anesthesia with isoflurane that can decrease fetal blood pressure. On the other hand, it has been shown that uterine and umbilical artery volume blood flows prior to and after the induction of general anesthesia are similar, suggesting conditions close to physiologic circulatory state in the

placenta (Acharya et al. 2004). Furthermore, the cardiovascular system of the newborn lamb is capable of increasing oxygen delivery in response to stress of hypoxemia during isoflurane anesthesia. Thus, at reasonable anesthetic depth, and in the absence of myocardial or peripheral cardiovascular disease, the newborn lamb can coordinate neural, endocrine, and local tissue responses to increase cardiovascular performance in response to hypoxemia (Brett et al. 1989). Validation studies in fetal sheep have shown that invasive and Doppler echocardiographic volume blood flow calculations correlate well (Schmidt et al. 1991). Importantly, the indirectly estimated Q_{FO} at the baseline condition in the present study is practically identical to that published by Anderson et al. (1985). The intraobserver variabilities of the Doppler ultrasonographic parameters of fetal sheep cardiovascular hemodynamics, as well as tissue Doppler-derived indices, have been shown to be comparable with those in previous human fetal studies during the second half of gestation (Bernard et al. 2012). In addition, our sample size ($n=9$) may affect the statistical power of the present study.

Our study has several important clinical implications. It seems that at near term gestation the capacity of foramen ovale to increase its volume blood flow is limited, at least in acute conditions. In normal circumstances, fetal pulmonary circulation is under acquired vasoconstriction during the last weeks of pregnancy that is sensitive to fetal oxygenation level. However, this regulatory mechanism can be overridden by acutely increased pulmonary arterial pressure. On the other hand, an increase in the proportion of Q_P of LV output can lead to decreased pO_2 and oxygen saturation in the blood entering the fetal cerebral circulation. From clinical standpoint, our results suggest that fetal pulmonary circulation is far more important in the maintenance of LV output than previously expected. This can be critical in fetuses with severe placental insufficiency or right-sided obstructive cardiac lesion and a small foramen ovale. In order to maintain or increase LV output, pulmonary circulation must provide more blood supply to the left atrium and ventricle. It has been shown that in infants born before 32 gestational weeks with placental insufficiency, suboptimal neurodevelopmental outcome is related to diminished cardiac output (Kaukola et al. 2005). We observed that the LV was unable to increase its systolic performance even when subjected to a sudden increase in preload. This can reflect the intrinsic properties of the LV, especially when the RV could maintain its function even in conditions with supra-systemic pulmonary arterial pressure. It can be proposed that the fetal RV is more optimal to function in hypoxemic environment than the LV.

Conclusions

Prolonged ductal occlusion in fetal sheep significantly increased pulmonary, but not foramen ovale volume blood flow. This resulted in increased left ventricular output. However, right ventricular and systemic cardiac outputs decreased. During ductal occlusion, left ventricular systolic performance did not improve and it exhibited signs of diminished relaxation. On the other hand, right ventricle was able to maintain its systolic and diastolic functions. Fetal arterial blood pressure was maintained during ductal occlusion.

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Figure 1.

Tissue Doppler-derived right ventricular longitudinal myocardial velocities at the level of tricuspid valve annulus. Isovolumic contraction velocity (IVCV), isovolumic contraction time (IVCT), velocity during ventricular systole (S'), isovolumic relaxation velocity (IVRV), isovolumic relaxation time (IVRT), velocities during early ventricular filling (E'), and atrial contraction (A') phases of the cardiac cycle.

Figure 2.

Mean (SE) changes from baseline for right (RVCO) and left (LVCO) ventricular cardiac outputs, pulmonary (Q_P) and foramen ovale (Q_{FO}) volume blood flows and systemic cardiac output (SCO).

Figure 3. Right pulmonary artery blood flow velocity waveform pattern at baseline (A) and after 15-minute ductal occlusion (B). At baseline, diastolic blood flow component is mainly retrograde (indicated by *). During ductal occlusion antegrade diastolic blood flow is markedly increased.

Table 1

Fetal arterial blood gas and blood pressure measurements (n = 9). Values represent mean (SD).

	Baseline	DO 15 minutes	DO 60 minutes	DO release	P-time
pH	7.34 (0.05)	7.31 (0.07)	7.27 (0.09)	7.29 (0.08)	0.06
pO ₂ (mmHg)	23.3 (3.9)	21.8 (4.1)	20.4 (3.5) *	26.1 (4.3) §	0.01
pCO ₂ (mmHg)	54.8 (6.4)	58.5 (10.7)	61.8 (13.7)	54.6 (8.0)	0.10
O ₂ saturation (%)	58 (13)	50 (13) *	43 (11) †	62 (9) §	0.0002
MAP (mmHg)	32 (5)	31 (7)	30 (5)	33 (5)	0.30
CVP (mmHg)	2 (1)	3 (2)	4 (2)	4 (3)	0.45

MAP = mean arterial blood pressure; CVP = central venous pressure.

* P < 0.05 different than Baseline;

† P < 0.001 different than Baseline;

‡ P < 0.05 DO 60 minutes different than DO 15 minutes;

§ P < 0.05 DO release different than DO 60 minutes.

Table 2

Fetal cardiovascular parameters (n = 9). Values represent mean (SD).

	Baseline	DO 15 minutes	DO 60 minutes	DO release	P-time
Heart rate (bpm)	160 (39)	174 (41)	175 (29)	155 (27)	0.16
LV stroke volume (ml)	4.5 (1.0)	5.7 (0.9) [‡]	5.2 (0.6) [*]	4.3 (1.2) [§]	0.0001
LVCO (ml/min)	725 (278)	1013 (332) [‡]	913 (206) [*]	653 (172) [§]	0.0001
RV stroke volume (ml)	7.6 (2.3)	3.1 (1.7) [‡]	2.1 (1.3) [‡]	5.0 (1.4) ^{*§}	0.0002
RVCO (ml/min)	1185 (387)	552 (330) [‡]	362 (215) ^{‡‡}	735 (210) [‡]	0.0001
Q _p (ml/min)	153 (49)	552 (330) [*]	362 (215) [*]	111 (24) ^{*§}	0.005
Q _{FO} (ml/min)	572 (237)	460 (318)	551 (250)	542 (155)	0.53
SCO (ml/min)	1757 (558)	1013 (332) [‡]	913 (206) ^{‡§}	1277 (275) [*]	0.0001

LV = left ventricle; LVCO = left ventricular cardiac output; RV = right ventricle; RVCO = right ventricular cardiac output; QP = pulmonary volume blood flow; QFO = foramen ovale volume blood flow; SCO = systemic cardiac output.

^{*} P < 0.05 different than Baseline;

[‡] P < 0.001 different than Baseline;

^{‡‡} P < 0.05 DO 60 minutes different than DO 15 minutes;

[§] P < 0.05 DO release different than DO 60 minutes.

Table 3

Fetal left ventricular tissue Doppler parameters (n = 9). Values represent mean (SD).

	Baseline	DO 15 minutes	DO 60 minutes	DO release	P-time
E' (cm/s)	10.34 (2.05)	11.65 (2.74)	9.03 (2.36)	8.83 (1.54)	0.11
A' (cm/s)	12.03 (3.01)	18.35 (5.11) [‡]	18.03 (4.70) [‡]	13.12 (4.01)	0.0003
S' (cm/s)	7.83 (1.99)	8.66 (2.11)	8.62 (3.08)	7.65 (1.87)	0.43
IVCV (cm/s)	7.41 (1.92)	8.06 (3.31)	9.14 (2.88)	7.78 (2.99)	0.20
IVCV acceleration (m/s ²)	5.01 (1.40)	4.12 (1.09)	4.42 (2.11)	4.04 (1.28)	0.23
IVRV (cm/s)	2.53 (0.51)	1.93 (1.15)	2.52 (0.24)	2.96 (0.74)	0.15
IVRV deceleration (m/s ²)	2.71 (0.90)	2.01 (1.24)	1.95 (0.32) [*]	2.77 (0.28)	0.003
IVCT (%)	8.2 (1.6)	9.7 (3.1)	9.9 (4.6)	10.4 (2.6) [*]	0.04
IVRT (%)	11.1 (3.2)	11.2 (4.3)	12.4 (3.1)	12.5 (2.5)	0.56
MPI	0.49 (0.11)	0.51 (0.18)	0.49 (0.08)	0.55 (0.10)	0.43

E' = early ventricular filling; A' = atrial contraction; S' = ventricular systole; IVCV = isovolumic contraction velocity; IVRV = isovolumic relaxation velocity; IVCT = isovolumic contraction time; IVRT = isovolumic relaxation time; MPI = myocardial performance index.

^{*} P < 0.05 different than Baseline;

[‡] P < 0.001 different than Baseline;

[‡] P < 0.05 DO 60 minutes different than DO 15 minutes;

[§] P < 0.05 DO release different than DO 60 minutes.

Table 4

Fetal right ventricular tissue Doppler parameters (n = 9). Values represent mean (SD).

	Baseline	DO 15 minutes	DO 60 minutes	DO release	P-time
E' (cm/s)	6.76 (2.48)	6.91 (1.77)	5.67 (2.82)	6.45 (2.38)	0.90
A' (cm/s)	9.32 (5.05)	14.30 (6.66)*	13.61 (5.53)*	9.28 (3.49) [§]	0.01
S' (cm/s)	8.25 (2.50)	7.64 (2.28)	6.63 (2.07)	6.85 (1.83)	0.18
IVCV (cm/s)	7.42 (2.79)	7.07 (1.61)	6.44 (3.87)	5.63 (2.52)	0.31
IVCV acceleration (m/s ²)	4.95 (1.38)	5.02 (1.38)	4.68 (1.42)	4.01 (1.83)	0.39
IVRV (cm/s)	4.13 (1.22)	3.50 (1.64)	3.36 (1.43)	2.87 (0.66)	0.16
IVRV deceleration (m/s ²)	3.19 (1.02)	2.88 (1.54)	3.14 (1.34)	2.14 (0.81)	0.13
IVCT (%)	9.3 (1.4)	10.9 (3.9)	9.4 (2.3)	10.3 (2.2)	0.39
IVRT (%)	14.2 (3.2)	17.9 (5.0)	17.4 (5.1)	17.8 (4.0)	0.12
MPI	0.60 (0.11)	0.64 (0.18)	0.64 (0.21)	0.71 (0.13)	0.37

E' = early ventricular filling; A' = atrial contraction; S' = ventricular systole; IVCV = isovolumic contraction velocity; IVRV = isovolumic relaxation velocity; IVCT = isovolumic contraction time; IVRT = isovolumic relaxation time; MPI = myocardial performance index.

* P < 0.05 different than Baseline;

[†] P < 0.001 different than Baseline;

[‡] P < 0.05 DO 60 minutes different than DO 15 minutes;

[§] P < 0.05 DO release different than DO 60 minutes.

Table 5

Fetal peripheral hemodynamics (n = 9). Values represent mean (SD).

	Baseline	DO 15 minutes	DO 60 minutes	DO release	P-time
Pulsatility Index					
DA	1.88 (0.40)			1.88 (0.46)	0.36
RPA	37.81 (41.64)	6.30 (13.02) *	6.94 (8.25) *	22.76 (16.67)	0.03
P _{vein}	5.88 (6.85)	2.39 (2.33)	5.56 (3.23) [‡]	22.70 (15.40) ^{*§}	0.007
UA	1.25 (0.25)	1.27 (0.24)	1.40 (0.20)	1.16 (0.25)	0.08
IVC	1.08 (0.41)	2.97 (1.69) *	3.48 (1.74) *	1.59 (1.15) [§]	0.006
DV	0.61 (0.14)	1.16 (0.24) *	1.65 (1.07) ^{‡‡}	0.73 (0.29) [§]	0.0003

DA = ductus arteriosus; RPA = right pulmonary artery; P_{vein} = pulmonary vein; UA = umbilical artery; IVC = inferior vena cava; DV = ductus venosus.

* P < 0.05 different than Baseline;

[‡] P < 0.001 different than Baseline;

[‡] P < 0.05 DO 60 minutes different than DO 15 minutes;

[§] P < 0.05 DO release different than DO 60 minutes.

Table 6

Fetal cardiac dimensions and right ventricular contractility (n = 9). Values represent mean (SD).

	Baseline	DO 15 minutes	DO 60 minutes	DO release	P-time
Left Ventricle					
Diastole (cm)	1.52 (0.17)	1.50 (0.24)	1.42 (0.18)	1.43 (0.24)	0.23
Systole (cm)	0.98 (0.09)	0.77 (0.19) [†]	0.69 (0.12) [†]	0.91 (0.16) [§]	0.0001
FS (%)	35.1 (4.0)	48.8 (7.7) [†]	51.7 (3.7) [†]	36.6 (5.5) [§]	0.0001
Right Ventricle					
Diastole (cm)	1.28 (0.36)	1.38 (0.34)	1.54 (0.17)	1.49 (0.16)	0.20
Systole (cm)	1.02 (0.15)	1.33 (0.46)	1.60 (0.27) [†]	1.22 (0.30) [§]	0.0008
FS (%)	25.9 (7.5)	10.2 (30.4)	-3.9 (9.3) [†]	19.0 (13.2) [§]	0.0001
dP/dT (mmHg/s)	1073 (142)	1128 (608)	1330 (628)	1110 (175)	0.85

FS = fractional shortening.

* P < 0.05 different than Baseline;

[†] P < 0.001 different than Baseline;

[‡] P < 0.05 DO 60 minutes different than DO 15 minutes;

[§] P < 0.05 DO release different than DO 60 minutes.