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Characteristics and physiological basis of falls in ventricular outputs after immediate cord clamping at delivery in preterm fetal lambs

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Running head: Immediate cord clamping and ventricular outputs in preterm fetal lambs

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Key points summary

- Controversy exists about the physiological mechanism(s) underlying decreases in cardiac output after immediate clamping of the umbilical cord at birth
- To define these mechanisms, the four major determinants of ventricular output (afterload, preload, heart rate and contractility) were measured concurrently in fetal lambs at 15s intervals over a 2-minute period after cord clamping and before ventilation following delivery
- After cord clamping, right (but not left) ventricular output fell by 20% in the initial 30s, due to increased afterload associated with higher arterial blood pressures, but both outputs then halved over 45s, due to a falling heart rate and deteriorating ventricular contractility accompanying rapid declines in arterial oxygenation to asphyxial levels
- Ventricular outputs subsequently plateaued from 75-120s, associated with rebound rises in ventricular contractility accompanying asphyxia-induced surges in circulating catecholamines
- These findings provide a physiological basis for the clinical recommendation that effective ventilation should occur within 60s after immediate cord clamping

Abstract

Controversy exists about the physiological mechanism(s) underlying large decreases in cardiac output after immediate clamping of the umbilical cord at birth. To define these mechanisms, anaesthetized preterm fetal lambs (127(1)d, n=12) were instrumented with flow probes and catheters in major central arteries, and a left ventricular (LV) micromanometer-conductance catheter. Following immediate cord clamping at delivery, haemodynamics, LV and right ventricular (RV) outputs, and LV contractility were measured at 15s intervals during a 2 minute non-ventilatory period, with aortic blood gases and circulating catecholamine (noradrenaline and adrenaline) concentrations measured at 30s intervals. After cord clamping, 1) RV (but not LV) output fell by 20% in the initial 30s, due to a reduced stroke volume associated with increased arterial blood pressures, 2) both outputs then halved over the next 45s, associated with falls in heart rate, arterial blood pressures and ventricular contractility accompanying a rapid decline in arterial oxygenation to asphyxial levels, 3) reduced outputs subsequently plateaued from 75-120s, associated with rebound rises in blood pressures and ventricular contractility accompanying exponential surges in circulating catecholamines. These findings are consistent with a time-dependent decline of ventricular outputs after immediate cord clamping, which comprised *1)* an initial, minor fall in RV output related to altered loading conditions, *2)* ensuing large decreases in both LV and RV outputs related to the combination of bradycardia and ventricular dysfunction during emergence of an asphyxial state, and *3)* subsequent stabilization of reduced LV and RV outputs during ongoing asphyxia, supported by cardiovascular stimulatory effects of marked sympathoadrenal activation.

Keywords: fetus, immediate cord clamping, ventricular outputs, left ventricular contractility, circulating catecholamines,

Introduction

Controversy presently exists about the physiological mechanism(s) underlying substantial reductions in cardiac output evident in the interval between immediate clamping of the umbilical cord and the onset of ventilation during the birth transition. Thus, based on findings from a study where changes in right ventricular (RV) output were measured during a two minute period of cord clamping prior to ventilation in preterm fetal lambs (Bhatt *et al.*, 2013), it is widely believed that cord clamping produces large falls in both left ventricular (LV) and RV outputs, and that these falls are entirely due to altered cardiac loading conditions which comprise an increased arterial blood pressure and decreased cardiac venous return that follow removal of the low resistance placental circulation from the fetal vascular circuit (Bhatt *et al.*, 2014; Hooper *et al.*, 2015a; Hooper *et al.*, 2015b; Kluckow & Hooper, 2015; Hooper *et al.*, 2016; Hooper *et al.*, 2019).

On the other hand, results of birth transition studies from our laboratory which measured RV output and ascending aortic/aortic trunk (AoT) blood flow as a surrogate of LV output (Smolich *et al.*, 2015; Smolich *et al.*, 2020) indicated that haemodynamic accompaniments of reductions in ventricular outputs after immediate cord clamping and prior to ventilation changed with the duration of cord clamping, suggesting that the aetiology of these reductions also changed over time. Thus, cord clamping initially produced rapid-onset but relatively minor falls in AoT blood flow and RV output, which could have resulted from an abrupt increase in arterial blood pressures and decrease in venous return. However, ensuing and larger falls in AoT blood flow and RV output were associated with a decline of arterial blood pressures and a pronounced bradycardia, and thus more likely to be related to a deterioration of cardiac function accompanying a rapid arterial blood de-oxygenation that reached asphyxial levels by 45-60 seconds after cord clamping (Smolich *et al.*, 2015; Smolich *et al.*, 2017; Smolich *et al.*, 2020). Furthermore, this asphyxial state was associated with surges in circulating concentrations of the catecholamines noradrenaline and adrenaline that were exponentially related to falling levels of aortic oxygenation (Smolich *et al.*, 2017), reflecting a marked degree of sympathoadrenal activation (Cohen *et al.*, 1982; Jensen & Berger, 1991). As catecholamines exert potent inotropic and vasoconstrictor effects (Jones & Ritchie, 1978; Padbury *et*

al., 1987; Seri, 2001), it is likely that these surges contributed to rebound rises evident in ventricular contractility and arterial blood pressure (Smolich *et al.*, 2017), with possible associated effects on ventricular outputs.

As in the adult, levels of ventricular output in the fetal circulation are dependent on four major determinants, namely *1)* preload (i.e. the degree of ventricular filling arising from venous return, reflected in the levels of ventricular end-diastolic or mean atrial blood pressures, and ventricular end-diastolic volume), *2)* afterload (i.e. the arterial load against which ventricular ejection occurs, reflected in parameters such as mean arterial blood pressure and downstream vascular resistance), *3)* heart rate and *4)* ventricular contractility (Rudolph, 1985; Anderson, 1996). Elucidation of the underlying physiological basis of changes in ventricular output after immediate cord clamping therefore requires concurrent assessment of all these determinants, which has not been undertaken in previous studies (Bhatt *et al.*, 2013; Smolich *et al.*, 2015; Smolich *et al.*, 2017; Smolich *et al.*, 2020).

The aim of this study was therefore to specifically test the hypothesis that changes in ventricular outputs during a two minute period of cord clamping after delivery at birth consisted of three distinct temporal phases, namely *1)* an initial circulatory re-organization phase, which reflected the direct physiological effects of umbilical cord clamping, *2)* a subsequent depressant phase associated with manifestations of ventricular dysfunction and related to development of an asphyxial state, and *3)* an ensuing compensatory phase supported by the cardiovascular stimulatory effects of marked asphyxia-related sympathoadrenal activation. Experiments were performed in anaesthetized preterm fetal lambs subjected to immediate cord clamping after delivery, with measurement of haemodynamics, LV and RV outputs and stroke volumes, LV pressure, LV volume and LV contractility at 15s intervals after cord clamping, as well as aortic blood gas status and circulating noradrenaline and adrenaline concentrations at 30s intervals. LV contractility was assessed with LV dP/dt_{max}, the maximal rate of rise of LV blood pressure (Stein *et al.*, 1993), as well as LV maximal chamber elastance (E_{max}), a relatively load-independent index obtained from LV pressure-volume (P-V) loops (Suga, 1990; Burkhoff *et al.*, 2005).

Methods

Studies conformed to guidelines of the National Health and Medical Council of Australia and were approved by the Murdoch Children's Research Institute Animal Ethics Committee (Project A816). This manuscript is compliant with the ARRIVE guidelines for reporting of animal research (Kilkenny *et al.*, 2010; Grundy, 2015).

Surgical preparation Studies were performed using 12 Border-Leicester cross ewes with one singleton and 11 twin pregnancies at a gestation of 127(1) days (mean(SD), term = 147 days), a time-point which is associated with a substantial degree of perinatal immaturity of both the cardiovascular and respiratory systems, with newborn lambs unable to survive without respiratory support. The general features of the anaesthetic and monitoring procedures in ewes were as previously described (Smolich *et al.*, 2015; Smolich *et al.*, 2017; Smolich *et al.*, 2020). Briefly, ewes were anaesthetized with an i.m. injection of ketamine (5 mg·kg^{-1}) and xylazine (0.1 mg·kg^{-1}), followed by 4% isoflurane delivered by mask. After intubation of the trachea with a cuffed endotracheal tube, anaesthesia was maintained with isoflurane (1-2%) and nitrous oxide (10-20%) delivered by ventilator in O_2-enriched air, supplemented with an i.v. infusion of ketamine (1-1.5 mg·kg^{-1}·hr^{-1}), midazolam (0.1-0.15 mg·kg^{-1}·hr^{-1}) and fentanyl (2-2.5 μg·kg^{-1}·hr^{-1}). Transcutaneous oxygen saturation (S_pO_2) was monitored continuously with a pulse-oximetry sensor applied to the ear or cheek. The right common carotid artery was cannulated for continuous monitoring of blood pressure and regular blood gas analysis (ABL800, Radiometer, Copenhagen, Denmark), with ventilation of the ewe targeted to maintain arterial O_2 tension (P_aO_2) at 100-120 mmHg and CO_2 tension (P_aCO_2) at 35-40 mmHg.

The uterus was exposed via a midline laparotomy. With a twin pregnancy, *1)* the position of fetuses was assessed by palpation, and *2)* the presence and degree of any meconium staining determined via a small uterine keyhole incision, usually over a hindlimb in a uterine horn. The fetus that was most accessible and with no or the least amount of meconium staining was then chosen for surgical preparation. Before any surgery on this fetus, the other fetus was completely delivered from

the uterus and euthanized with an intracardiac injection of sodium pentobarbitone (100 mg·kg^{-1}) after the umbilical cord had been clamped and cut.

The head of the fetus undergoing surgical preparation was exteriorized via a hysterectomy and placed in a saline-filled glove to prevent loss of lung liquid. The neck was incised in the midline and a fluid-filled catheter passed into the superior vena cava via the left external jugular vein for fluid and drug administration. The left common carotid artery was cannulated with *1)* a 6-Fr self-sealing vascular sheath that was passed into the distal portion of the brachiocephalic trunk (BCT) for pressure measurement and blood sampling, and *2)* a 3.5-Fr micromanometer (model SPR-524, Millar Instruments, Houston, TX, USA) that was positioned in the AoT to obtain high-fidelity blood pressure. The left forelimb and thorax were exteriorized and a left thoracotomy performed in the 3rd interspace, with access to the heart and major central arteries increased by resection of the third and fourth ribs. Following careful dissection, non-constrictive perivascular transit-time flow probes (Transonic Systems, Ithaca, NY, USA) were placed around the BCT (4 or 6 mm), aortic isthmus (AI, 6 mm), ductus arteriosus (8 or 10 mm) and left pulmonary artery (PA, 4 or 6 mm) in all animals. To measure pressures, *1)* a fluid-filled catheter and another 3.5-Fr micromanometer were inserted via purse-string sutures into the pulmonary trunk (PT), and *2)* a fluid-filled catheter was inserted into the left atrial (LA) appendage in all fetuses, and into an accessible right atrial (RA) appendage in a subgroup of 3 fetuses. Subsequently, a dual-field, multi-segment 3-Fr conductance-micromanometer catheter (model SPR-877, Millar Instruments) was inserted into the carotid artery sheath and advanced across the aortic valve to obtain high-fidelity LV P-V signals. Correct placement of the conductance catheter was inferred from the presence of in-phase segmental volume waveforms and square-like counterclockwise whole-chamber P-V loops. Finally, a clamped 4.5 mm endotracheal tube filled with normal saline was inserted via a tracheostomy into a proximal intercartilaginous space and tied into place.

Experimental protocol Following removal of the glove from the fetal head, the endotracheal tube was unclamped to allow lung liquid to drain passively via gravity for 20-30s, and then re-clamped, taking care to ensure that the endotracheal tube remained entirely filled with fluid and that

no air bubbles entered the lungs. While physiological data were continuously recorded onto computer, fetal AoT blood samples were withdrawn for measurement of blood resistivity (Rho calibration cuvette, Millar Instruments) and gas analysis (ABL800, Radiometer) in all fetuses, and catecholamine assay in a subgroup of 8 fetuses. Approximately 0.5 ml of hypertonic (10%) saline was then injected via the LA catheter for estimation of parallel conductance, i.e. the offset in the conductance catheter signal related to conductivity arising from structures surrounding the blood pool in the LV cavity (Baan *et al.*, 1984; Steendijk *et al.*, 2001).

Subsequently, fetuses (6 male and 6 female) were completely delivered from the uterus, placed on the ewe's abdomen and covered with warmed towels, taking care to avoid tension on the umbilical cord. AoT samples were again collected ~30s later for blood gas and catecholamine analyses. The umbilical cord was then clamped and cut, with recording of physiological data continued for a period of 120s post-cord clamping, after which lambs proceeded into various other protocols that involved immediate ventilation, or ongoing periods of cord clamping prior to ventilation. AoT samples were collected at 30, 60 and 90s after cord clamping for blood gas and catecholamine analyses (n = 8), or for blood gas analysis only (n = 4). Euthanasia was performed with i.v. sodium pentobarbitone (100 mg·kg^{-1}) in ewes after cord clamping, and in lambs after completion of study protocols, with lambs then weighed (4.16(0.47) kg).

Physiological data AoT, PT, LA and RA fluid-filled catheter pressures were measured with transducers calibrated against a water manometer before each study and referenced to atmospheric pressure at LA level. Instantaneous high-fidelity LV pressure and volume signals were obtained from the micromanometer-conductance catheter via an interfacing P-V signal processing system (MV Ultra, Millar Instruments). Signals from fluid-filled, micromanometer and conductance catheters, as well as flow probes, were digitized at a sampling rate of 1 kHz and displayed using programmable acquisition and analysis software (Spike2, Cambridge Electronic Design, Cambridge, UK). A 48 Hz low-pass filter was applied to digitized data at the time of analysis to remove any 50 Hz mains electrical interference.

Steady-state fetal data before and after delivery were analyzed over a period of ~20s. As rapid haemodynamic changes occur following immediate cord clamping after delivery (Smolich *et al.*, 2015; Smolich *et al.*, 2017; Smolich *et al.*, 2020), data during the 120s period of cord clamping were analyzed in 5-7s epochs at 15s intervals. Ensemble-averaged signals were generated from these data for subsequent analysis.

During data analysis, mean AoT and PT micromanometer pressures were matched to corresponding mean fluid-filled catheter pressures. LV pressure was subsequently matched to AoT micromanometer pressure over a period in mid-to-late systole where both LV and AoT differentials were near-zero (Smolich *et al.*, 2021). LV pressure was then measured at end-diastole, identified at the foot of the upstroke in the LV pressure waveform using an automated curvature-based feature extraction algorithm (Mynard *et al.*, 2008), with LV end-diastolic volume obtained from the corresponding time-point in the LV volume signal (Smolich *et al.*, 2021).

AoT flow (i.e. LV output minus coronary blood flow) was obtained as the sum of flows in the BCT, the only major cephalic branch of the AoT in sheep (Sizarov *et al.*, 2014), and the AI (Smolich *et al.*, 2015; Smolich *et al.*, 2020). RV output was calculated as the sum of ductal and total (i.e. the combined left and right) PA flows, with the latter computed as the product of measured left PA flow and the post-mortem total-to-left lung weight ratio (Smolich *et al.*, 2015; Smolich *et al.*, 2020). AoT and RV stroke volumes were derived as the corresponding flow or output divided by heart rate.

To assess alterations in ventricular contractility, the rates of change of high fidelity LV, AoT and PT pressures were calculated using 3-point differentiation to obtain their corresponding dP/dt_{max}, with AoT and PT dP/dt_{max} used as surrogate measures of LV and RV contractility respectively (Masutani *et al.*, 2009; Morimont *et al.*, 2012; Smolich *et al.*, 2017). As dP/dt_{max} can be influenced by heart rate and cardiac loading conditions (Broughton & Korner, 1980; Fisher & Gross, 1983; Monge Garcia *et al.*, 2018), LV, AoT and PT dP/dt_{max} were normalized to heart rate, while LV and AoT dP/dt_{max} were also normalized to LV end-diastolic volume (Smolich *et al.*, 2021).

The relationship between mean LA and RA blood pressures in fetal lambs is highly linear with a slope of near-unity (Thornburg & Morton, 1986; Morton *et al.*, 1987; Smolich & Mynard, 2019). As a similar finding was confirmed in the present study using 30 data-points from the three experiments where both mean LA (X) and RA blood pressure (Y) were measured, with Y = 1.1X -0.4 (R^2 = 0.84, P < 0.001), the latter relationship was used to estimate mean RA blood pressure in studies where only LA pressure was available.

AoT O_2 content before and after cord clamping was computed as (1.36·S_aO_2·Hb/100) + 0.003·P_aO_2, where Hb = haemoglobin concentration (g/dl).

Systemic vascular resistance Vascular resistance (VR) of the fetal upper body region, which is perfused almost exclusively by LV output, and from where blood returning via the superior vena cava passes almost entirely to the right atrium and ventricle (Rudolph, 1985), was calculated as (mean AoT pressure - mean RA pressure) / BCT flow. On the basis that mean blood pressures in the ascending and descending aorta of the fetus are almost identical (Rudolph, 1985), and that venous return from the lower body and placenta passes not only into the right atrium, but also the left atrium via the foramen ovale (Rudolph, 1976), the combined lower fetal body and placental VR was estimated as [mean AoT pressure – (0.5·RA pressure + 0.5·LA pressure)] / DTA flow, where DTA (descending thoracic aortic) flow was obtained as the sum of AI and ductal flows (Smolich & Mynard, 2019).

Left ventricular pressure-volume analysis LV volume was computed from the conductance catheter signal using standard methodology incorporating estimates of blood resistivity, parallel conductance and gain constant (Baan *et al.*, 1984; Baan & Van der Velde, 1988; Steendijk *et al.*, 2001; Wo *et al.*, 2019). The gain constant was calculated as the ratio of conductance catheter and AoT flow-derived stroke volumes under steady-state conditions, both before and after fetal delivery, with the post-delivery value also used throughout the period of cord clamping. LV output before and after cord clamping was calculated as the product of LV stroke volume derived from the conductance catheter, and heart rate. Note that LV output and AoT flow were thus equivalent under steady-state conditions before cord clamping, but not during the period of cord clamping, as

changes in LV output then included alterations in coronary artery blood flow, whereas AoT flow did not.

LV elastance was calculated as the P-V ratio of ensemble-averaged P-V loops, with maximal elastance (E_{max}), which occurs at end-systole, used as an index of LV contractility (Suga, 1990). Note that assessment of LV contractility via P-V analysis is usually performed by generation of an end-systolic P-V relationship obtained from a series of P-V loops during a transient change in LV load produced by an intervention such as a brief (e.g. 10-15s) occlusion of the inferior vena cava or descending aorta, with end-systolic elastance determined from the slope of this relationship (Baan *et al.*, 1984; Baan & Van der Velde, 1988; Suga, 1990; Burkhoff *et al.*, 2005; Smolich *et al.*, 2021). However, this approach was clearly not possible during the very rapid haemodynamic changes occurring after cord clamping. The reported E_{max} was thus equivalent to LV end-systolic elastance estimated with a single-beat approach (Wo *et al.*, 2019) where the volume intercept of the end-systolic P-V relation (i.e. V_0) in fetal lambs was assumed to be zero, an assumption consistent with available experimental data (Smolich *et al.*, 2021).

Catecholamine analysis Blood samples for catecholamine assay were immediately transferred into ice-chilled tubes containing an anticoagulant and antioxidant mixture (EGTA plus reduced glutathione). Withdrawn blood was replaced with an equal volume of heparinized fetal blood delivered via an infusion pump. Tubes were kept on ice until centrifugation at 4°C, with plasma then stored at -80°C until assay. During batch assay, endogenous noradrenaline and adrenaline were extracted from 1 ml plasma samples using alumina adsorption and separated by high performance liquid chromatography, with quantitation of peaks using coulometric detection (Lambert & Jonsdottir, 1998; Smolich *et al.*, 2017; Smolich *et al.*, 2019).

Statistical analysis Arterial blood gas, general haemodynamic and catecholamine data from three of the 12 animals in the study group have been included in a previous publication (Smolich *et al.*, 2017). Study results were analyzed using GraphPad Prism 8 (GraphPad Software Inc., La Jolla, CA), preceded by logarithmic transformation of data with a non-normal distribution. Temporal changes in variables were analyzed using repeated measures one-way analysis of variance (ANOVA)

and specific comparisons evaluated by partitioning the within-animal sum of squares into individual degrees of freedom, with a Bonferroni correction applied as required for multiple comparisons. Post-cord clamping data between sites were analyzed with two-way repeated measures ANOVA. Data are expressed as mean(SD) and significance was taken at $P < 0.05$.

Results

Blood gases Minor falls in pH, S_aO_2, P_aO_2 and O_2 content, and a slight rise in P_aCO_2, occurred following delivery ($P \leq 0.013$), but after cord clamping, such changes became much more pronounced ($P < 0.0001$), with asphyxial levels of oxygenation (S_aO_2 <10% and P_aO_2 <10 mmHg) evident at ≥60s (Table 1).

Plasma catecholamines Circulating concentrations of noradrenaline rose 47% and adrenaline almost 3-fold with delivery ($P \leq 0.002$). Catecholamine concentrations were progressively higher at longer time-intervals after cord clamping, with noradrenaline increasing 17-fold and adrenaline 98-fold by 90s ($P < 0.0001$; Table 2). Moreover, clear exponential relationships were evident between a decreasing AoT O_2 content and increasing concentrations of circulating noradrenaline and adrenaline (Fig. 1).

Pressures and heart rate Fetal AoT and PT mean blood pressures increased by 5.8(5.2) mmHg with delivery ($P = 0.012$), and a further 9.6(5.9) mmHg (to 57.5(9.0) mmHg) by 15s after cord clamping ($P = 0.0001$). These pressures were stable in the initial 30s after cord clamping, but then declined to 40.1(12.5) mmHg by 75s ($P < 0.0001$), before rising to 60.7(6.6) mmHg by 120s ($P < 0.0001$), a value not different from the initial post-cord clamp level ($P = 0.217$, Fig. 2A).

With delivery, atrial pressures fell from 2.1(1.9) to 0.6(1.2) mmHg ($P = 0.008$), but LV end-diastolic pressure was unchanged ($P = 0.609$, average 4.3(3.1) mmHg). Neither atrial nor LV end-diastolic pressures changed significantly by 15s after cord clamping ($P \geq 0.460$), and were relatively stable for 45-60s. However, atrial pressures then rose progressively to 2.9(2.2) mmHg ($P < 0.0001$) and LV end-diastolic pressure increased to 7.4(3.4) mmHg ($P < 0.0001$) by 120s (Fig. 2B).

Heart rate rose from 143(18) to 155(16) beats/min with delivery ($P = 0.004$), and was

unchanged for 30s after cord clamping, but then fell to a stable plateau of 117(13) beats/min at ≥75s ($P < 0.0001$; Fig. 2C).

Ventricular outputs and aortic trunk blood flow With delivery, LV output increased from 379(120) to 432(138) ml/min ($P = 0.021$), RV output rose from 728(199) to 821(183) ml/min ($P = 0.036$) and AoT blood flow increased from 383(122) to 439(137) ml/min ($P = 0.016$). LV output was unchanged at 15s after cord clamping (423(179) ml/min, $P = 0.754$) and remained stable to 30s, but then fell by 45% ($P < 0.0001$) to a plateau of 232(88) ml/min at ≥75s. On the other hand, AoT blood flow fell 16% to 367(124) ml/min by 15s after cord clamping ($P = 0.006$), was unaltered at 30s, and then fell progressively by another 75% to 91(61) ml/min at 120s ($P < 0.0001$). AoT flow comprised 83-87% of LV output from 15-45s after cord clamping, but divergence increased with longer durations of cord clamping (2-way ANOVA, time x flow, $P < 0.0001$) so that, by 120s after cord clamping, AoT flow constituted only 36% of LV output ($P = 0.0005$). Unlike LV output, RV output declined by 19% at 15s after cord clamping (to 668(147) ml/min, $P = 0.0001$), but like LV output, was then unchanged at 30s before falling by a further 51% to a plateau of 325(51) ml/min at ≥75s ($P < 0.0001$, Fig. 3A).

Stroke volumes LV, RV and AoT stroke volumes were unaltered with delivery ($P \geq 0.197$). At 15s after cord clamping, LV stroke volume was unchanged but AoT stroke volume fell by 15% ($P = 0.002$) and RV stroke volume decreased by 17% ($P = 0.0003$), with all stroke volumes then stable to 30s. However, both LV and RV stroke volume then steadily declined to a plateau at ≥75 s ($P \leq 0.001$), while AoT stroke volume decreased progressively to 120s ($P < 0.0001$, Fig. 3B).

Systemic vascular resistance Prior to cord clamping, upper body VR was more than 3-fold that of lower body plus placental VR ($P < 0.0001$), with neither VR affected by delivery. Upper body VR was unchanged at 15s after cord clamping, and was initially similar to lower body VR. Subsequently, both upper and lower body VR remained stable to 75s, but then increased to 120s ($P \leq 0.0003$), with the rise progressively greater in the lower body region (2-way ANOVA, time x flow, $P < 0.0001$, Fig. 4).

dP/dt$_{max}$ With delivery, LV dP/dt$_{max}$ increased 19% (P = 0.004), but AoT and PT dP/dt$_{max}$ were unaltered. However, while LV, AoT and PT dP/dt$_{max}$ did not change statistically at 15s after cord clamping and were stable to 30s, these variables then fell progressively to reach a nadir by 75s (P < 0.0001). All variables then rose (P < 0.0001), with LV and AoT dP/dt$_{max}$ at 120s not statistically different from the 15s values, while PT dP/dt$_{max}$ was 21% lower (P = 0.0006, Fig. 5A). Furthermore, *1)* the pattern of changes in LV, AoT and PT dP/dt$_{max}$ were similar after normalization to heart rate (Fig. 5B), *2)* changes in patterns of LV and AoT dP/dt$_{max}$ were not altered after normalization to LV end-diastolic volume (Fig. 5C), *3)* LV dP/dt$_{max}$ (X) and AoT dP/dt$_{max}$ (Y) were closely related after cord clamping (Y = 0.45X - 42, R^2 = 0.89, P < 0.0001) and *4)* AoT dP/dt$_{max}$ (X) and PT dP/dt$_{max}$ (Y) were also related after cord clamping (Y = 0.85X - 47, R^2 = 0.71, P < 0.0001).

Left ventricular pressure-volume data The size and shape of LV P-V loops were similar before and after delivery, but changed rapidly and appreciably after cord clamping, with relatively minor alterations in the first 30s, followed by a noticeable decrease in P-V loop rectilinearity (particularly during the phase of isovolumic relaxation) and area to 75s, and then an ensuing increase in these features to 120s (Fig. 6).

LV end-diastolic volume tended to rise with delivery (from 4.5(1.5) to 5.1(1.2) ml, P = 0.131), and then remained unchanged throughout the period of cord clamping (Fig. 7A). LV E$_{max}$ was unaltered at delivery or 15s after cord clamping (P = 0.397, average 23.7(11.6) mmHg/ml), and was initially stable for 45s, but then decreased to 14.1(8.3) mmHg/ml at 75s (P = 0.0002), before rising to 21.9(11.2) mmHg/ml by 120s (P = 0.002; Fig. 7B), a value not different from the 15s post-cord clamp level (P = 0.418).

a) Discussion

This study, which has evaluated the characteristics and physiological basis of changes in ventricular outputs within a two minute non-ventilatory period following delivery and immediate cord clamping in preterm fetal lambs, has produced four main findings. First, both LV and RV outputs increased with delivery, primarily related to a rise in heart rate. Second, in association with an

increase in arterial blood pressure occurring immediately after cord clamping, RV output decreased by ~20% due to a reduction in stroke volume, but LV output was unchanged. Third, LV and RV outputs were maintained in the initial 30s after cord clamping, but while arterial oxygenation decreased rapidly to asphyxial levels and arterial blood pressures declined, both these outputs then approximately halved over the next 45s, in association with falls in heart rate and ventricular contractility. Finally, reductions in LV and RV outputs then plateaued from 75-120s, accompanied by exponential surges in concentrations of circulating noradrenaline and adrenaline, a rebound in ventricular contractility, a recovery of arterial blood pressures and an increase in ventricular filling pressures. These findings are consistent with a time-dependent response of ventricular outputs to immediate cord clamping after delivery that comprised *1)* an initial and minor fall in RV (but not LV) output related to altered loading conditions, *2)* ensuing large decreases in both LV and RV outputs during emergence of an established asphyxial state, related to the combination of bradycardia and a deterioration of ventricular contractile function, and *3)* a subsequent stabilization of these reduced levels of LV and RV output during ongoing asphyxia, supported by the cardiovascular stimulatory effects of a marked sympathoadrenal activation.

The results of the present study were in accord with prior findings indicating that complete delivery of fetal lambs was accompanied by increased concentrations of circulating catecholamines (Padbury *et al.*, 1981; Smolich *et al.*, 2017; Smolich *et al.*, 2019), as well as rises in resting heart rate and blood pressures (Smolich *et al.*, 2017; Smolich *et al.*, 2019). Moreover, as ventricular stroke volumes did not change, elevations in LV and RV outputs at delivery (Fig. 3) were primarily related to an increase in heart rate, a potent determinant of fetal cardiac output (Rudolph & Heymann, 1976; Anderson *et al.*, 1987). However, a fall in atrial blood pressures (Fig. 2B) and a trend to an increased LV end-diastolic volume evident with fetal delivery (Fig. 7A) may be indicative of an additional effect arising from a diminution of external cardiac constraint (Smolich *et al.*, 2021), related to removal of surrounding amniotic fluid and maternal abdominal pressures (Grant *et al.*, 1992; Grant, 1999).

Given an accompanying lack of changes in heart rate (Fig. 2C), LV end-diastolic and atrial pressures (Fig. 2B), LV contractility (Figs. 5 & 7B) and upper body VR (Fig. 4), the initial stepwise

increase in mean arterial blood pressures evident with cord clamping (Fig. 2A) was primarily attributable to a mechanical effect directly linked to an abrupt loss of the low resistance placental circulation from the vascular circuit (Smolich *et al.*, 2016). In conjunction with any active secretion/release of catecholamines from the adrenal medulla or other sympathetically-innervated fetal tissues, it is likely that removal of the placenta also underlie the initial rise in circulating concentrations of noradrenaline and adrenaline after cord clamping (Table 2). Thus, plasma concentrations of catecholamines are dependent on the balance between their entry into and clearance from the circulation (Esler *et al.*, 1990), so cord clamping would be expected to raise these concentrations via a combination of *1)* reduced size of the vascular compartment into which fetal catecholamine release occurred, and *2)* loss of the placenta, a major site of catecholamine clearance (Jones, 1980; Smolich *et al.*, 1996; Smolich & Esler, 1999). Indeed, study of noradrenaline and adrenaline kinetics during the birth transition in chronically-instrumented fetal lambs suggested that these two effects arising from removal of the placenta accounted for almost half of the rise in circulating catecholamine concentrations evident after birth (Smolich *et al.*, 1996).

Contrary to the presumption that umbilical cord clamping affects LV and RV outputs similarly (Bhatt *et al.*, 2014; Hooper *et al.*, 2015a; Hooper *et al.*, 2015b; Kluckow & Hooper, 2015; Hooper *et al.*, 2016; Hooper *et al.*, 2019), our study results pointed to a clear difference in the initial response of these outputs to such clamping. Thus, RV output decreased by ~20% with cord clamping (Fig. 3A), with the accompanying rise in PT blood pressure but unaltered mean RA pressure (Fig. 2) implying that increased RV afterload was the predominant factor underlying this decrease, with no substantial effect attributable to a reduced RV preload occurring secondary to loss of umbilical venous return. This proposition was in accord with *1)* a pronounced sensitivity of the fetal right ventricle to rises in afterload, with incremental increases in mean PT blood pressure producing striking linear reductions of RV output (Thornburg & Morton, 1983, 1986; Reller *et al.*, 1987; Kamitomo *et al.*, 1992; Reller *et al.*, 1992) and *2)* the recent conclusion that cardiac preload was not reduced following complete occlusion of the umbilical cord in chronically-instrumented fetal lambs (Lear *et al.*, 2021).

In contrast to a fall in RV output, LV output was initially unchanged after cord clamping, a finding that was not unexpected for at least three reasons. First, the left ventricle is less sensitive to increases in afterload than the right ventricle, with LV output displaying either a lesser reduction (Reller *et al.*, 1987) or no significant change (Thornburg & Morton, 1986; Kamitomo *et al.*, 1992) during elevations in mean arterial blood pressure. Second, a rise in blood pressure associated with cord clamping increases ventricular pressure work, a potent stimulus for elevations in fetal coronary blood flow (Reller *et al.*, 1992), which is derived entirely from LV output. Third, cord clamping may be accompanied by an initial rise in PA blood flow (Smolich *et al.*, 2015), and therefore pulmonary venous return, which would offset a reduction in LV filling arising from any fall in foramen ovale flow secondary to loss of umbilical venous return. Note that the lack of significant changes in LV end-diastolic and mean LA pressure (Fig. 2B), as well as LV end-diastolic volume (Fig. 7A), at the first (15s) time-point after immediate cord clamping suggested that this intervention also did not reduce LV preload.

In support of our main hypothesis, study data after cord clamping were consistent with changes in ventricular outputs having three distinct phases during an ensuing two minute non-ventilatory period. In the first phase, which lasted for ~30s after cord clamping, the stability of ventricular outputs (Fig. 3A), as well as heart rate (Fig. 2C), arterial/atrial/LV end-diastolic blood pressures (Figs. 2A &B), upper/lower body VR (Fig. 4) and measures of ventricular contractility (Figs. 5 & 7B), suggested that this phase primarily reflected a continuation of the mechanical effects of cord clamping per se. It is important to note, however, that levels of oxygenation were falling and concentrations of circulating catecholamines increasing in this period (Tables 1 & 2). Nonetheless, average circulating catecholamine concentrations of ~2,600 pmol/l for noradrenaline and ~940 pmol/l for adrenaline at the end of this phase (Table 2) were still below the thresholds of ~3,600-4,700 pmol/l for noradrenaline and ~2,700-4,400 pmol/l for adrenaline required to produce rises in heart rate, blood pressures and cardiac contractility in preterm fetal lambs (Padbury *et al.*, 1987).

In the second phase, evident between ~45-75s after cord clamping, changes in cardiovascular variables increasingly reflected the effects of a supervening asphyxial state, with progressive and

large (~50%) falls in LV and RV outputs (Fig. 3A) that were accompanied by the emergence of a pronounced bradycardia (Fig. 2C), declining arterial blood pressures (Fig. 2A), decreases in LV E_{max} (Fig. 7B) and LV/arterial dP/dt_{max} (Fig. 5), but a relatively stable upper/lower body VR (Fig. 4). Taken together, such data suggested that these large falls in LV and RV outputs primarily had a cardiac origin, and arose from a combination of bradycardia and a deterioration in ventricular contractility (i.e. ventricular dysfunction). Bradycardia is a well-recognized accompaniment of fetal asphyxia and arises from marked parasympathetic activation mediated via the peripheral chemoreceptor reflex (Galinsky *et al.*, 2016; Lear *et al.*, 2016b; Lear *et al.*, 2020b). It is likely that this parasympathetic activation, via the myocardial actions of vagal stimulation (Degeest *et al.*, 1965), as well as the direct myocardial effects of decreased tissue oxygenation (Allen & Orchard, 1987), contributed to an observed decline in ventricular contractility. Note that declines in LV and arterial dP/dt_{max} during this phase (Fig. 5A) were not simply due to bradycardia, as similar patterns were also present after normalizing these dP/dt_{max} to heart rate (Fig. 5B).

During this second phase, average circulating concentrations of noradrenaline (~8,600 pmol/l) and adrenaline (~8,500 pmol/l; Table 2) were 2 to 3-fold higher than concentrations required to increase heart rate and arterial blood pressures in normoxaemic preterm fetal lambs (Padbury *et al.*, 1987). That heart rate and arterial blood pressures did not increase during this phase may have been related to two factors. First, it is likely that the stimulatory actions of elevated concentrations of circulating catecholamines were countered by the parasympathetic activation occurring during fetal asphyxia (Galinsky *et al.*, 2014; Lear *et al.*, 2020d). Second, asphyxia appears to inhibit the chronotropic and pressor actions of circulating catecholamines in the immature circulation, particularly in the presence of acidaemia (Preziosi *et al.*, 1993).

In the third phase, evident during the ongoing asphyxial state present beyond ~75s after immediate cord clamping, a stabilization of markedly reduced levels of LV and RV output (Fig. 3A) was accompanied by increases in arterial blood pressures (Fig. 2A), LV/arterial dP/dt_{max} Fig. 5), LV E_{max} (Fig. 7B), atrial/LV end-diastolic pressures (Fig. 2B) and upper/lower body VR (Fig. 4), as well as extremely high concentrations of circulating catecholamines (Table 2). These changes were

suggestive of an activation of at least three cardiovascular support mechanisms. Thus, consistent with rises in LV/arterial dP/dt_{max} and LV E_{max}, the first was an increase in LV contractility that was most likely related to the stimulatory effects of circulating catecholamines (Padbury *et al.*, 1987) and activation of the cardiac sympathetic nervous system (Eisenhofer *et al.*, 1992). The second was increased peripheral vasoconstriction, suggested by a rise in arterial blood pressures and systemic VR, that most likely arose from a generalized sympathetic activation and very high levels of circulating catecholamines (Galinsky *et al.*, 2014), as well as asphyxia-induced rises in other circulating factors such as angiotensin II, arginine vasopressin and neuropeptide Y (Jensen & Lang, 1992; Giussani, 2016; Lear *et al.*, 2020c). Interestingly, the rise in lower body VR was more pronounced than upper body VR in this phase (Fig 4), which was consistent with activation of mechanisms directed towards preservation of cerebral perfusion during fetal asphyxia (Jensen & Hanson, 1995). The third mechanism, suggested by rises in atrial and LV end-diastolic pressures (Fig 2B), was a recruitment of ventricular preload reserve to sustain ventricular output (Ross, 1976). Note that rises in these pressures, which accord with the prior finding that LA blood pressure increased during fetal asphyxia (Adamson *et al.*, 1970), were accompanied by marked alterations in the shape and position of the isovolumic relaxation limb of P-V loops (Fig. 6), implying that impaired LV relaxation during the asphyxial period after cord clamping was a major factor contributing to these pressure rises (Pagel *et al.*, 1993). Surprisingly, however, LV end-diastolic volume was not increased, whereas dilatation occurs in the adult heart with impairment of myocardial oxygen availability (McCans & Parker, 1973). One possible explanation for the difference is that increases in LV end-diastolic volume were limited because of the substantial external constraint exerted on the heart by the fluid-filled lungs in the fetus (Grant, 1999; Smolich *et al.*, 2021).

While our evaluation of mechanisms underlying falls in ventricular output after immediate cord clamping at delivery mainly focused on the left ventricle, it is likely that similar mechanisms pertained to the right ventricle, given that *1)* changes in PT dP/dt_{max} correlated with those of AoT dP/dt_{max}, which in turn mirrored the pattern of alterations in LV dP/dt_{max} (Figs. 5A & B) and E_{max} (Fig. 7B), and *2)* changes in AoT and PT blood pressures were similar, as were those of LA and RA blood pressures (Figs. 2A & B). However, our finding that, by 120s after cord clamping, LV and AoT dP/dt_{max}

had returned to their initial post-cord clamp values, whereas PT dP/dt_{max} was ~20% lower (Fig. 5A), implies that rebound increases in contractility with ongoing asphyxia were greater in the LV than RV.

A noteworthy finding of our study was that changes in LV output and AoT blood flow did not mirror one another after cord clamping, but instead progressively diverged following development of an asphyxial state (Fig. 3A). This divergence can be explained on the basis of coronary blood flow changes which accompany fetal asphyxia. Thus, in the normoxaemic state, the difference between LV output and AoT blood flow is relatively small, as coronary blood flow in fetal lambs constitutes <10% of LV output (Rudolph, 1985). However, fetal coronary blood flow increases progressively with falls in arterial oxygenation, culminating in approximately a 4-fold increment at asphyxial levels of oxygenation (Peeters *et al.*, 1979; Jensen & Lang, 1992; Jensen & Hanson, 1995). As changes in coronary blood flow after cord clamping were included in conductance catheter measurements of LV output, but not in flow-probe estimates of AoT blood flow, AoT blood flow therefore underestimated true LV output to a progressively greater degree as the duration of cord clamping lengthened.

A potential limitation of our study was that the nature and extent of the implanted instrumentation (i.e. four flow probes, two rigid micromanometer catheters and one stiff combined conductance-micromanometer catheter), as well as the frequency of required arterial blood sampling, necessitated acute instrumentation and an experimental protocol performed under general anesthesia. However, resting baseline blood gas variables, haemodynamics and catecholamine concentrations in our preparation were similar to those of unanaesthetized, chronically-instrumented pre-term fetal lambs (Padbury *et al.*, 1987; Cheung & Brace, 1988; Hunter *et al.*, 2003; Wassink *et al.*, 2007; Crossley *et al.*, 2009; Bhatt *et al.*, 2013; Galinsky *et al.*, 2014). Furthermore, the pattern of changes in arterial blood gases and catecholamine concentrations observed with a 2 min period of in utero asphyxia also resembled those of chronically-instrumented fetal lambs (Jensen & Lang, 1992; Jensen & Hanson, 1995; Galinsky *et al.*, 2014; Lear *et al.*, 2020a).

Differences appeared to be present, however, in the temporal pattern of changes in heart rate and arterial blood pressure seen after cord clamping, compared to inflation of an umbilical cord

balloon occluder in chronically-instrumented fetal lambs. Thus, in our study, immediate cord clamping resulted in *1)* a relatively delayed fall in heart rate and *2)* a rapid initial and large increase in arterial blood pressure that was followed by a fall and then a subsequent rise in blood pressure (Fig, 2). However, cord occlusion via a balloon occluder in chronically-instrumented fetuses was accompanied by *1)* a brisk fall in heart rate, e.g. Fig. 1 in (Lear *et al.*, 2021) and Fig. 3 in (Lear *et al.*, 2016a), but *2)* an initial rapid and small rise in arterial blood pressure, followed by a more gradual and larger increase in this pressure, e.g. Fig. 1 in (Lear *et al.*, 2021) and Fig. 1 in (Lear *et al.*, 2020b). At present, it is unclear to what extent these differences were related to factors such as *1)* effects of acute surgical preparation under general anaesthesia, *2)* an in utero versus ex utero location of the fetus (Sobotka *et al.*, 2014), or *3)* abrupt clamping of the cord (which will occlude umbilical veins and arteries simultaneously), versus a relatively slower inflation of a balloon occluder with fluid (which will occlude low pressure umbilical veins prior to umbilical arteries). It is noteworthy, however, that the pattern of heart rate and blood pressure changes in our study with 2 minutes of immediate cord clamping were quite similar to those reported by Bhatt et al (Bhatt *et al.*, 2013), where fetal lambs were chronically-instrumented 3 days before the birth transition.

Taken in conjunction with our previous findings (Smolich *et al.*, 2015; Smolich *et al.*, 2017; Smolich *et al.*, 2020), the experimental results of the present study do not support a widely-promoted view, based solely on measurement of RV output and mean arterial blood pressure in preterm lambs (Bhatt *et al.*, 2013), without any assessment of LV output, ventricular contractility or atrial/ventricular filling pressures, that large falls in both LV and RV outputs during a two-minute interval after immediate cord clamping at delivery can be entirely accounted for by an increase in ventricular afterload and a decrease in ventricular preload (Bhatt *et al.*, 2014; Hooper *et al.*, 2015a; Hooper *et al.*, 2015b; Kluckow & Hooper, 2015; Hooper *et al.*, 2016; Hooper *et al.*, 2019). Instead, our data suggested that *1)* the contribution of altered cardiac loading conditions was confined to a relatively minor (~20%) reduction in RV (but not LV) output evident within the initial 30s after cord clamping, and *2)* this reduction in RV output was primarily related to a stepwise increase in PT blood pressure (and thus RV afterload) associated with cord clamping, without any appreciable contribution from a diminution in RV preload. Furthermore, the main factor contributing to large

falls in LV and RV output after immediate cord clamping was not altered cardiac loading conditions, but the combination of a profound bradycardia and a decline in ventricular contractility that accompanied rapid emergence of an asphyxial state within 45-60s after cord clamping. A subsequent stabilization of these reduced levels of LV and RV output occurred against a background of exponential rises in concentrations of circulating catecholamines that were accompanied by rebound increases in arterial blood pressures and ventricular contractility.

Two main clinical implications arose from our study. First, in conjunction with prior observations that *1)* AoT S_aO_2 fell linearly at a rate of ~1.3%/s and P_ao_2 at a rate of ~0.3 mmHg/s after immediate cord clamping prior to ventilation (Smolich *et al.*, 2015), and *2)* reductions in cerebral oxygenation measured with near-infrared spectroscopy were detectable within 5s after umbilical cord occlusion in chronically-instrumented fetal lambs (Lear *et al.*, 2020b), our findings re-iterated the sometimes under-appreciated rapidity with which falls in arterial oxygenation can occur after cord clamping. Second, a rapid decline of arterial blood oxygenation to asphyxial levels by ~60s after immediate cord clamping in the absence of ventilation was accompanied by an equally rapid deterioration of ventricular contractility (i.e. development of myocardial dysfunction) that was associated with a halving of ventricular outputs and a marked drop in arterial blood pressures. With persistence of this asphyxial state, however, the presence of this dysfunction was masked, as measures of ventricular contractility recovered due to stimulatory effects of a marked sympathoadrenal activation, in conjunction with a stabilization of reduced levels of ventricular outputs, a rebound rise in arterial blood pressures and increases in ventricular filling pressures. The duration of this compensatory circulatory phase remains to be determined, as does the nature of changes in LV and RV outputs, as well as associated alterations in ventricular function and concentrations of circulating catecholamines, which occur with more prolonged non-ventilatory periods after immediate cord clamping that culminate in overt manifestations of birth asphyxia in the newborn. Nonetheless, from the perspective of preventing or minimizing myocardial dysfunction in the birth transition, the findings of this study reinforce and provide a physiological basis for the recommendation of the International Liaison Committee on Resuscitation that effective ventilation should occur within 60s after immediate cord clamping at birth (Wyckoff *et al.*, 2015).

Author contributions

Experimental studies were performed within the Large Animal Facility of the Murdoch Children's Research Institute. JJS conceptualized and designed the study, and drafted the manuscript. JJS and KRK performed the physiological studies, and acquired and analyzed physiological data. SEP and GWL performed the catecholamine assays. JJS, KRK, SEP, JPM, MMC and GWL were involved in data interpretation and critical review of the manuscript, and approved the final version of the manuscript.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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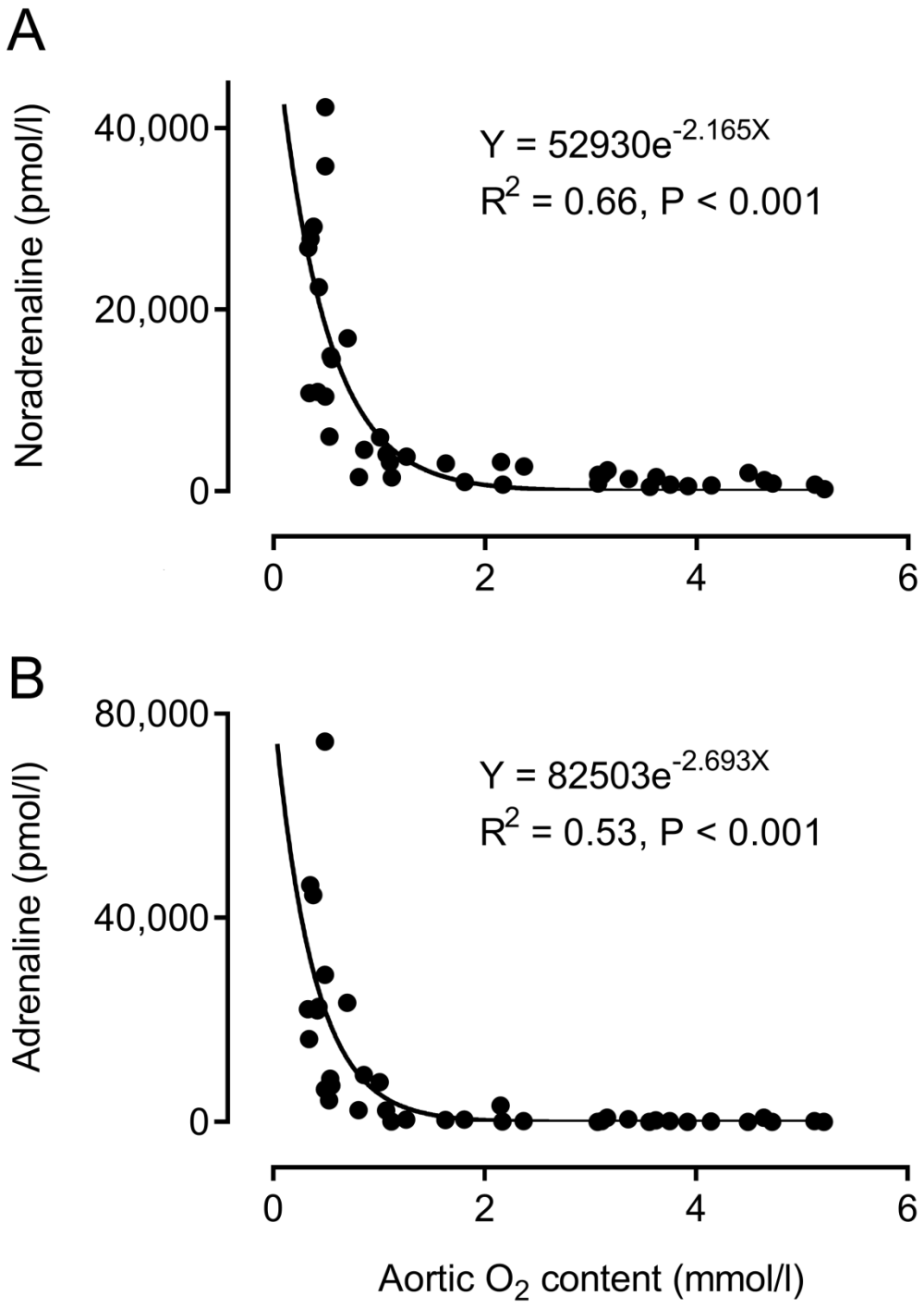

Figure 1. Relation between aortic O_2 content and circulating noradrenaline (panel A) and adrenaline (panel B) after umbilical cord clamping.

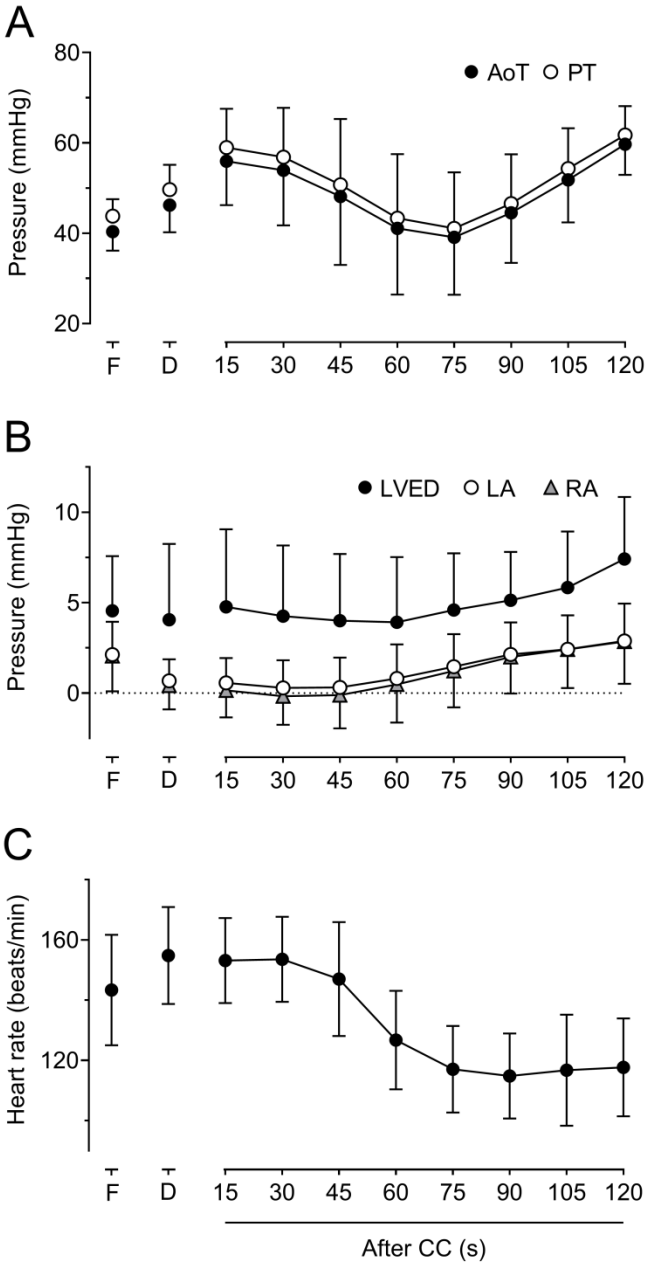

Figure 2. Mean aortic trunk (AoT) and pulmonary trunk (PT) blood pressures (panel A), left ventricular end-diastolic (LVED), mean left atrial (LA) and mean right atrial (RA) blood pressures (panel B), and heart rate (panel C) in the fetus before delivery (F), after delivery (D) and at 15s intervals after umbilical cord clamping (CC). Results are expressed as mean (SD); n = 12, with only one limb of the bidirectional SD displayed in panels A & B to aid visualization.

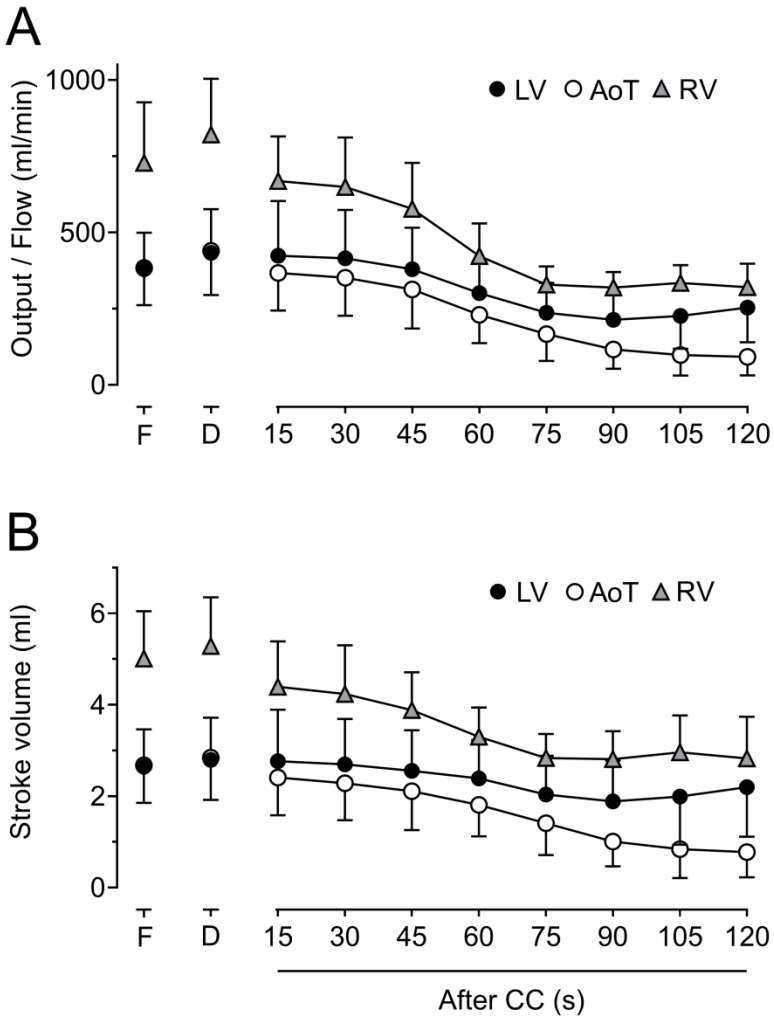

Figure 3. Left ventricular (LV) output, aortic trunk blood flow (AoT) and right ventricular (RV) output (panel A), and corresponding stroke volumes (panel B) using the same format and time-points defined in Fig. 2. Results are expressed as mean(SD); n = 12, with only one limb of bidirectional SD displayed to aid visualization. Note that the before and after delivery AoT data-points in both panels are hidden by the corresponding LV data-points.

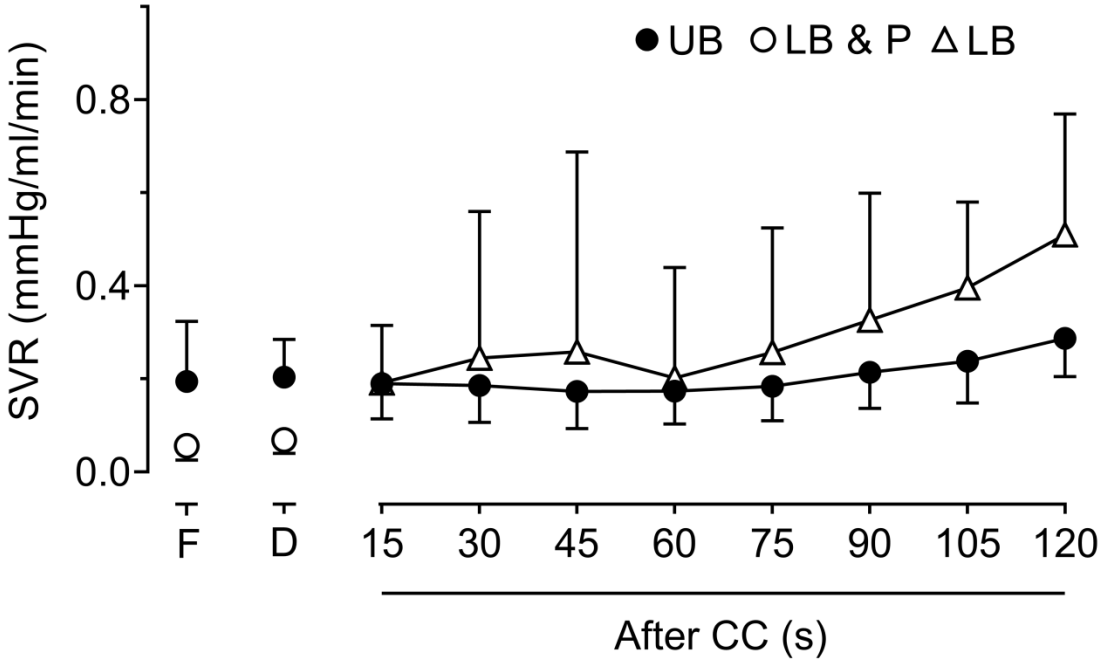

Figure 4. Systemic vascular resistance (SVR) of upper body (UB) region, the combined lower body and placenta (LB&P) and the post-cord clamp lower body region (LB) using the same format and time-points defined in Fig. 2. Results are expressed as mean(SD); n = 12, with only one limb of bidirectional SD displayed to aid visualization.

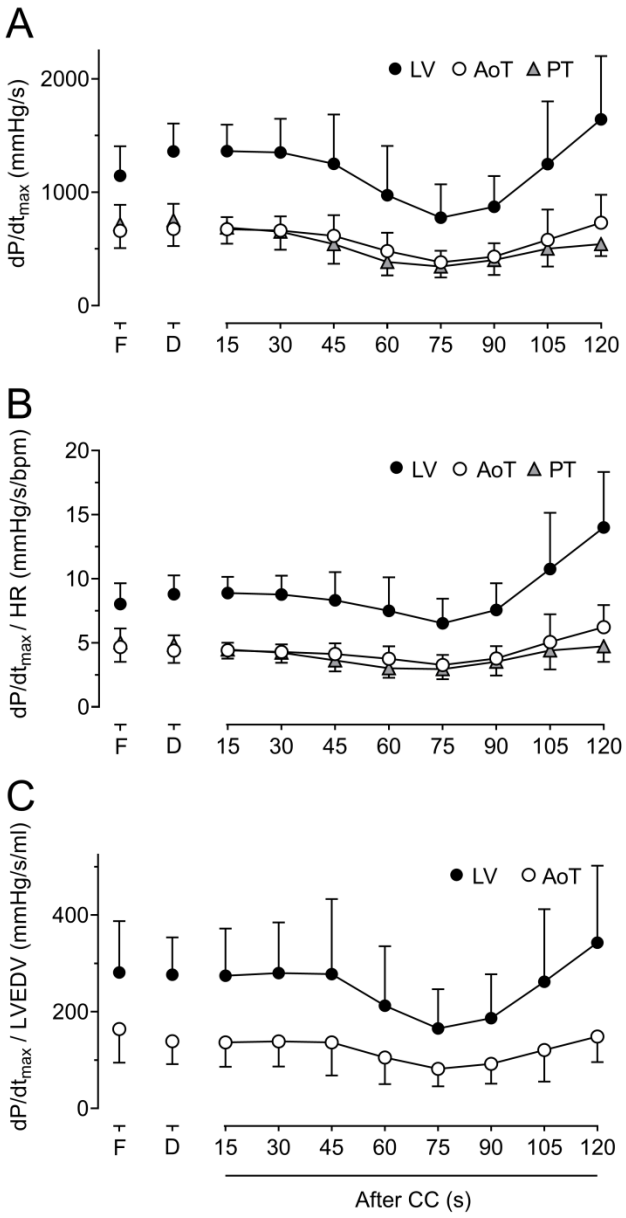

Figure 5. Maximal rate of rise of blood pressure (dP/dt_{max}) in the left ventricle (LV), aortic trunk (AoT) and pulmonary trunk (PT, panel A), the corresponding dP/dt_{max} normalized for heart rate in beats/min (bpm, panel B), and LV and AoT dP/dt_{max} normalized for left ventricular end-diastolic volume (LVEDV, panel C) using the same format and time-points defined in Fig. 2. Results are expressed as mean(SD); n = 12, with only one limb of bidirectional SD displayed to aid visualization.

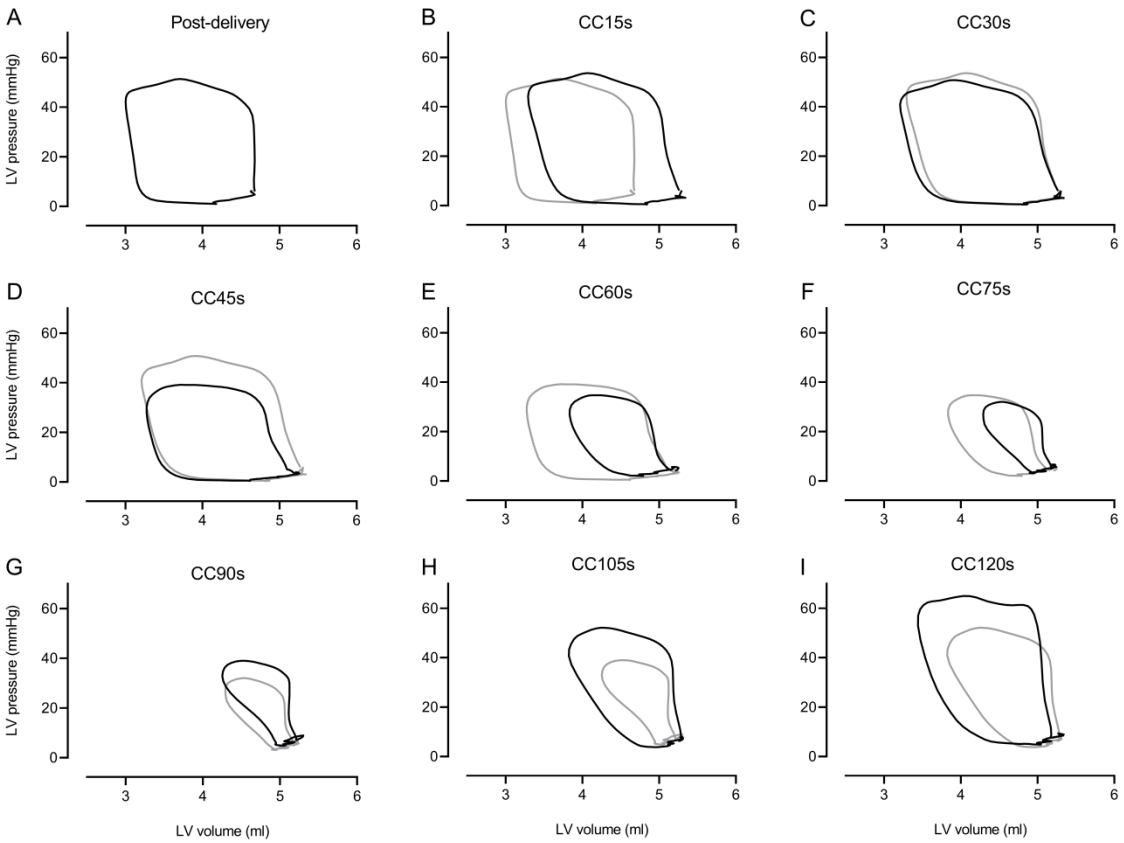

Figure 6. Illustrative example of sequential changes in morphology of left ventricular (LV) pressure-volume loops following delivery (panel A), and then at 15s intervals to 120s after umbilical cord clamping (CC15s to CC120s, panels B to I). To demonstrate the rapidity of changes, the grey pressure-volume loop in panels B to I is identical to the black pressure-volume loop of the immediate preceding panel.

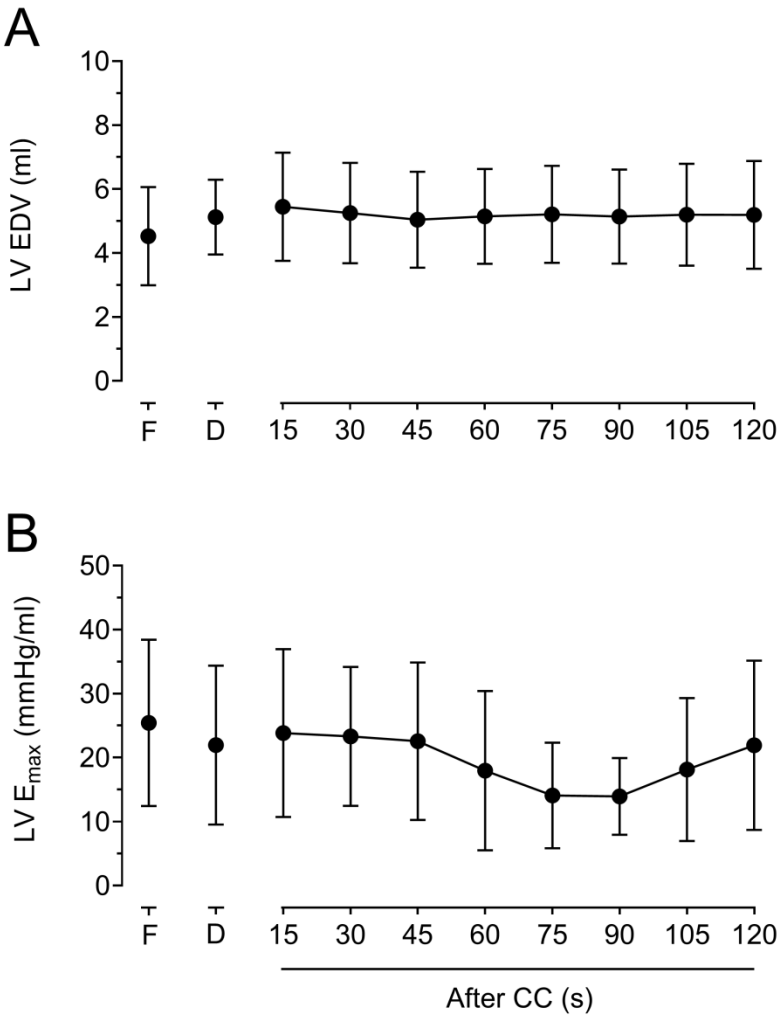

Figure 7. Left ventricular end-diastolic volume (LV EDV, panel A) and LV maximal elastance (E_{max}, panel B) using the same format and time-points defined in Fig. 2. Results are expressed as mean(SD); n = 12.

Table 1. Aortic trunk blood gas sample variables before and after umbilical cord clamping.

Variable	Fetus	Post-delivery	30s CC	60s CC	90s CC	*P*
Hb (g/dl)	11.1(1.1)	11.2(0.9)	11.3(1.1)	11.3(1.2)	11.3(1.2)	0.811
pH	7.299(0.021)[c]	7.284(0.027)	7.252(0.025)	7.239(0.023)	7.215(0.029)	<0.0001
SaO_2 (%)	63.6(12.8)[b]	54.1(15.2)	21.5(7.8)	8.8(3.1)	6.1(1.8)	<0.0001
P_aO_2 (mmHg)	25.3(5.9)[b]	22.2(5.3)	12.8(2.8)	8.2(1.7)	6.6(1.4)	<0.0001
P_aCO_2 (mmHg)	49.2(2.9)[a]	51.2(3.7)	58.0(3.9)	59.9(3.6)	61.9(4.9)	<0.0001
O_2 content (mmol/l)	4.26(0.72)[a]	3.67(0.86)	1.47(0.50)	0.61(0.21)	0.42(0.11)	<0.0001

Data are expressed as mean(SD); n = 12. Abbreviations: CC, umbilical cord clamping; Hb, haemoglobin concentration; SaO_2, haemoglobin oxygen saturation. [a]$P \leq 0.013$, [b]$P \leq 0.004$, [c]$P = 0.001$, fetus vs. post-delivery, one-way repeated measures analysis of variance. *P* value refers to one-way repeated measures analysis of variance across interval between post-delivery and 90s CC.

Table 2. Aortic trunk catecholamine concentrations before and after umbilical cord clamping.

Variable	Fetus	Post-delivery	30s CC	60s CC	90s CC	*P*
Noradrenaline (pmol/l)	1006(638)[a]	1483(756)	2576(1302)	8621(4839)	26502(10001)	<0.0001
Adrenaline (pmol/l)	122(174)[a]	353(322)	939(1144)	8437(5882)	34806(19379)	<0.0001

Data are expressed as mean(SD); n = 8. Abbreviation: CC, umbilical cord clamping. [a]$P \leq 0.002$, fetus vs. post-delivery, one-way repeated measures analysis of variance. *P* value refers to one-way repeated measures analysis of variance across interval between post-delivery and 90s CC.

Author photo and profile

Joe Smolich is a clinician turned scientist who is a Principal Research Fellow within the Heart Research Group of the Murdoch Children's Research Institute in Melbourne, Australia. Over the past 10 years he has focused on the application of sophisticated methodology such as wave intensity and ventricular pressure-volume analysis to the assessment of cardiovascular function in the fetal and perinatal periods, with an emphasis on the effects of immediate and delayed cord clamping during the birth transition.