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Foramen ovale blood flow and cardiac function after main pulmonary artery occlusion in fetal sheep

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Abstract

The foramen ovale (FO) accounts for the majority of fetal left ventricular (LV) output. Increased right ventricular afterload can cause a redistribution of combined cardiac output between the ventricles. To understand the capability of the FO to increase its volume blood flow and thus LV output, we mechanically occluded the main pulmonary artery in seven chronically instrumented near-term sheep fetuses. We hypothesized that FO volume blood flow and LV output would increase during main pulmonary artery occlusion. Fetal cardiac function and haemodynamics were assessed by pulsed and tissue Doppler at baseline, 15 and 60 min after occlusion of the main pulmonary artery and 15 min after occlusion was released. Fetal ascending aorta and central venous pressures and blood gas values were monitored. Main pulmonary artery occlusion initially increased fetal heart rate ($P < 0.05$) from [mean (SD)] 158 (7) to 188 (23) beats min^{-1} and LV cardiac output ($P < 0.0001$) from 629 (198) to 776 (283) ml min^{-1}. Combined cardiac output fell ($P < 0.0001$) from 1524 (341) to 720 (273) ml min^{-1}. During main pulmonary artery occlusion, FO volume blood flow increased ($P < 0.001$) from 507 (181) to 776 (283) ml min^{-1}. This increase was related to fetal tachycardia, because LV stroke volume did not change. Fetal ascending aortic blood pressure remained stable. Central venous pressure was higher ($P < 0.05$) during the occlusion than after it was released. During the occlusion, fetal pH decreased and P_{CO_2} increased. Left ventricular systolic dysfunction developed while LV diastolic function was preserved. Right ventricular systolic and diastolic function deteriorated after the occlusion. In conclusion, the FO has a limited capacity to increase its volume blood flow at near-term gestation.

KEYWORDS

blood flow, echocardiography, physiology

1 | INTRODUCTION

Fetal right (RV) and left (LV) ventricles pump in parallel into the systemic circulation. The RV is mainly responsible for lower body and placental blood flow and perfusion, whereas myocardial, brain and upper body blood flow is provided by the LV. In physiological conditions in near-term fetal sheep, ~70% of combined cardiac output (CCO) is ejected by the right ventricle (RVCO), and almost 90% of RVCO is directed through the ductus arteriosus (DA) towards the lower body and placenta, and the rest to the pulmonary circulation (Anderson, Bissonnette, Faber, & Thornburg, 1981; Rudolph, 1985). Given that the fetal pulmonary circulation is under acquired vasoconstriction at near-term gestation (Lewis, Heymann, & Rudolph, 1976), blood flow across the foramen ovale (FO) accounts for 34% of CCO and 85% of LV output (LVCO) (Anderson et al., 1981). However, changes in ventricular loading conditions can lead to redistribution of CCO between the two ventricles and disturb the RV dominance, because RVCO is particularly sensitive to increased afterload (Reller, Morton, Reid, & Thornburg, 1987; Thornburg & Morton, 1983). During prolonged DA occlusion in near-term fetal sheep, RVCO and CCO decreased, while LVCO increased (Hashima et al., 2015). Interestingly, an increase in LVCO was attributable to increased pulmonary volume blood flow while FO volume blood flow did not change (Hashima et al., 2015). This suggests that at near-term gestation the fetal pulmonary circulation is an important regulator of LVCO, whereas FO blood flow might be at or near its maximal capacity.

To investigate the capability of the fetal FO to increase its volume blood flow and thus improve LV filling and output, we performed a complete mechanical main pulmonary artery occlusion in chronically instrumented near-term fetal sheep. During the occlusion, there is no forward blood flow across the DA, and the LV alone accounts for systemic and placental circulation. Furthermore, LV preload is preferentially supplied by the FO blood flow. We hypothesized that FO volume blood flow, hence LVCO, would increase during main pulmonary artery occlusion in order to maintain adequate systemic and placental perfusion. The specific aims of this study were to investigate the effect of main pulmonary artery occlusion on the following fetal parameters: (i) LVCO, CCO and systemic arterial and central venous pressures; (ii) RV and LV systolic and diastolic function; (iii) peripheral venous blood flow patterns; and (iv) placental volume blood flow and fetal oxygenation.

New Findings

- **What is the central question of this study?**
  At near-term gestation, foramen ovale blood flow accounts for a significant proportion of fetal left ventricular output. Can the foramen ovale increase its volume blood flow when right ventricular afterload is increased by main pulmonary artery occlusion?
- **What is the main finding and its importance?**
  Foramen ovale volume blood flow increased during main pulmonary artery occlusion. However, this increase was attributable to an increase in fetal heart rate, because left ventricular stroke volume remained unchanged. These findings suggest that the foramen ovale has a limited capacity to increase its volume blood flow.

2 | METHODS

2.1 | Ethical approval

The study protocol was approved by the National Animal Experiment Board of Finland (ESAVI/3510/04.10.03/2011). The animal transport, husbandry and experimental procedures were performed in compliance with the national legislation (Finnish Government 2013; Parliament of Finland 2013) and the EU directive (The European Parliament and the Council of the European Union 2010). The investigators acknowledge the ethical principles of *Experimental Physiology* and confirm that the study was conducted in compliance with the animal ethics checklist (Grundy, 2015). A total of seven time-mated pregnant 1- to 7-year-old Aland landrace sheep weighing between 41 and 53 kg (Lammastila Sikka Talu, University of Turku, Rymättylä, Finland) were used in the experiment. The sheep were transported from the breeder to the Laboratory Animal Centre at the University of Oulu, Finland 2 weeks before the experiment. During the adaptation period, the sheep were group housed in two pens of 10.8 m^2 in area and during the experiment in individual pens of 3.6 m^2, with straw bedding. Adjacent sheep were able to be in contact with each other through the windows between the pen walls, and no individual sheep was left alone in the animal room. The room temperature was 18 ± 2°C, ventilation rate 15 times h^{-1}, and humidity 45 ± 5%. The light–dark cycle was 12 h–12 h, with the lights off at 18.00 h. The sheep were given tap water and hay *ad libitum*, and they had a salt block in the pen. Individually rationed oat grains, turnip rape-based protein supplement (Farmarin rypsi; Hankkija-Maatalous Oy, Seinäjoki, Finland) and mineral and vitamin supplement (Lammas Hertta; Hankkija-Maatalous Oy) were given twice daily, and the rations were increased gradually towards the end of the pregnancy. When needed, supportive doses of calcium were given either orally or I.V. Animals were monitored several times daily by a veterinarian, animal technicians and the investigators for signs of pain, distress, injury or disease. The focus was set to ensure the well-being of animals and to minimize pain and suffering (see methodological description below).

2.2 | Surgical protocol

The sheep with either singleton or twin pregnancies were operated on at 120–130 gestational days (term 145 days). In the event of twin pregnancy, only one fetus was instrumented. The sheep were premedicated with ketamine (2 mg kg^{-1}, I.M.; Ketaminol vet; Intervet, Boxmeer, The Netherlands) and midazolam (0.2 mg kg^{-1}, I.M.; Midazolam Hameln; Hameln Pharmaceuticals, Hameln, Germany). The left external jugular vein was cannulated for I.V. access, and

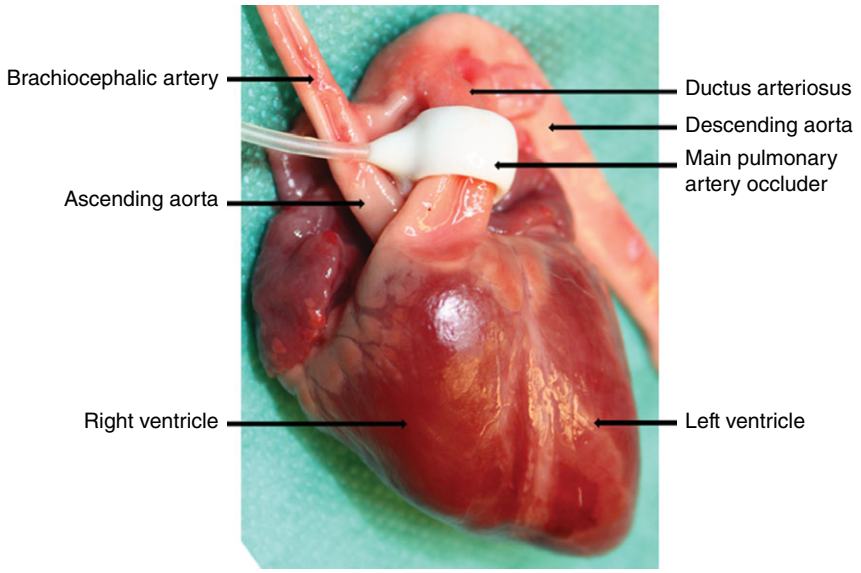

FIGURE 1 Location of the main pulmonary artery occluder

lactated Ringer solution was infused at a rate of 200 ml h^{-1}. General anaesthesia was induced with propofol (4–7 mg kg^{-1}, I.V.; Propofol-Lipuro; Braun, Melsungen, Germany) and maintained with isoflurane (1.5–2.5%; Isofluran Baxter; Baxter S.A., Lessines, Belgium) in an oxygen–air mixture delivered via an endotracheal tube. Mechanical ventilation was provided with a Siemens 730 ventilator (Siemens-Elema AB, Solna, Sweden). Maternal heart rate and arterial blood pressure were monitored invasively via a cannulated auricular artery. For pain relief, I.V. boluses of fentanyl (0.05–0.15 mg; Fentanyl-Hameln; Hameln Pharma plus, Hameln, Germany) were administered on the basis of changes in maternal heart rate and arterial blood pressure during surgical stimuli as deemed necessary by an experienced anaesthetist.

A mid-line 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, with the catheter tips in the ascending aorta and superior vena cava, respectively. A left lateral thoracotomy was performed, and the pericardial sac was opened to expose the great arteries. The main pulmonary artery was isolated, and a 6 mm vascular occluder (In Vivo Metric, Healdsburg, CA, USA) was placed around it between pulmonary valve and the bifurcation of the left and right pulmonary arteries (Figure 1). A three-lead 28-gauge silver-coated copper ECG wire (New England Wire Tech., Lisbon, NH, USA) was placed subcutaneously on the fetal chest. Thereafter, the fetal chest was closed. A separate polyvinyl catheter was placed in the amniotic cavity. Lost amniotic fluid was replaced with warm saline solution, and an intra-amniotic injection of penicillin G (1 million units; Geepenil; Orion Oyj, Espoo, Finland) was administered. The surgical incisions were closed. All catheters were tunnelled subcutaneously and exteriorized through a small incision in the ewe's flank. Postoperative analgesia was provided with transdermal fentanyl patches (Fentanyl ratiopharm; Ratiopharm, Ulm, Germany), at the dose rate of 2 μg kg^{-1} h^{-1}, applied to the ewe's antebrachium before surgery.

2.3 | Experimental protocol

After a 4 day recovery period at 124–134 gestational days, general anaesthesia was induced with a single bolus of propofol and maintained with isoflurane only. The isoflurane concentration was titrated to keep the ewe's heart rate and blood pressure normal and allow for ultrasound imaging without discomfort while minimizing the physiological alterations associated with its use. Before induction, each ewe was prehydrated with 1 litre of lactated Ringer solution, followed by a fixed infusion of lactated Ringer solution at a rate of 200 ml h^{-1} throughout the experiment. A 16-gauge polyurethane catheter was inserted into the maternal femoral artery in order to measure maternal arterial blood pressure and to obtain arterial blood gas samples. The ewe was placed supine with a right lateral tilt and allowed to stabilize for 30 min before the baseline measurements were taken. Thereafter, the main pulmonary artery occluder was inflated with saline until resistance was met. Complete occlusion was confirmed by colour Doppler ultrasonography, with no blood flow across the occluder. Ultrasonographic data, identical to baseline measurements, were obtained 15 and 60 min after the main pulmonary artery occlusion. After the 60 min occlusion data were collected, the main pulmonary artery occluder was completely deflated to restore the main pulmonary artery blood flow. The last set of ultrasonographic measurements was taken 15 min after the main pulmonary artery occluder was released. At each phase, the ultrasonographic data acquisition took ~15–20 min, and the data were collected in random order. The ultrasonographic data were stored and analysed afterwards in a blind manner. At the end of the experiment, the fetus and ewe were killed with an I.V. overdose (100 mg kg^{-1}) of pentobarbital sodium (Mebunat vet; Orion Oyj), and fetal weight was determined.

2.4 | Monitoring protocol

Fetal and maternal blood pressures were continuously monitored with disposable pressure transducers (DT-XX; Ohmeda, Hatfield, UK). Fetal

TABLE 1 Fetal arterial blood gas and blood pressure measurements

Parameter	Baseline	PaO 15 min	PaO 60 min	PaO release	*P* value for time
pH	7.33 (0.04)	7.30 (0.02)	7.26 (0.04)*	7.24 (0.09)*	0.043
P_{O_2} (kPa)	2.6 (0.6)	2.3 (0.4)	2.1 (0.6)	2.3 (0.5)	0.176
P_{CO_2} (kPa)	6.3 (0.6)	6.9 (0.9)	7.5 (0.8)*,†	7.1 (1.3)	0.033
Base excess (mmol l^{-1})	−0.9 (2.9)	−0.6 (3.2)	−1.4 (4.2)	−3.3 (6.8)	0.265
Lactate (mmol l^{-1})	3.36 (1.33)	3.07 (1.16)	3.40 (1.69)	3.91 (2.28)	0.199
Ascending aortic blood pressure					
Systolic (mmHg)	47 (6)	46 (5)	45 (4)	47 (4)	0.719
Mean (mmHg)	38 (3)	37 (4)	37 (3)	38 (5)	0.928
Diastolic (mmHg)	32 (3)	32 (4)	32 (3)	32 (4)	0.996
CVP (mmHg)	4 (3)	6 (3)	5 (3)	4 (3)‡	0.012

Values are means (SD); $n = 7$. Abbreviations: CVP, central venous pressure; and PaO, main pulmonary artery occlusion. *Different from baseline, $P < 0.05$. †Different from preceding phase, $P < 0.05$. ‡Different from PaO 15 min phase, $P < 0.05$.

blood pressures were referenced to intra-amniotic pressure. Heart rates were determined from the arterial pressure waveforms. Fetal ECG leads were connected to the ultrasound equipment. Maternal and fetal blood gas values were corrected to 39°C and analysed at each study point using an Abbot i-Stat 1 arterial blood gas analyser (i-Stat, East Windsor, NJ, USA).

Ultrasonographic measurements were taken by a single investigator (J.R.) using a Vivid 7 Dimension ultrasound system (GE Vingmed Ultrasound, Horten, Norway) with a 10 MHz phased-array transducer. Pulmonary and aortic valve diameters were measured, and their cross-sectional areas were calculated. Blood flow velocity waveforms across the pulmonary and aortic valves were obtained with pulsed Doppler. The angle of insonation was kept at <15 deg. Volume blood flows across the pulmonary (RVCO) and aortic (LVCO) valves were calculated (Rasanen, Wood, Weiner, Ludomirski, & Huhta, 1996). Previous fetal sheep studies have shown that the proportion of pulmonary volume blood flow ($\dot{Q}_P$) of the CCO is ~8% at near-term gestation (Rudolph & Heymann, 1970). This estimate was used to calculate fetal $\dot{Q}_P$ in the baseline and recovery phases. Foramen ovale volume blood flow ($\dot{Q}_{FO}$) was determined by subtracting $\dot{Q}_P$ from LVCO in the baseline and recovery phases. During main pulmonary artery occlusion, $\dot{Q}_{FO}$ equals LVCO. The RV and LV fractional shortenings were calculated from M-mode recordings (DeVore, Siassi, & Platt, 1984).

Longitudinal velocities of the RV and LV free wall during the cardiac cycle were assessed using pulsed-wave tissue Doppler imaging. The sample volume (1–1.5 mm) was placed at the level of the atrio-ventricular valve annuli and aligned as parallel as possible (<15 deg) to the myocardial wall. Myocardial velocities were recorded during three to six cardiac cycles at a sweep speed of 100 mm s^{-1}. The frame rate was maximized. Isovolumic relaxation (IVRV), early ventricular filling (E′), atrial contraction (A′), isovolumic contraction (IVCV) and ventricular systolic peak (S′) velocities were measured. The isovolumic myocardial acceleration and deceleration were calculated (Acharya et al., 2008). The isovolumic contraction (IVCT) and relaxation times (IVRT) were measured, and their proportions (as percentages) of the total cardiac cycle were calculated (Acharya et al., 2008). Global ventricular function was evaluated by the myocardial performance index [MPI = (IVRT + IVCT)/ejection time] (Tei, Nishimura, Seward, & Tajik, 1997).

Blood flow velocity waveforms for the ductus arteriosus (DA), umbilical artery (UA), right pulmonary artery (RPA), pulmonary vein, ductus venosus (DV) and inferior vena cava (IVC) were obtained for calculation of their pulsatility index (PI) values. To estimate volume blood flow in the placenta ($\dot{Q}_{Plac}$), umbilical venous volume blood flow was calculated (Acharya, Wilsgaard, Rosvold Berntsen, Maltau, & Kiserud, 2005).

2.5 | Statistical analysis

The summary measurements are presented as means (SD). Repeatedly measured variables were analysed using repeated-measures ANOVA. Pairwise comparisons between different time points were performed only if the overall change over time according to ANOVA was significant ($P < 0.05$). The least significant difference adjustment for multiple comparisons was used, and if statistical significance was reached, mean differences with 95% confidence intervals (CIs) were calculated. Student's paired two-tailed *P* tests were used. All analyses were performed using the SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

3 | RESULTS

Maternal heart rate, arterial blood pressure, blood gas values and lactate concentration remained within the normal physiological range during the entire experiment (data not shown). Maternal mean (SD) end-tidal isoflurane concentration was 1.3 (0.2)% at baseline, 1.5 (0.3)% at 15 min of occlusion, 1.4 (0.4)% at 60 min of occlusion and 1.4 (0.3)% after the occlusion was released ($P = 0.247$). Fetal mean (SD) weight was 2093 (439) g. Fetal invasively measured parameters are shown in Table 1. Fetal ascending aortic blood pressure was stable throughout the experiment. Central venous pressure was higher (mean difference 2.0 mmHg, 95% CI 0.8–3.2 mmHg) during the occlusion than after it was released. Fetal pH was lower (mean difference 0.07, 95% CI 0.05–0.09) and P_{CO_2} was higher (mean difference 1.2 kPa, 95% CI

TABLE 2 Fetal cardiovascular parameters

Parameter	Baseline	PaO 15 min	PaO 60 min	PaO release	*P* value for time
FHR (beats min^{-1})	158 (7)	188 (23)*	177 (20)	160 (18)	0.022
LVSV (ml)	4.14 (1.40)	4.14 (1.47)	3.95 (1.48)	3.53 (1.04)	0.067
LVCO (ml min^{-1})	629 (198)	776 (283)*	720 (273)	541 (172)*†	0.0001
RVSV (ml)	5.85 (1.75)	0	0	1.88 (1.37)*	0.002
RVCO (ml min^{-1})	895 (259)	0	0	303 (229)*	0.003
CCO (ml min^{-1})	1524 (341)	776 (283)*	720 (273)*	844 (290)*	0.0001
$\dot{Q}_P$ (ml min^{-1})	122 (27)	0	0	68 (23)*	0.002
$\dot{Q}_{FO}$ (ml min^{-1})	507 (181)	776 (283)*	720 (273)*	473 (159)†	0.001

Values are means (SD); $n = 7$. Abbreviations: CCO, combined cardiac output; FHR, fetal heart rate; LVCO, left ventricular cardiac output; LVSV, left ventricular stroke volume; PaO, main pulmonary artery occlusion; $\dot{Q}_{FO}$, foramen ovale volume blood flow; $\dot{Q}_P$, pulmonary volume blood flow; RVCO, right ventricular cardiac output; and RVSV, right ventricular stroke volume. *Different from baseline, $P < 0.05$. †Different from preceding phase, $P < 0.05$.

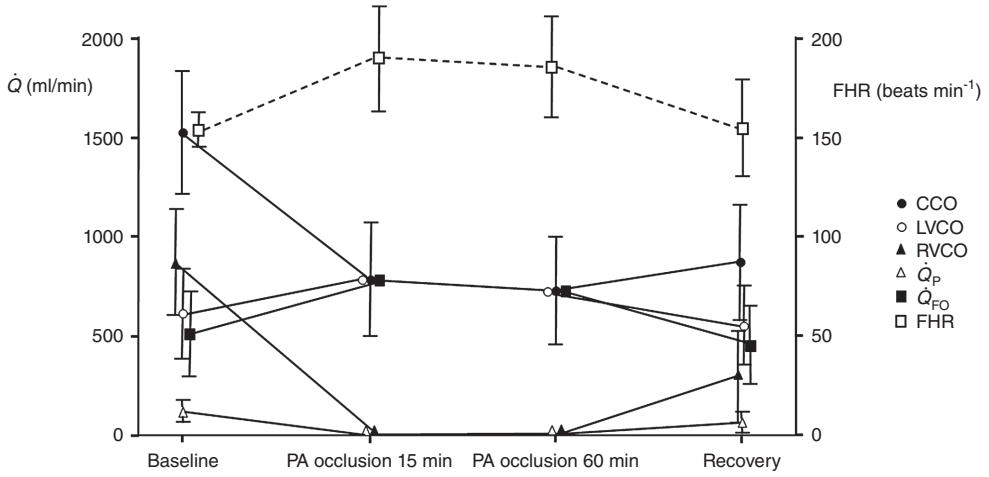

FIGURE 2 Fetal combined (CCO), left ventricular (LVCO) and right ventricular (RVCO) cardiac outputs, pulmonary ($\dot{Q}_P$) and foramen ovale ($\dot{Q}_{FO}$) volume blood flows, and fetal heart rate (FHR) during the main pulmonary artery (PA) occlusion

0.4–2.1 kPa) at 60 min of occlusion than at baseline. Fetal P_{O_2}, base excess and lactate concentrations did not show any statistically significant changes during the entire experiment.

During main pulmonary artery occlusion, no retrograde blood flow across the DA or blood flow in the pulmonary circulation could be identified by colour Doppler ultrasound. At 15 min of occlusion, fetal heart rate (mean difference 31 beats min^{-1}, 95% CI 6–55 beats min^{-1}) and LVCO (mean difference 147 ml min^{-1}, 95% CI 54–239 ml min^{-1}) were greater than at baseline (Table 2; Figure 2). However, at 60 min of occlusion fetal heart rate and LVCO did not differ significantly from baseline values. After the occlusion was released, LVCO was lower than at baseline (mean difference 89 ml min^{-1}, 95% CI 44–133 ml min^{-1}) or during the occlusion (mean difference at 15 min of occlusion 235 ml min^{-1}, 95% CI 122–348 ml min^{-1}). Main pulmonary artery occlusion increased $\dot{Q}_{FO}$ (mean difference at 15 min of occlusion 268 ml min^{-1}, 95% CI 100–436 ml min^{-1}). However, the increase in $\dot{Q}_{FO}$ could only compensate for the lack of $\dot{Q}_P$, because LV stroke volume did not change significantly during the entire experiment. During the occlusion, CCO decreased ~50% from baseline values. After the occlusion was released, CCO remained lower than at baseline (mean difference 680 ml min^{-1}, 95% CI 356–1005 ml min^{-1}). In addition, RV stroke volume (mean difference 3.97 ml, 95% CI 2.04–5.91 ml), RVCO (mean difference 592 ml min^{-1}, 95% CI 299–885 ml min^{-1}) and $\dot{Q}_P$ (mean difference 54 ml min^{-1}, 95% CI 29–80 ml min^{-1}) were lower after the release of occlusion than at baseline (Table 2; Figure 2).

An increase in RV afterload by main pulmonary artery occlusion decreased both RV IVCV (mean difference at 15 min of occlusion 1.84 cm s^{-1}, 95% CI 0.15–3.54 cm s^{-1}) and its acceleration (mean difference 1.71 m s^{-2}, 95% CI 0.29–3.12 m s^{-2}), in addition to IVRV (mean difference 3.25 cm s^{-1}, 95% CI 2.51–3.99 cm s^{-1}) and its deceleration (mean difference 3.51 m s^{-2}, 95% CI 2.91–4.12 m s^{-2}) (Table 3). In addition, E′ (mean difference at 60 min of occlusion 3.82 cm s^{-1}, 95% CI 2.04–5.61 cm s^{-1}) and S′ (mean difference 3.31 cm s^{-1}, 95% CI 1.11–5.52 cm s^{-1}) were lower than at baseline. Both IVRT% (mean difference at 60 min of occlusion 5.4%, 95% CI 1.7–9.1%) and MPI (mean difference 17.51, 95% CI 8.44–26.59) increased. None of these parameters was restored to the baseline level after the occluder was released. However, A′ and IVCT% did not change significantly over the experiment ($P = 0.053$).

During main pulmonary artery occlusion, LV IVCV increased (mean difference at 15 min of occlusion 2.59 cm s^{-1}, 95% CI 0.74–4.45 cm s^{-1}; Table 4). After the occluder was released, it was comparable to baseline values. The value of A′ was greater during the occlusion

TABLE 3 Fetal right ventricular tissue Doppler parameters

Parameter	Baseline	PaO 15 min	PaO 60 min	PaO release	*P* value for time
E′ (cm s^{-1})	5.71 (1.10)	2.86 (2.07)*	1.88 (1.51)*	2.47 (1.72)*	0.002
A′ (cm s^{-1})	9.69 (1.74)	11.15 (3.91)	9.72 (1.72)	9.08 (1.41)	0.373
S′ (cm s^{-1})	8.38 (2.11)	5.78 (1.46)*	5.07 (0.75)*	5.08 (0.98)*	0.001
IVCV (cm s^{-1})	5.31 (1.35)	3.46 (0.94)*	3.27 (0.96)*	2.93 (0.54)*	0.001
IVCV acceleration (m s^{-2})	5.39 (1.10)	3.68 (1.15)*	3.60 (1.16)*	3.42 (1.14)*	0.002
IVRV (cm s^{-1})	3.25 (0.80)	0*	0.24 (0.64)*	1.86 (0.27)*†	0.0001
IVRV deceleration (m s^{-2})	3.51 (0.66)	0*	0.16 (0.43)*	2.14 (0.55)*†	0.0001
IVCT (%)	6.8 (1.4)	10.5 (5.4)	11.2 (2.8)	9.9 (3.4)	0.078
IVRT (%)	11.9 (2.5)	16.9 (2.8)*	17.3 (3.5)*	18.2 (2.3)*	0.0001
MPI	0.45 (0.07)	0.61 (0.25)	0.63 (0.15)*	0.73 (0.25)*	0.004

Values are means (SD); $n = 7$. Abbreviations: A′, atrial contraction velocity; E′, early ventricular filling velocity; IVCT, isovolumic contraction time; IVCV, isovolumic contraction velocity; IVRT, isovolumic relaxation time; IVRV, isovolumic relaxation velocity; MPI, myocardial performance index; PaO, main pulmonary artery occlusion; and S′, ventricular systolic peak velocity. *Different from baseline, $P < 0.05$. †Different from preceding phase, $P < 0.05$.

TABLE 4 Fetal left ventricular tissue Doppler parameters

Parameter	Baseline	PaO 15 min	PaO 60 min	PaO release	*P* value for time
E′ (cm s^{-1})	8.05 (2.26)	5.33 (2.64)	5.28 (2.88)	6.32 (2.64)	0.7
A′ (cm s^{-1})	11.73 (1.91)	15.57 (3.13)*	16.41 (3.85)*	14.59 (3.05)*	0.03
S′ (cm s^{-1})	7.07 (1.13)	7.64 (1.96)	7.01 (1.78)	6.62 (1.24)	0.116
IVCV (cm s^{-1})	5.72 (1.39)	8.32 (2.49)*	8.02 (3.05)	6.66 (1.70)	0.017
IVCV acceleration (m s^{-2})	4.65 (1.04)	4.00 (0.98)	3.45 (0.84)*	3.65 (1.18)*	0.043
IVRV (cm s^{-1})	2.70 (0.37)	1.86 (1.32)	1.63 (1.13)	2.38 (0.34)	0.131
IVRV deceleration (m s^{-2})	3.37 (0.82)	2.30 (1.67)	1.90 (1.36)	2.80 (0.58)	0.076
IVCT (%)	7.2 (0.9)	10.7 (1.3)*	10.7 (2.3)*	10.3 (2.9)*	0.006
IVRT (%)	11.7 (2.0)	13.4 (2.2)	13.4 (2.6)	13.8 (1.4)	0.212
MPI	0.43 (0.04)	0.57 (0.13)	0.53 (0.13)	0.50 (0.22)	0.353

Values are means (SD); $n = 7$. Abbreviations: A′, atrial contraction velocity; E′, early ventricular filling velocity; IVCT, isovolumic contraction time; IVCV, isovolumic contraction velocity; IVRT, isovolumic relaxation time; IVRV, isovolumic relaxation velocity; MPI, myocardial performance index; PaO, main pulmonary artery occlusion; and S′, ventricular systolic peak velocity. *Different from baseline, $P < 0.05$.

(mean difference at 15 min of occlusion 3.83 cm s^{-1}, 95% CI 1.52–6.14 cm s^{-1}) and after main pulmonary artery occlusion was released (mean difference 2.85 cm s^{-1}, 95% CI 0.75–4.96 cm s^{-1}) when compared with baseline values. Furthermore, IVCT% increased (mean difference at 60 min of occlusion 3.5%, 95% CI 1.2–5.8%) and IVCV acceleration decreased (mean difference 1.20 m s^{-2}, 95% CI 0.16–2.24 m s^{-2}) during the experiment and did not return to baseline after the occluder was released (mean difference 1.00 m s^{-2}, 95% CI 0.09–1.91 m s^{-2}). No statistically significant changes in E′, S′, IVRV and its deceleration, and in IVRT% and MPI were found during the experiment.

Right ventricular fractional shortening decreased (mean difference at 60 min of occlusion 36.3%, 95% CI 24.7–47.9%) and did not show any recovery to baseline level after the occluder was released (mean difference 25.1%, 95% CI 15.6–34.6%) (Table 5). In contrast, LV fractional shortening increased during the occlusion (mean difference at 60 min of occlusion 13.2%, 95% CI 4.0–22.4%), and it returned to the baseline level after release of the occluder.

In the fetal venous circulation, DV (mean difference at 60 min of occlusion 1.54, 95% CI 0.53–2.55) and IVC PI (mean difference 6.37, 95% CI 1.59–11.14) values increased during main pulmonary artery occlusion, and DV PI values also remained higher after the occlusion was released when compared with baseline (mean difference 0.94, 95% CI 0.17–1.71) (Table 6). After the release of occlusion, both RPA (mean difference 20.16, 95% CI 3.27–37.05) and pulmonary vein PI (mean difference 8.52, 95% CI 3.62–13.41) values were greater than at baseline, whereas DA PI did not show a statistically significant difference from baseline. In the placental circulation, UA PI values decreased (mean difference at 15 min of occlusion 0.24, 95% CI 0.11–0.38) during the occlusion. After the occlusion was released, UA PI values returned towards the baseline. However, $\dot{Q}_{Plac}$ did not show a statistically significant change over the experiment.

4 | DISCUSSION

As we hypothesized, main pulmonary artery occlusion significantly increased LVCO. However, this increase was only ~20% from baseline values, consequently causing ~50% reduction in CCO. Most interestingly, LV stroke volume did not increase during the occlusion, and an increase in LVCO was attributable to increased heart rate. Nevertheless, fetal arterial blood pressure remained stable, whereas central venous pressure was higher during the occlusion than after it was released. Unexpectedly, signs of LV systolic dysfunction developed

TABLE 5 Fetal cardiac dimensions

Parameter	Baseline	PaO 15 min	PaO 60 min	PaO release	*P* value for time
Left ventricle					
Diastole (cm)	1.25 (0.15)	1.22 (0.11)	1.17 (0.17)	1.21 (0.18)	0.654
Systole (cm)	0.80 (0.10)	0.62 (0.19)	0.59 (0.11)*	0.75 (0.14)	0.029
FS (%)	35.8 (6.2)	49.0 (12.1)	49.0 (9.6)*	37.6 (8.3)	0.024
Right ventricle					
Diastole (cm)	1.14 (0.16)	1.37 (0.26)	1.41 (0.19)*	1.33 (0.19)*	0.001
Systole (cm)	0.81 (0.22)	1.42 (0.30)*	1.51 (0.25)*	1.27 (0.25)*†	0.001
FS (%)	30.0 (10.4)	−4.0 (13.4)*	−6.3 (7.4)*	4.9 (6.5)*†	0.0001

Values are means (SD); $n = 7$. Abbreviations: FS, fractional shortening; and PaO, main pulmonary artery occlusion. *Different from baseline, $P < 0.05$. †Different from preceding phase, $P < 0.05$.

TABLE 6 Fetal peripheral haemodynamics and placental volume blood flow

Parameter	Baseline	PaO 15 min	PaO 60 min	PaO release	*P* value for time
Pulsatility index					
DA	2.71 (0.86)	—	—	4.56 (3.14)	0.286
RPA	4.78 (1.85)	—	—	24.94 (18.11)*	0.027
P_{vein}	1.23 (0.26)	—	—	9.75 (4.60)*	0.007
UA	1.41 (0.25)	1.17 (0.17)*	1.26 (0.34)*	1.28 (0.33)	0.017
IVC	1.95 (1.24)	10.80 (9.57)	8.32 (4.88)*	4.93 (4.31)	0.023
DV	0.86 (0.27)	2.14 (0.91)*	2.40 (0.96)*	1.80 (0.70)*	0.003
$\dot{Q}_{Plac}$ (ml min^{-1})	156 (66)	88 (27)	105 (41)	125 (69)	0.165

Values are means (SD); $n = 7$. Abbreviations: DA, ductus arteriosus; DV, ductus venosus; IVC, inferior vena cava; $\dot{Q}_{Plac}$, placental volume blood flow; PaO, main pulmonary artery occlusion; P_{vein}, pulmonary vein; RPA, right pulmonary artery; and UA, umbilical artery. *Different from baseline, $P < 0.05$.

while diastolic function was preserved. In the RV, a sudden increase in afterload immediately led to severe systolic and diastolic dysfunction. Fetal cardiac functional abnormalities persisted during the recovery period. Even though fetal P_{O_2} was maintained, an increase in P_{CO_2} suggested a disturbance in placental perfusion during the occlusion. Altogether, our findings support the concept that the FO has a limited capacity to increase its volume blood flow.

During the main pulmonary artery occlusion, LV preload was entirely dependent on volume blood flow across the FO, because we could not detect any retrograde blood flow across DA. It is obvious that there was a slight increase in FO volume blood flow, because LV stroke volume was maintained during the occlusion. An increase in LVCO was attributable to increased fetal heart rate. Although the Frank–Starling mechanism is functional in the fetal heart (Kirkpatrick, Pitlick, Naliboff, & Friedman, 1976), the fetal ventricles seem to operate near the plateau of their function curves and have limited capacity to respond to volume loading by increasing stroke volume (Reller et al., 1987; Thornburg & Morton, 1983, 1986). Furthermore, tachycardia shortens LV filling time and may thus decrease stroke volume (Anderson, Glick, Killam, & Mainwaring, 1986). In the present study, the lack of an increase in LV stroke volume could be caused by either an inability of the LV to increase its stroke volume or a limited capacity of the FO to increase its volume blood flow. In a previous study with DA occlusion, we found a significant increase in LV stroke volume and LVCO that was caused by increased $\dot{Q}_P$. No change in FO volume blood flow was noted (Hashima et al., 2015). Furthermore, the percentage increase (40%) in LVCO was greater than in the present study. These observations suggest that the fetal LV can increase its stroke volume in response to volume loading. In addition, previous experimental work has suggested that FO blood flow cannot fully compensate for impaired pulmonary venous return (Erkinaro et al., 2007, 2013). All these findings suggest that in fetal sheep the FO volume blood flow is close to its maximal capacity at near-term gestation.

Increased heart rate during the occlusion was most probably associated either with the Bainbridge reflex owing to atrial stretch or with a chronotropic effect induced by circulating catecholamines released in excess in response to haemodynamic stress, or both. Noradrenaline-induced peripheral vasoconstriction could explain the stable fetal arterial pressures during a remarkable decrease in CCO.

In normal physiological conditions, the kinetic energy of blood in the IVC is a more important determinant of FO blood flow than the pressure gradient between the two atria or that between the IVC and the left atrium (Anderson et al., 1981, 1985). There are two distinct blood flow streams within the intrathoracic portion of the IVC: the high-velocity DV stream, which predominantly flows through the FO, and the low-velocity caudal IVC stream, which is preferentially directed into the right atrium (Schmidt, Silverman, & Rudolph, 1996). In the present study, pulsatility increased in the IVC and DV, most probably reflecting elevated RV end-diastolic pressure (Hecher, Campbell, Doyle, Harrington, & Nicolaides, 1995). In addition, severe cardiac dysfunction has been shown to constrain central venous blood velocity even during ventricular systole (Ghio et al., 2001).

Increased pulsatility in the DV and IVC blood flow velocity also confirm that FO volume blood flow could not be increased substantially.

Tissue Doppler-derived cardiac functional parameters showed that LV A′ velocity increased during the main pulmonary artery occlusion. Augmented left atrial contraction was most probably caused by an increase in circulating catecholamines. We observed a decrease in IVCV acceleration during prolonged main pulmonary artery occlusion. Given that IVCV acceleration is a load-independent index of myocardial contractility (Vogel et al., 2002), this indicates that LV systolic dysfunction developed during the experiment. However, IVRV and its deceleration were not affected, suggesting well-preserved LV diastolic function. The declining LV systolic function without signs of LV diastolic dysfunction was in contrast to our expectations. We anticipated that increased levels of circulating catecholamines during the occlusion would enhance LV contractility. In addition, increased atrial pressure is known rapidly to induce atrial natriuretic peptide secretion in fetal sheep (Jaekle, Sheikh, Berry, Washburn, & Rose, 1995), which could potentially have a positive effect on both systolic and diastolic function (Ozawa et al., 2015). As expected, a severe increase in RV afterload resulted in significant RV systolic and diastolic dysfunction, with increased RV dimensions and a leftward shift of the interventricular septum during systole, as verified by negative RV fractional shortening values during the occlusion. Consequently, we propose that the concomitant RV dysfunction and altered interventricular septal movement, through ventricular interdependence, is at least one possible mechanism for the decrease in LV contractility in the present study. After the main pulmonary artery occlusion was released, RV systolic and diastolic dysfunction and LV systolic dysfunction persisted. The most likely explanation is that increased right ventricular end-diastolic pressure during main pulmonary artery occlusion could impair coronary artery blood flow and limit the oxygen delivery to the subendocardial area of the myocardium.

The umbilicoplacental circulation lacks significant autoregulation and is directly proportional to perfusion pressure (Berman, Goodlin, Heymann, & Rudolph, 1976). A 50% reduction in CCO during the main pulmonary artery occlusion deteriorated placental circulation to some extent. Although the decrease in placental volume blood flow was not statistically significant, it resulted in derangement of placental gas exchange, because the decrease in fetal pH was associated with respiratory acidaemia without a metabolic component. Umbilical artery PI values decreased during the occlusion, demonstrating that the UA blood flow velocity waveform is not a direct measure of placental vascular resistance; instead, it reflects the number of tertiary villous arterioles (Giles, Trudinger, & Baird, 1985). Our findings demonstrate that sufficient placental perfusion for fetal survival can be maintained by fetal compensatory mechanisms that aim to direct blood flow and perfusion to vital fetal organs.

The present study was designed to investigate the capability of the FO to increase its volume blood flow when main pulmonary artery is obstructed. We recognize that an acute complete occlusion of the main pulmonary artery is not a physiological event. However, fetuses with right ventricular outflow tract obstruction or tricuspid atresia depend mainly on blood flow across the FO. In human fetuses with pulmonary outflow tract obstruction, retrograde blood flow in the DA that is directed to the pulmonary circulation helps to support LVCO (Peyvandi et al., 2014). It is known that during the last trimester, human fetal pulmonary circulation becomes responsive to changes in fetal oxygenation, and in the normal physiological environment fetal pulmonary vascular resistance increases, thus limiting pulmonary venous return to the left atrium (Rasanen et al., 1996, 1998). If the fetus becomes hypoxaemic, vasoconstriction in the pulmonary circulation reduces lung blood flow further. Thus, in the fetus with right ventricular outflow tract obstruction or tricuspid atresia, $\dot{Q}_{FO}$ becomes a crucial factor in order to maintain systemic blood flow and perfusion. Our results have significant clinical value. Fetuses with pulmonary outflow tract obstruction or tricuspid atresia could be at higher risk for intrauterine demise, if hypoxaemia develops, especially during the last trimester of pregnancy.

There are limitations in our study. We acknowledge that the sample size is relatively small, which might limit the power of our study. In this type of experimental study, with complete elimination of cardiac output from one of the ventricles, it is virtually impossible to make any reliable power calculations. The sample size is comparable to that of previous experiments on large laboratory animals. The fetuses underwent surgical procedures that might constitute a major stress. Nevertheless, the recovery period after surgery should be long enough for proper recovery of fetal myocardial function (De Muylder, Fouron, Bard, & Urfer, 1983). The experiments were performed under general anaesthesia that could modify fetal cardiovascular adaptation. It has been shown that the cardiovascular system of the newborn lamb can increase oxygen delivery in response to hypoxaemic stress during isoflurane anaesthesia. Therefore, at reasonable anaesthetic depth and without myocardial or peripheral cardiovascular disease, the newborn lamb can coordinate neural, endocrine and local tissue responses to increase cardiovascular performance in response to hypoxaemia (Brett, Teitel, Heymann, & Rudolph, 1989). There are some differences in cardiovascular physiology and anatomy between human and sheep fetuses. However, ovine experiments have provided invaluable information on fetal haemodynamic regulation. Previous Doppler ultrasonographic studies have suggested that the phasic flow events associated with the cardiac cycle are comparable in human and sheep fetuses (Kiserud, Eik-Nes, Blaas, & Hellevik, 1992; Schmidt et al., 1996). In addition, validation studies in sheep fetuses have proved that invasive and Doppler echocardiographic volume blood flow calculations are well correlated (Schmidt, Di Tommaso, Silverman, & Rudolph, 1991). The intra-observer variabilities of Doppler ultrasonographic parameters of fetal sheep cardiovascular haemodynamics and tissue Doppler-derived indices are comparable to those found in human fetuses during the second half of pregnancy (Bernard et al., 2012; Rasanen et al., 1998). Finally, FO volume blood flow was calculated by subtracting $\dot{Q}_P$ from LVCO in the baseline and recovery phases. Unfortunately, direct measurement of volume blood flow across the FO is impossible.

In conclusion, complete main pulmonary artery occlusion led to an increase of ~20% in LVCO and a reduction of ~50% in CCO. During the occlusion, LV stroke volume did not change. An increase in LVCO was related to an increase in fetal heart rate. Fetal arterial blood pressure

was maintained during the occlusion, wheras central venous pressure was higher during the occlusion than after it was released. During main pulmonary artery occlusion, LV systolic dysfunction developed, while diastolic function was preserved. Severe RV dysfunction was reflected as increased pulsatility in systemic venous blood flow patterns. Fetal P_{O_2} was maintained; however, an increase in P_{CO_2} suggested placental perfusion disturbance during the occlusion. Altogether, our findings show that the FO has a limited capacity to increase its volume blood flow.

AUTHOR CONTRIBUTIONS

Conception and design of the experiments: L.E.D., A.R.H., G.A. and J.R. Acquisition, analysis or interpretation of data for the work and drafting the work or revising it critically for important intellectual content: all authors. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

COMPETING INTERESTS

None declared.

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