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Extended period of ventilation before delayed cord clamping augments left-to-right shunting and decreases systemic perfusion at birth in preterm lambs

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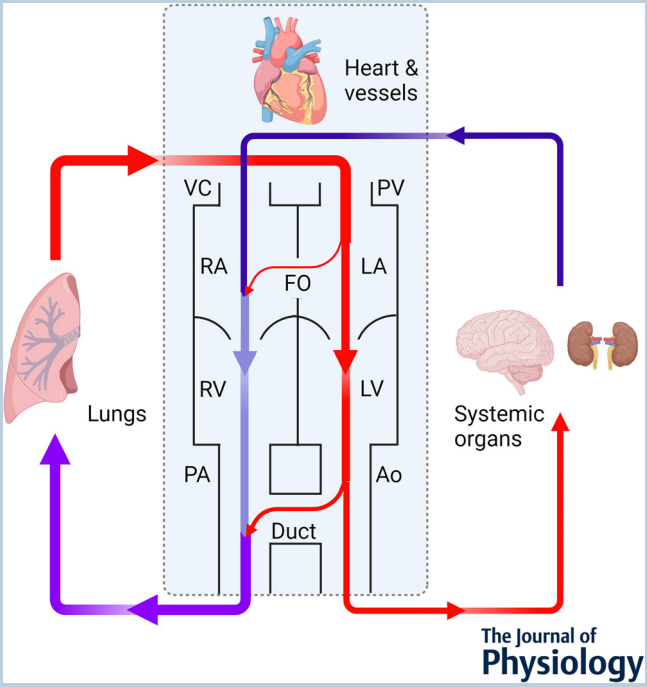

Abstract Previous studies have suggested that an extended period of ventilation before delayed cord clamping (DCC) augments birth-related rises in pulmonary arterial (PA) blood flow. However, it is unknown whether this greater rise in PA flow is accompanied by increases in left ventricular (LV) output and systemic arterial perfusion or whether it reflects enhanced left-to-right shunting across

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the ductus arteriosus and/or foramen ovale (FO), with decreased systemic arterial perfusion. Using an established preterm lamb birth transition model, this study compared the effect of a short (∼40 s, $n = 11$), moderate (∼2 min, $n = 11$) or extended (∼5 min, $n = 12$) period of initial mechanical lung ventilation before DCC on flow probe-derived perinatal changes in PA flow, LV output, total systemic arterial blood flow, ductal shunting and FO shunting. The LV output was relatively stable during initial ventilation but increased after DCC, with similar responses in all groups. Systemic arterial flow patterns displayed only minor differences during brief and moderate periods of initial ventilation and were similar after DCC. However, an increase in PA flow was augmented with an extended initial ventilation ($P < 0.001$), owing to an earlier onset of left-to-right ductal and FO shunting ($P < 0.001$), and was accompanied by a pronounced reduction in total systemic arterial flow ($P = 0.005$) that persisted for 4 min after DCC ($P \leq 0.039$). These findings suggest that, owing to increased left-to-right shunting and a greater reduction in systemic arterial perfusion, an extended period of ventilation before DCC does not result in greater perinatal circulatory benefits than shorter periods of initial ventilation in the birth transition.

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Abstract figure legend Soon after birth, two essential vascular channels that shunt blood from the right to the left side of the fetal circulation, namely the foramen ovale (FO) and the ductus arteriosus (Duct), reverse their direction of flow. The effect of these flow reversals is to increase the amount of blood flowing to the lungs but to decrease blood flow to vital systemic organs, such as the brain and kidneys. With conventional 'delayed cord clamping' (DCC), where clamping of the umbilical cord is typically delayed for ≤2 min after delivery and the start of lung ventilation, significant left-to-right shunting via the FO and Duct does not occur until after the cord is clamped. However, with an extended (5 min) period of ventilation before DCC that augments the level of lung blood flow before birth, this shunting commences sooner, resulting in a greater reduction of systemic organ perfusion during the birth transition. Abbreviations: Ao, aorta; LA, left atrium; LV, left ventricle; PA pulmonary artery; PV, pulmonary veins; RA, right atrium; RV right ventricle; VC, vena cavae.

Key points

- Previous studies suggest that an extended period of initial ventilation before delayed cord clamping (DCC) augments birth-related rises in pulmonary arterial (PA) blood flow.
- It is unknown whether this greater rise in PA flow is accompanied by an increased left ventricular output and systemic arterial perfusion or whether it reflects enhanced left-to-right shunting across the ductus arteriosus and/or foramen ovale, with decreased systemic arterial perfusion.
- Anaesthetized preterm fetal lambs instrumented with central arterial flow probes underwent a brief (∼40 s), moderate (∼2 min) or extended (∼5 min) period of ventilation before DCC.
- Perinatal changes in left ventricular output were similar in all groups, but extended initial ventilation augmented both perinatal increases in PA flow, owing to earlier onset and greater left-to-right ductal and foramen ovale shunting, and perinatal reductions in total systemic arterial perfusion.
- Extended ventilation before DCC does not confer a greater perinatal circulatory benefit than shorter periods of initial ventilation.

Introduction

Considerable effort has been directed over recent years towards encouraging use of delayed cord clamping (DCC), rather than the decades-long practice of immediate/early cord clamping, after delivery at birth (McAdams, 2014; Rabe et al., 2022). As part of this effort, professional bodies such as the American College of Obstetrics and Gynaecology (American College of Obstetrics & Gynaecology, 2020), the European Resuscitation Council (Madar et al., 2021), the European Society for Paediatric Research (Sweet et al., 2019) and the International Liaison

Council on Resuscitation (Wyckoff et al., 2022) have recommended that cord clamping be deferred for intervals ranging from 30 to ≥60 s after delivery in preterm or term births not requiring resuscitation. Consistent with these recommendations, the interval between delivery and DCC in the majority of published randomized controlled trials has ranged from 30 s to 2 min (Fogarty et al., 2018; Jasani et al., 2021; McDonald et al., 2013; Seidler et al., 2021).

Given that most non-compromised preterm and term newborns start breathing within 15 s after delivery (Badurdeen et al., 2022; Boere et al., 2015; Ersdal et al., 2014; Kilicdag et al., 2022; Perretta et al., 2020), DCC is therefore usually preceded by a variable period of lung ventilation. Such ventilation appears to be beneficial, because each 10 s of delay before cord clamping after the onset of breathing can reduce the risk of neonatal death or hospital admission within the first 24 h by 20% (Ersdal et al., 2014). Initial ventilation is also a key component of 'physiological based cord clamping' (PBCC), a widely promoted DCC variant in which an extended period of ventilation, typically ranging from 3 to 6 min, precedes cord clamping (Bhatt et al., 2013; Blank et al., 2018; Boere et al., 2015; Brouwer et al., 2019; Knol et al., 2020; Polglase et al., 2015). The rationale advanced for this extended initial ventilation is that it augments increases in pulmonary arterial (PA) blood flow and therefore pulmonary venous return to the left atrium, which preserves left ventricular (LV) preload and thereby sustains LV output and systemic arterial perfusion after reduction of right-to-left (R→L) shunting across the foramen ovale (FO) with abolition of umbilical venous return by cord clamping (Bhatt et al., 2014; Hooper et al., 2015, 2019; Kluckow & Hooper, 2015; Te Pas et al., 2019).

Whether an extended period of ventilation before cord clamping has greater circulatory benefits than shorter ventilation-to-cord clamp intervals of conventional DCC has not been established experimentally, however, because earlier studies have focused primarily on comparison of PBCC with immediate/early cord clamping (Bhatt et al., 2013; Blank et al., 2018; Polglase et al., 2015). Indeed, the fundamental assumption that a larger increase in PA blood flow during an extended period of ventilation before PBCC is accompanied by a higher LV output and greater systemic arterial perfusion has not been validated. This issue is of particular relevance because, although PA blood flow rose progressively during an extended (∼4 min) period of ventilation before PBCC in preterm fetal lambs, carotid arterial flow fell steadily (Bhatt et al., 2013). Although this fall could have represented the initial phase of a recognized homeostatic decline in cerebral perfusion that maintains brain O_2 delivery with rises in arterial oxygenation during the birth process (Iwamoto et al., 1987), a more likely possibility is that it reflected enhanced left-to-right (L→R) shunting, which characteristically increases pulmonary blood flow, but decreases systemic perfusion (Kluckow, 2005; Kluckow & Evans, 2001; Kulik & Levy, 2022).

The potential sources of L→R shunting in the perinatal period are the two structures essential for R→L shunting in the fetus. The most-studied source arises from reversal of phasic arterial blood flow across the ductus arteriosus (Crossley et al., 2009; Smolich & Kenna, 2022; Smolich et al., 2016, 2020), which initially originates almost entirely via an abrupt onset of retrograde diastolic discharge from a descending aortic reservoir ('windkessel') that is initiated by umbilical cord clamping (Smolich & Mynard, 2021b; Smolich et al., 2016). The resultant shunting of blood flow from the thoracic aorta to the PA vasculature increases LV output (Clyman, 2022), becomes prominent within minutes after birth and, following immediate/early cord clamping, can provide up to half of lung perfusion in the neonatal period (Crossley et al., 2009; Smolich et al., 2016, 2020).

The second source of L→R shunting occurs from the left to the right atrium via reversal of flow across the FO (Evans & Iyer, 1994b; Hannu et al., 1989; Hiraishi et al., 1991), which increases right ventricular (RV) output but decreases LV output (Kluckow, 2005). Surprisingly, such reversal has received scant attention in experimental studies of the birth transition to date, presumably in part because prominent developmental physiologists have considered that closure of the FO occurs almost immediately after birth (Dawes, 1968; Rudolph, 2009b). Nonetheless, the presence of L→R FO shunting has been observed in fetal lambs during *in utero* ventilation (Anderson et al., 1985) and in the early newborn period in both spontaneously breathing (Baik et al., 2016; Evans & Archer, 1990; Hannu et al., 1989; Hiraishi et al., 1991; Markhorst et al., 1995; Noori et al., 2012; Ozcelik et al., 2006) and ventilated human infants (Evans & Iyer, 1994a, b). Indeed, L→R FO shunting is detectable with Doppler echocardiography in 75–94% of human neonates in the first hour after birth (Baik et al., 2016; Hiraishi et al., 1991; Noori et al., 2012). However, particularly in the setting of DCC preceded by different durations of initial ventilation, very little information is available about the following factors: (i) the time course of perinatal changes in FO shunt flow patterns; (ii) the extent to which L→R FO shunting, in addition to the combination of L→R ductal and FO shunting, contributes to perinatal increases in pulmonary perfusion; or (iii) the nature of any relationship between levels of L→R shunting and systemic arterial perfusion.

To characterize the physiological basis of perinatal changes in pulmonary and systemic arterial blood flows associated with differing durations of ventilation preceding DCC, this study therefore compared the effect of a short (∼40 s), moderate (∼2 min) or extended (∼5 min) period of lung ventilation prior to DCC on ventricular outputs, PA and major systemic arterial blood flows, and

upper body O_2 delivery, in addition to ductal and FO shunting patterns, during the birth transition in preterm lambs. The main hypothesis tested was that any greater increase in PA blood flow associated with an extended period of ventilation prior to DCC was accompanied by augmented L→R shunting through the ductus arteriosus and FO, to the detriment of systemic arterial perfusion.

Methods

Studies conformed to guidelines of the National Health and Medical Research Council of Australia (National Health & Medical Research Council, 2013) and were approved by the Murdoch Children's Research Institute Animal Ethics Committee (Projects A765, A872 and A928). This manuscript is compliant with the ARRIVE guidelines for reporting of animal research (Grundy, 2015; Kilkenny et al., 2010).

Surgical preparation

The general features of the anaesthetic and monitoring procedures were as previously described (Smolich & Kenna, 2022; Smolich et al., 2015, 2017, 2020). Briefly, 34 Border Leicester cross ewes were premedicated at a gestation of 127 (2) days [mean (SD), term = 147 days] with an I.M. injection of ketamine (5 mg kg^{-1}) and xylazine (0.1 mg kg^{-1}), then anaesthetized with 4% isoflurane administered by mask. After intubation of the trachea with a cuffed endotracheal tube, anaesthesia was maintained with isoflurane (0.5–2%) and nitrous oxide (10–20%) delivered by ventilator in O_2-enriched air, supplemented with ketamine (1–1.5 mg kg^{-1} h^{-1}), midazolam (0.1–0.15 mg kg^{-1} h^{-1}) and fentanyl (2–2.5 μg kg^{-1} h^{-1}) infused through a right external jugular venous cannula. Transcutaneous oxygen saturation (S_{pO_2}) was monitored continuously via pulse oximetry of an 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 adjusted 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. In the event of a multiple pregnancy, the position of all fetuses was assessed by palpation, with the presence and degree of any meconium staining in each fetus determined via a small keyhole incision, usually over a hindlimb in a uterine horn. The most accessible fetus in the best condition was then chosen for surgical preparation, with any other fetus(es) first delivered completely from the uterus and humanely killed 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 accessed via a hysterotomy and placed in a saline-filled glove to prevent loss of lung liquid. Through a midline neck incision, fluid-filled catheters were passed into the superior vena cava via the left external jugular vein for fluid and drug administration. A 6 Fr sheath was inserted into the brachiocephalic trunk (BCT) via the left common carotid artery for pressure measurement and blood sampling and for passage of a 3.5 Fr micromanometer catheter (SPR-524, Millar Instruments, Houston, TX, USA) into the ascending aorta/aortic trunk (AoT) to obtain a high-fidelity pressure measurement. After exteriorization of the left forelimb and adjacent thorax, a thoracotomy was performed in the third interspace, with resection of the third and fourth ribs to permit adequate access to all major central arteries. After careful dissection, transit-time flow probes (Transonic Systems, Ithaca, NY, USA) were placed non-constrictively around the BCT (4 or 6 mm), aortic isthmus (AI; 6 mm), ductus arteriosus (8 or 10 mm) and left PA (4 or 6 mm). To measure pressures: (i) a fluid-filled catheter and another 3.5 Fr micromanometer (SPR-524, Millar Instruments) were inserted via purse-string sutures into the pulmonary trunk (PT) close to its junction with the ductus and common PA; and (ii) a fluid-filled catheter was inserted into the left atrial (LA) appendage. Finally, a clamped 4.5 mm endotracheal tube filled with isotonic saline was inserted through a proximal tracheostomy and tied into place. Owing to the presence of multiple catheters and space-occupying flow probes, thoracic structures were positioned in their normal anatomical location at the end of surgery, but the thoracotomy was not surgically closed. Haemodynamics were allowed to stabilize for ∼10 min after completion of surgery.

Experimental protocol

The overall experimental protocol in lambs, with timing of haemodynamic measurements and AoT blood sampling for gas analysis, is depicted schematically in Fig. 1. After removal of the glove from the fetal head, the endotracheal tube was unclamped to allow lung liquid to drain passively via gravity for 20–30 s, simulating the reduction in lung liquid volume that normally occurs during the birth process (Berger et al., 1998; Pfister et al., 2001; Stockx et al., 2007), then reclamped. Subsequently, fetuses were delivered completely from the uterus, placed on the abdomen of the ewe under an overhead heating lamp and covered with warmed towels, avoiding tension on the umbilical cord. While haemodynamics were recorded continuously, an AoT sample was collected ∼30 s later for blood gas analysis (ABL800, Radiometer).

Lambs then underwent positive-pressure mechanical ventilation via the endotracheal tube using warmed and

humidified gases at the initial settings described below, in one of three protocols allocated before surgery. These were as follows: (i) ventilation for 42 (7) s before DCC (40sV, $n = 11$; 5 males and 6 females; 3 singletons, 7 twins and 1 triplet), with an AoT sample collected for blood gas analysis at 20 s after the onset of ventilation; (ii) ventilation for 122 (2) s before DCC (120sV, $n = 11$; 7 males and 4 females; 2 singletons and 9 twins), with AoT samples collected for blood gas analysis at 30, 60 and 90 s after the start of ventilation; or (iii) ventilation for 302 (3) s before DCC (300sV, $n = 12$; 6 males and 6 females; 2 singletons, 9 twins and 1 triplet), with AoT samples collected for blood gas analysis at 30, 60, 90, 150, 210 and 270 s after ventilation onset.

After birth, which was defined as the point when both ventilation and cord clamping had occurred, AoT samples were collected at 0.5, 1, 2, 3, 5 and 10 min for gas analysis, with blood samples taken immediately before and during the initial 3 min after birth stored on ice before analysis. Anaesthesia in lambs after birth was continued with an I.V. infusion of ketamine (4 mg kg^{-1} h^{-1}) and midazolam (0.05 mg kg^{-1} h^{-1}).

Lambs received volume-targeted ventilation (SLE5000, SLE Ltd, Croydon, UK), with initial ventilator settings consisting of a positive end-expiratory pressure (PEEP) of 8 cm H_2O, a maximum positive inspiratory pressure of 50 cmH_2O, a respiratory rate of 60 inflations min^{-1}, an inspiratory time of 0.4 s, a tidal volume of 7 ml kg^{-1} estimated body weight and an inspired fractional O_2 concentration of 0.3. After birth, ventilation was adjusted to increase preductal S_{pO_2}, measured with a pulse oximetry sensor on the left forelimb, to 85–95% by the 10 min time point (Wyckoff et al., 2015).

The recording of data that commenced with delivery of the fetus was continued for ∼10 min after birth. After completion of this recording, lambs were carefully transferred onto a heated neonatal bed. Haemodynamic data were then recorded at 15 and 30 min after birth, with each recording preceded by withdrawal of an AoT sample for blood gas analysis. After the 5 min post-birth time point, ventilator settings were adjusted on the basis of blood gas results, with a target haemoglobin O_2 saturation (S_{aO_2}) of 90–95% and P_{aCO_2} of 45–50 mmHg.

Animals were humanely killed with an I.V. overdose of sodium pentobarbitone (100 mg kg^{-1}), administered to ewes after cord clamping and to lambs after completion of the study protocol. At post-mortem examination, no differences were present between lambs of the three study groups in body weight [average = 3.74 (0.49) kg, $P = 0.992$, one-way ANOVA], total lung weight [average = 122 (24) g, $P = 0.701$], left lung weight [average = 50 (10) g, $P = 0.768$], total-to-left lung weight

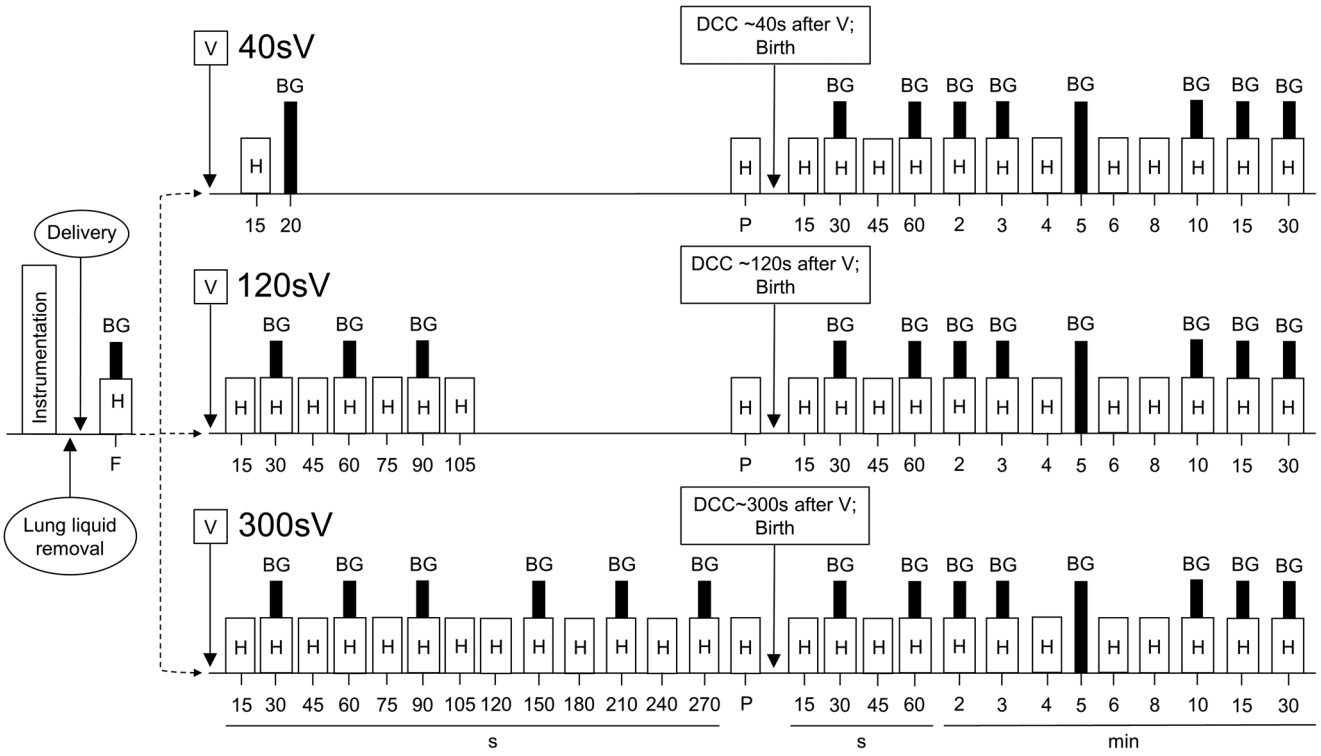

Figure 1. Schematic depiction of the experimental protocol
After fetal delivery, initial ventilation (V) was followed by delayed cord clamping (DCC) ∼40 s (40sV), ∼120 s (120sV) or ∼300 s (300sV) later. Abbreviations: BG, aortic trunk blood gas sample; H, haemodynamic measurement. Time points: F, fetus after lung liquid removal and delivery; P, immediately prior to cord clamping.

ratio [average = 2.47 (0.12), $P = 0.393$] or the total lung-to-body weight ratio [average = 32.7 (4.5) g kg^{-1}, $P = 0.418$].

Physiological data

The AoT, PT and LA fluid-filled catheter pressures were measured with transducers calibrated against a water manometer before each study and referenced to atmospheric pressure at LA level. Signals from fluid-filled and micromanometer catheters and from 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).

For detailed characterization of perinatal changes in central blood flow patterns, data epochs were extracted in subfiles from the main birth transition file at frequent time points. These comprised a 10–15 s epoch immediately before ventilation in all groups, followed by 5–7 s epochs at: (i) 15 s after onset of ventilation in the 40sV group; (ii) 15 s intervals after onset of ventilation to 105 s in the 120sV group; and (iii) 15 s intervals after onset of ventilation to 120 s and then 10–15 s epochs at 30 s intervals to 270 s in the 300sV group. In all groups, data were then obtained in: (i) 5–7 s epochs immediately before and at 15 s intervals in the first minute after birth; and (ii) 10–20 s epochs at 2, 3, 4, 6, 8, 10, 15 and 30 min after birth.

During data analysis, 50 Hz mains electrical interference from signals was removed with a 48 Hz low-pass filter, with measurements performed on ensemble-averaged signals typically generated from >10 beats in 5–7 s subfiles and >20 beats in 10–20 s subfiles. Mean AoT and PT micromanometer pressures were calibrated to the corresponding mean fluid-filled catheter pressures.

Right ventricular 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, 2020). On the basis that coronary blood flow accounts for ∼3% of the combined ventricular output in the perinatal period when ventilation precedes occlusion of the umbilical cord (Rudolph, 2009b), LV output was estimated as AoT flow + [0.03 × (AoT flow + RV output)], where AoT flow equalled the sum of the BCT and AI flows, noting that the BCT constitutes the only major cephalic branch of the AoT in sheep (Smolich et al., 2021). The relative distribution of LV output to the upper body region was assessed from the BCT flow-to-LV output ratio. Net FO shunt flow was estimated as LV output minus total PA blood flow (Anderson et al., 1981; Prsa et al., 2014; Smolich & Mynard, 2021a) and was positive if net FO shunting was R→L and negative if shunting was L→R (Anderson et al., 1985). Blood flow in the proximal descending thoracic aorta (DTA) was derived as the instantaneous sum of the AI and ductal flows (Smolich & Kenna, 2022; Smolich et al., 2016), and total systemic arterial blood flow was computed as the sum of the BCT and DTA flows. Pulmonary vascular conductance (PVC), the reciprocal of pulmonary vascular resistance, was calculated as (total PA flow)/(mean PT pressure − mean LA pressure).

To obtain total phasic L→R ductal shunt flow during and after the birth transition, the raw measured flows of all L→R (i.e. negative) segments in the ensemble-averaged ductal flow profile were multiplied by the quotient of segment duration and heart period, then summed (Smolich et al., 2016, 2020). The difference between total L→R ductal flow and mean ductal flow yielded total R→L ductal flow, and subtraction of L→R ductal flow from PA flow provided the contribution of RV output to PA flow (Smolich et al., 2020). The portion of this RV component arising from L→R FO flow was then equivalent to the product of total L→R FO flow and the fractional distribution of RV output to the lungs. The sum of L→R FO flow passing to the lungs and total phasic L→R ductal flow thus represented the overall contribution of L→R shunting to PA flow.

AoT O_2 content was computed as $(1.36 \times S_{aO_2} \times \text{Hb}/100) + 0.003 \times P_{aO_2}$, where Hb is the haemoglobin concentration (in grams per decilitre). Upper body O_2 delivery was calculated as the product of AoT O_2 content and BCT blood flow. Note that it was not possible to estimate lower body O_2 delivery in this study because: (i) umbilical arterial blood flow was not measured before cord clamping; and (ii) blood samples were not obtained from the post-ductal aorta before and after ventilation or cord clamping.

Statistical analysis

Results were analysed using GraphPad Prism v.9 (GraphPad Software, La Jolla, CA, USA), preceded by logarithmic transformation of data with a non-normal distribution. Given that the experimental protocol up to and including 15 s after the start of initial ventilation was identical in all animals, data from the three groups at these time points were combined and analysed with one-way repeated-measures (RM) ANOVA. Longitudinal data within individual study groups were also analysed using one-way RM ANOVA, and specific contrasts were evaluated by partitioning the within-animal sums of squares into individual degrees of freedom, with a Bonferroni correction applied as required for multiple comparisons. Haemodynamic and blood flow data immediately prior to and at 15 s after birth in the three groups were compared with two-way RM ANOVA.

Haemodynamic and blood flow data between the 40sV and 120sV groups were compared with either Student's unpaired *t* test before birth or two-way RM ANOVA after birth. If not statistically different, these data were then combined and compared with the 300sV group via Student's unpaired *t* test or two-way RM ANOVA with Fisher's *post hoc* least significant difference tests. The relationships between total L→R shunt flow passing to the lungs and either PVC or total systemic arterial blood flow were evaluated with least squares linear regression analysis. Data are expressed as means (SD), and significance was taken at $P < 0.05$.

Results

Blood gas variables

With initial ventilation, AoT pH, S_{aO_2}, P_{aO_2} and O_2 content rose in all groups, whereas P_{aCO_2} fell ($P \leq 0.033$; Table 1). Arterial oxygenation increased further after DCC, while pH decreased and P_{aCO_2} rose, with relatively minor differences between groups (Table 2).

Haemodynamics

Mean AoT and PT pressures decreased by 5 (2) mmHg after 15 s of ventilation ($P < 0.001$), then increased by 6 (2) mmHg following DCC ($P < 0.001$), with no subsequent differences between groups ($P \geq 0.807$; Fig. 2*A* and *B*). Mean LA pressure rose by 0.6 (0.5) mmHg with the onset of ventilation ($P < 0.001$), then increased further by 0.6 (0.5) and 1.6 (1.2) mmHg in the 120sV and 300sV groups, respectively ($P \leq 0.006$). Left atrial pressure was initially unaffected by DCC, but subsequently rose to peak at 8–10 min after birth ($P < 0.001$), with no overall difference between groups ($P = 0.710$; Fig. 2*C*). Heart rate fell by 7 (11) beats min^{-1} with the onset of ventilation ($P = 0.002$) and was unchanged by DCC, with no difference between groups after birth ($P = 0.625$; Fig. 2*D*).

Ventricular outputs

After rising by 18 (37) ml min^{-1} with the onset of ventilation ($P = 0.008$), LV output was unchanged during ongoing ventilation and initially after DCC, but then increased steadily ($P < 0.001$) to peak at 15 min after birth, with no overall difference between groups ($P = 0.911$; Fig. 3*A*). Right ventricular output was unaltered with the onset of ventilation and relatively stable with ongoing ventilation. However, RV output fell abruptly by 134 (87) ml min^{-1} after DCC ($P < 0.001$) and was then stable after birth ($P = 0.397$), with no difference between groups ($P = 0.724$; Fig. 3*B*).

Systemic arterial perfusion

Brachiocephalic trunk blood flow decreased by 39 (19) ml min^{-1} with the onset of ventilation ($P < 0.001$) and was then unchanged in the 40sV group, but fell by another 30 (31) ml min^{-1} in the 120sV group ($P = 0.009$) and by 52 (38) ml min^{-1} in the 300sV group ($P < 0.001$). Brachiocephalic trunk blood flow rose by 29 (20) ml min^{-1} after DCC ($P < 0.001$), with no difference in increments between groups ($P = 0.272$), but tended to be lower in the 300sV group at 15 s ($P = 0.054$) and 30 s after birth ($P = 0.063$). Brachiocephalic trunk blood flow then decreased progressively over 15 min ($P \leq 0.001$), with no overall difference between groups ($P = 0.371$; Fig. 4*A*). Perinatal changes in the BCT flow-to-LV output ratio were directionally similar, with a lower ratio in the 300sV group before DCC ($P = 0.028$) and for 2 min after birth ($P \leq 0.027$; Fig. 4*B*).

Total systemic arterial blood flow fell by 168 (72) ml min^{-1} with the onset of ventilation ($P < 0.001$) and was then unchanged in the 40sV group, but declined by a further 81 (62) ml min^{-1} in the 120sV group and 245 (140) ml min^{-1} in the 300sV group (both $P < 0.001$). Total systemic arterial flow fell substantially with DCC [average fall = 249 (88) ml min^{-1}, $P < 0.001$] and was lower in the 300sV group for the first 4 min after birth ($P \leq 0.039$; Fig. 4*C*). This perinatal pattern closely resembled that of DTA flow (Fig. 4*D*).

Upper body O_2 delivery

Upper body O_2 delivery rose by 2.7 (3.4) ml O_2 min^{-1} 20–30 s after the onset of ventilation ($P < 0.001$) and increased further at 60 s in the 120sV and 300sV groups ($P < 0.001$), before falling linearly in the 300sV group ($P < 0.001$) to a level similar to that of fetuses after delivery ($P = 0.667$). Upper body O_2 delivery decreased after birth ($P < 0.001$), with the level tending to be lower at 30 s in the 300sV group [19.0 (5.0) *vs.* 23.5 (9.2) ml O_2 min^{-1}, $P = 0.104$] and the subsequent decline more gradual than in the 40sV and 120sV groups (P for time × group interaction = 0.015; Fig. 5).

Pulmonary perfusion and vascular conductance

Pulmonary arterial blood flow tripled to 307 (109) ml min^{-1} by 15 s after the onset of ventilation ($P < 0.001$), then declined in the 40sV group ($P = 0.005$), but subsequently recovered to the initial post-ventilation value in the 120sV group ($P < 0.001$) and rose by 177 (151) ml min^{-1} in the 300sV group ($P < 0.001$). Pulmonary arterial flow increased with DCC ($P < 0.001$), with increments similar between groups [average = 107 (51) ml min^{-1}, $P = 0.806$], but was higher in the 300sV group in the first 4 min after birth ($P \leq 0.023$) and rose in all groups

Table 1. Aortic trunk blood gas variables during initial ventilation for ∼40 s (40sV), ∼120 s (120sV) or ∼300 s (300sV) before delayed cord clamping

Variable	Group	Post-delivery	20 s ventilation	30 s ventilation	60 s ventilation	90 s ventilation	150 s ventilation	210 s ventilation	270 s ventilation	*P*-value
Hb (g dl^{-1})	40sV	11.7 (0.8)	11.6 (0.8)							
	120sV	11.6 (0.9)		11.6 (0.9)	11.6 (1.1)	11.4 (0.9)				0.232
	300sV	11.3 (0.8)		11.3 (0.7)	11.2 (0.8)	11.2 (0.7)	11.2 (0.7)	11.1 (0.6)	11.2 (0.8)	0.663
pH	40sV	7.31 (0.03)[b]	7.32 (0.02)							
	120sV	7.29 (0.03)[a]		7.30 (0.03)	7.31 (0.03)	7.32 (0.03)				0.002
	300sV	7.31 (0.03)[b]		7.32 (0.03)	7.33 (0.04)	7.33 (0.03)	7.34 (0.03)	7.35 (0.03)	7.36 (0.03)	<0.001
S_{aO_2} (%)	40sV	59.3 (8.0)[c]	78.7 (7.3)							
	120sV	53.3 (6.9)[c]		74.5 (9.8)	79.9 (7.6)	83.8 (7.1)				<0.001
	300sV	50.9 (9.4)[c]		71.5 (13.5)	76.5 (10.4)	81.3 (9.8)	85.7 (8.7)	87.2 (8.4)	88.5 (7.4)	<0.001
P_{aO_2} (mmHg)	40sV	23.5 (2.1)[b]	32.7 (7.7)							
	120sV	21.3 (2.9)[c]		30.5 (8.5)	33.1 (7.3)	36.8 (10.3)				<0.001
	300sV	20.5 (2.0)[c]		28.5 (5.6)	31.0 (5.5)	34.5 (6.8)	39.3 (10.4)	43.3 (16.7)	47.4 (25.3)	0.021
P_{aCO_2} (mmHg)	40sV	48.3 (3.4)[c]	43.7 (2.1)							
	120sV	51.1 (2.4)[c]		46.7 (2.7)	44.5 (3.0)	42.6 (3.0)				<0.001
	300sV	51.3 (2.2)[c]		46.5 (3.7)	43.8 (3.9)	43.4 (4.1)	42.5 (3.4)	41.4 (3.6)	41.0 (3.3)	<0.001
O_2 content (mM)	40sV	4.22 (0.57)[c]	5.56 (0.69)							
	120sV	3.79 (0.63)[c]		5.30 (0.89)	5.69 (0.84)	5.86 (0.68)				<0.001
	300sV	3.53 (0.33)[c]		4.96 (1.08)	5.27 (0.90)	5.57 (0.87)	5.90 (0.84)	5.97 (0.72)	6.11 (0.74)	<0.001

Data are expressed as means (SD); n = 11 for the 40sV and 120sV groups and n = 12 for the 300sV group. Abbreviations: Hb, haemoglobin concentration; P_{aCO_2}, arterial CO_2 tension; P_{aO_2}, arterial O_2 tension; S_{aO_2}, haemoglobin O_2 saturation. [a]$P = 0.033$, [b]$P \leq 0.003$, [c]$P < 0.001$, post-delivery *versus* 20 or 30 s of ventilation, one-way repeated-measures ANOVA. The *P*-value in the right-hand column refers to one-way repeated-measures ANOVA in the interval between the 30 s and 90 s time points in the 120sV group or the 30 s and 270 s time points in the 300sV group.

Table 2. Aortic trunk blood gas variables after birth following initial ventilation for ∼40 s (40sV), ∼120 s (120sV) or ∼300 s (300sV) before delayed cord clamping

Variable	Group	0.5 min	1 min	2 min	3 min	5 min	10 min	15 min	30 min	*P*-value
Hb (g dl^{-1})	40sV	11.5 (1.0)	11.4 (0.9)	11.4 (0.8)	11.3 (1.0)	11.2 (0.9)	11.2 (0.9)	11.4 (1.0)	11.4 (1.1)	0.314
	120sV	11.4 (0.9)	11.2 (1.1)	11.0 (1.1)	11.0 (1.1)	11.2 (1.1)	11.2 (1.1)	11.4 (0.9)	11.4 (0.9)	0.016
	300sV	11.3 (0.8)	11.1 (0.8)	10.9 (0.7)	10.8 (0.4)	10.9 (0.6)	11.3 (0.6)	11.5 (0.7)	11.7 (0.7)	<0.001
pH	40sV	7.32 (0.02)	7.31 (0.03)	7.32 (0.03)	7.32 (0.03)	7.32 (0.04)	7.32 (0.05)	7.29 (0.04)	7.26 (0.04)	<0.001
	120sV	7.32 (0.02)	7.32 (0.02)	7.30 (0.02)	7.29 (0.03)	7.27 (0.04)	7.26 (0.04)	7.26 (0.04)	7.28 (0.02)	<0.001
	300sV	7.35 (0.03)	7.35 (0.03)	7.34 (0.03)	7.33 (0.03)	7.32 (0.04)	7.30 (0.04)	7.30 (0.04)	7.29 (0.04)	<0.001
S_{aO_2} (%)	40sV	82.7 (12.4)	83.1 (14.0)	85.3 (12.7)	88.4 (9.4)	91.2 (5.7)	94.0 (4.7)	92.8 (5.7)	92.8 (2.7)	0.037
	120sV	82.6 (11.6)	80.4 (13.8)	81.6 (15.6)	82.9 (16.0)	87.6 (9.3)	92.1 (3.0)	94.8 (1.8)	95.1 (3.0)	0.011
	300sV	85.7 (11.0)	84.9 (12.0)	85.9 (11.6)	87.3 (10.4)	89.2 (6.3)	92.2 (4.3)	94.0 (5.7)	90.4 (5.3)	0.019
P_{aO_2} (mmHg)	40sV	40.0 (16.9)	41.7 (17.9)	43.5 (16.3)	49.0 (21.5)	49.1 (15.4)	56.4 (16.3)	52.0 (10.2)	49.7 (9.0)	0.118
	120sV	43.7 (24.6)	44.4 (32.0)	49.1 (37.3)	49.3 (34.0)	46.1 (19.8)	47.7 (7.4)	55.1 (10.1)	60.6 (23.8)	0.386
	300sV	53.4 (41.0)	55.8 (45.9)	54.6 (39.0)	58.5 (49.1)	47.4 (24.0)	49.4 (10.8)	60.3 (21.0)	45.9 (9.6)	0.411
P_{aCO_2} (mmHg)	40sV	42.5 (3.9)	42.1 (3.7)	42.0 (3.0)	42.7 (3.1)	41.3 (4.2)	43.0 (5.0)	47.3 (4.9)	51.9 (3.8)	<0.001
	120sV	43.1 (3.8)	43.8 (3.2)	45.5 (4.3)	47.4 (4.3)	49.1 (5.0)	50.9 (7.1)	52.4 (6.0)	48.8 (2.5)	<0.001
	300sV	42.6 (3.9)	42.7 (4.0)	42.8 (3.7)	44.2 (4.2)	43.4 (5.3)	47.5 (4.7)	49.2 (4.8)	52.7 (6.2)	<0.001
O_2 content (mM)	40sV	5.82 (1.09)	5.81 (1.12)	5.96 (1.06)	6.14 (0.93)	6.25 (0.67)	6.50 (0.68)	6.47 (0.75)	6.46 (0.73)	0.071
	120sV	5.76 (0.95)	5.51 (1.12)	5.50 (1.20)	5.59 (1.14)	5.98 (0.66)	6.31 (0.56)	6.61 (0.56)	6.68 (0.51)	0.014
	300sV	5.99 (0.98)	5.85 (1.12)	5.76 (0.99)	5.79 (0.81)	5.96 (0.60)	6.37 (0.53)	6.63 (0.66)	6.47 (0.52)	0.005

Data are expressed as means (SD); $n = 11$ for 40sV and 120sV groups and $n = 12$ for 300sV group. Abbreviations are as in Table 1. The *P*-value in the right-hand column refers to one-way repeated-measures ANOVA across the interval between 0.5 and 30 min.

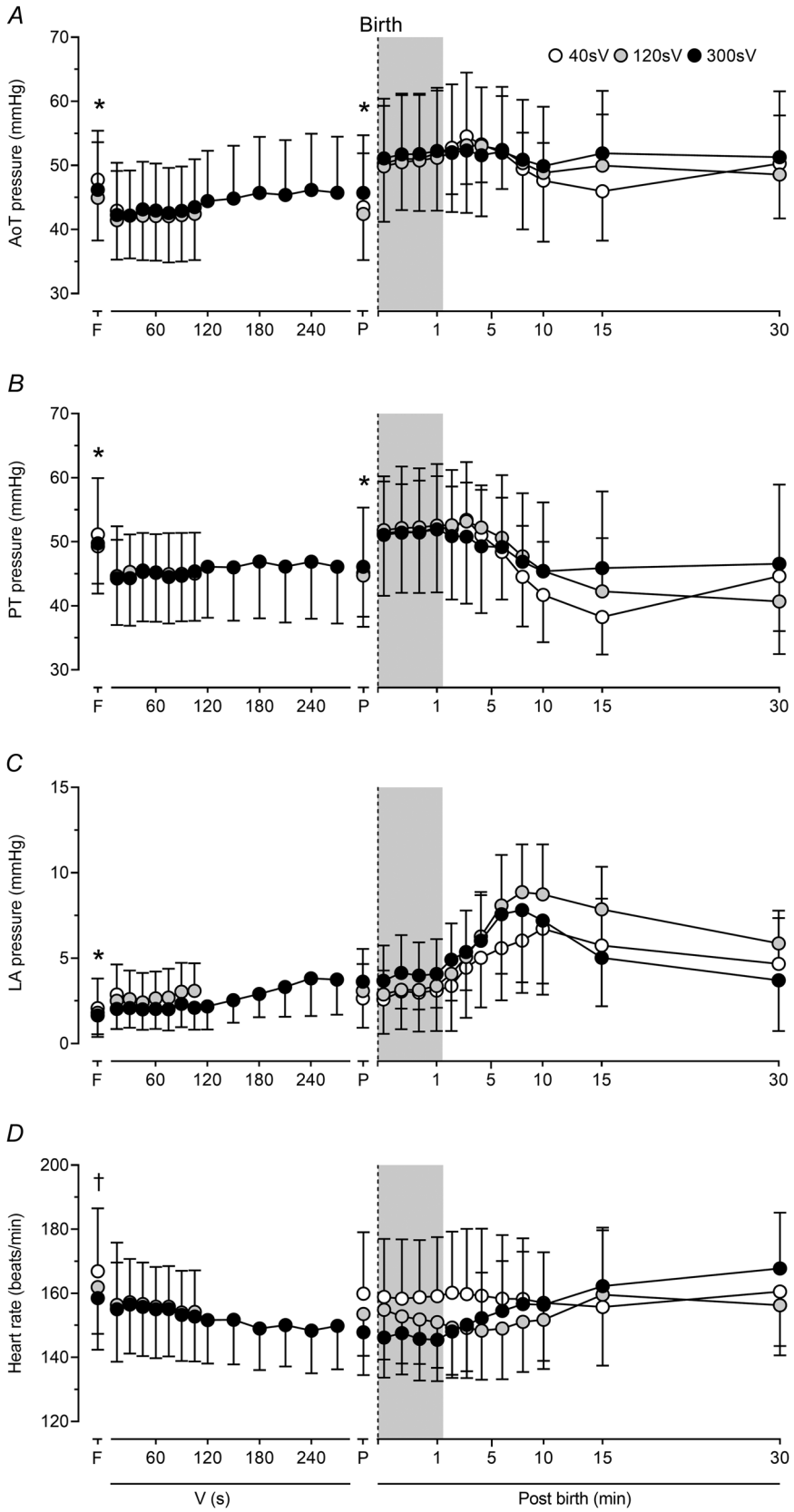

Figure 2. Mean aortic trunk (*A*), pulmonary trunk (*B*) and left atrial blood pressure (*C*) and heart rate (*D*)
Abbreviations: AoT, aortic trunk; LA, left atrial; PT, pulmonary trunk. Time points correspond to the fetus after delivery (F), after initial ventilation (V) for ∼40 s (40sV), ∼120 s (120sV) and ∼300 s (300sV) before delayed cord clamping at birth, and in the same groups immediately prior to birth (P) and over the initial 30 min after birth. Note that the time scale of the first minute after cord clamping (shaded area) is magnified for ease of visualization. Results are expressed as means (SD); n = 11 in the 40sV and 120sV groups and n = 12 in the 300sV group, with only one limb of the bidirectional SD displayed to aid visualization. $^{\dagger}P = 0.002$ and $^{*}P < 0.001$, compared with subsequent time point.

to peak at 10–15 min ($P < 0.001$; Fig. 6*A*). Pulmonary vascular conductance displayed a similar pattern of perinatal changes (Fig. 6*B*).

Ductal shunting

The R→L ductal flow fell by 175 (70) ml min^{-1} with ventilation ($P < 0.001$) and was then unaltered in the 40sV group, but it declined by an additional 67 (38) ml min^{-1} in the 120sV group and 217 (117) ml min^{-1} in the 300sV group (both $P < 0.001$). The R→L ductal flow dropped by a further 150 (68) ml min^{-1} after DCC ($P < 0.001$), but was lower in the 300sV group for 3 min after birth ($P \leq 0.017$) and reached near-zero in all groups by 10 min (Fig. 7*A*).

The magnitude of L→R ductal flow increased by 12 (16) ml min^{-1} with the onset of ventilation ($P < 0.001$) and was then unchanged in the 40sV and 120sV groups, but rose to 55 (22) ml min^{-1} in the 300sV group ($P < 0.001$). This shunting increased further after DCC ($P < 0.001$), with the increment in the 300sV group [118 (25) ml min^{-1}] exceeding that of the 40sV and 120sV groups [average = 76 (52) ml min^{-1}, $P = 0.003$]. As a result, L→R ductal shunting was greater in the 300sV group for the first 4 min after birth ($P \leq 0.003$) but peaked at 10–15 min in all groups ($P < 0.001$; Fig. 7*B*).

Foramen ovale shunting

Foramen ovale shunting was uniformly R→L in fetuses [average = 244 (102) ml min^{-1}], but after 15 s of ventilation it fell to 56 (97) ml min^{-1} ($P < 0.001$), where it remained in the 40sV and 120sV groups. However, in the 300sV group, net FO shunt flow became L→R at ≥270 s ($P \leq 0.031$). The L→R FO shunting increased by 114 (46) ml min^{-1} with DCC ($P < 0.001$), with similar increments between groups ($P = 0.299$), but was more pronounced in the 300sV group for 6 min ($P \leq 0.005$), and it rose progressively ($P < 0.001$) to peak at 10 min after birth in all groups (Fig. 8).

Perinatal sources of pulmonary perfusion

The proportion of RV output passing to the lungs rose from 14 (11) to 40 (11)% after 15 s of ventilation ($P < 0.001$), then decreased in the 40sV group ($P = 0.002$), but it returned to the initial post-ventilation value in the 120sV group ($P < 0.001$) and increased to 62 (14)% in the 300sV group ($P < 0.001$). This component rose by 15 (6)% with DCC ($P < 0.001$) and was higher in the 300sV group in the first 3 min after birth ($P \leq 0.026$; Fig. 9*A*).

The contribution of L→R ductal shunting to PA flow increased from 1 (4) to 4 (5)% after 15 s of ventilation ($P < 0.001$) and was then unaltered in the 40sV group,

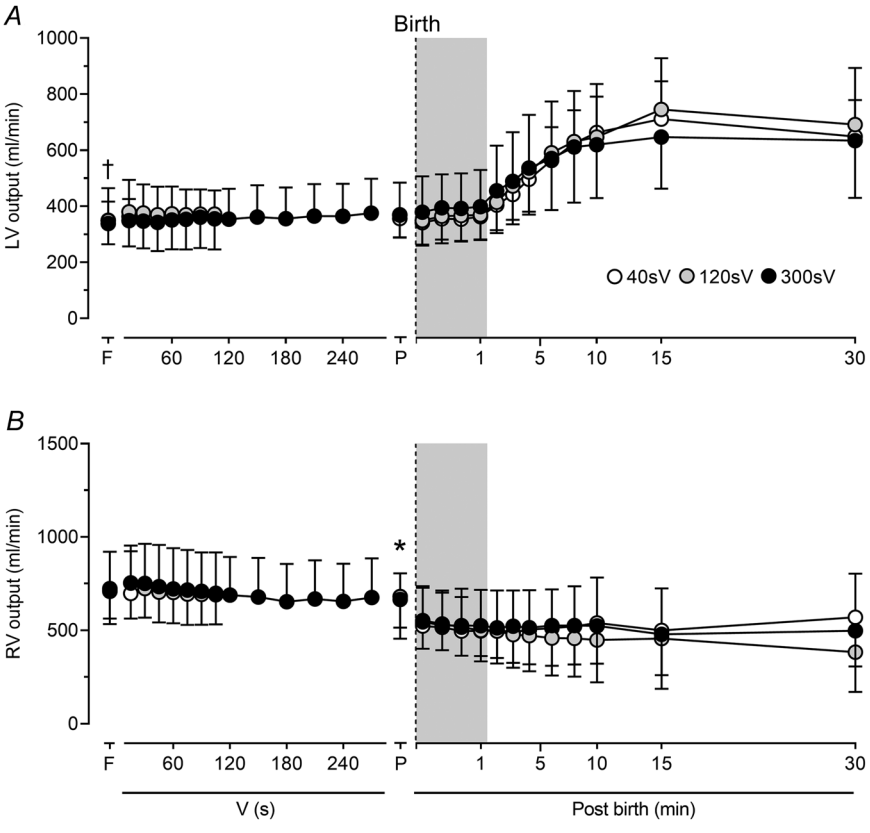

Figure 3. Left ventricular (*A*) and right ventricular output (*B*)
Abbreviations: LV, left ventricular; RV, right ventricular. Results are displayed using the same format and time points defined in Fig. 2 and expressed as means (SD); $n = 11$ in the 40sV and 120sV groups and $n = 12$ in the 300sV group, with only one limb of the bidirectional SD displayed to aid visualization. $^{\dagger}P = 0.008$ and $^{*}P < 0.001$, compared with subsequent time point.

but it rose to 7 (5)% in the 120sV group ($P = 0.003$) and 13 (7)% in the 300sV group ($P < 0.001$). This contribution rose further after DCC ($P < 0.001$), with increments not different between groups [average = 18 (8)%, $P = 0.824$], and was greater in the 300sV group for 2 min ($P \leq 0.012$), before increasing ($P < 0.001$) to peak by 15 min in all groups [average = 55 (14)%; Fig. 9*B*].

The L→R FO shunt component of PA flow, which resided within RV output, increased from 0 (0) to 2 (4)% after 15 s of ventilation ($P = 0.019$) and was then unaltered in the 40sV and 120sV groups, but after 120 s it rose to 13

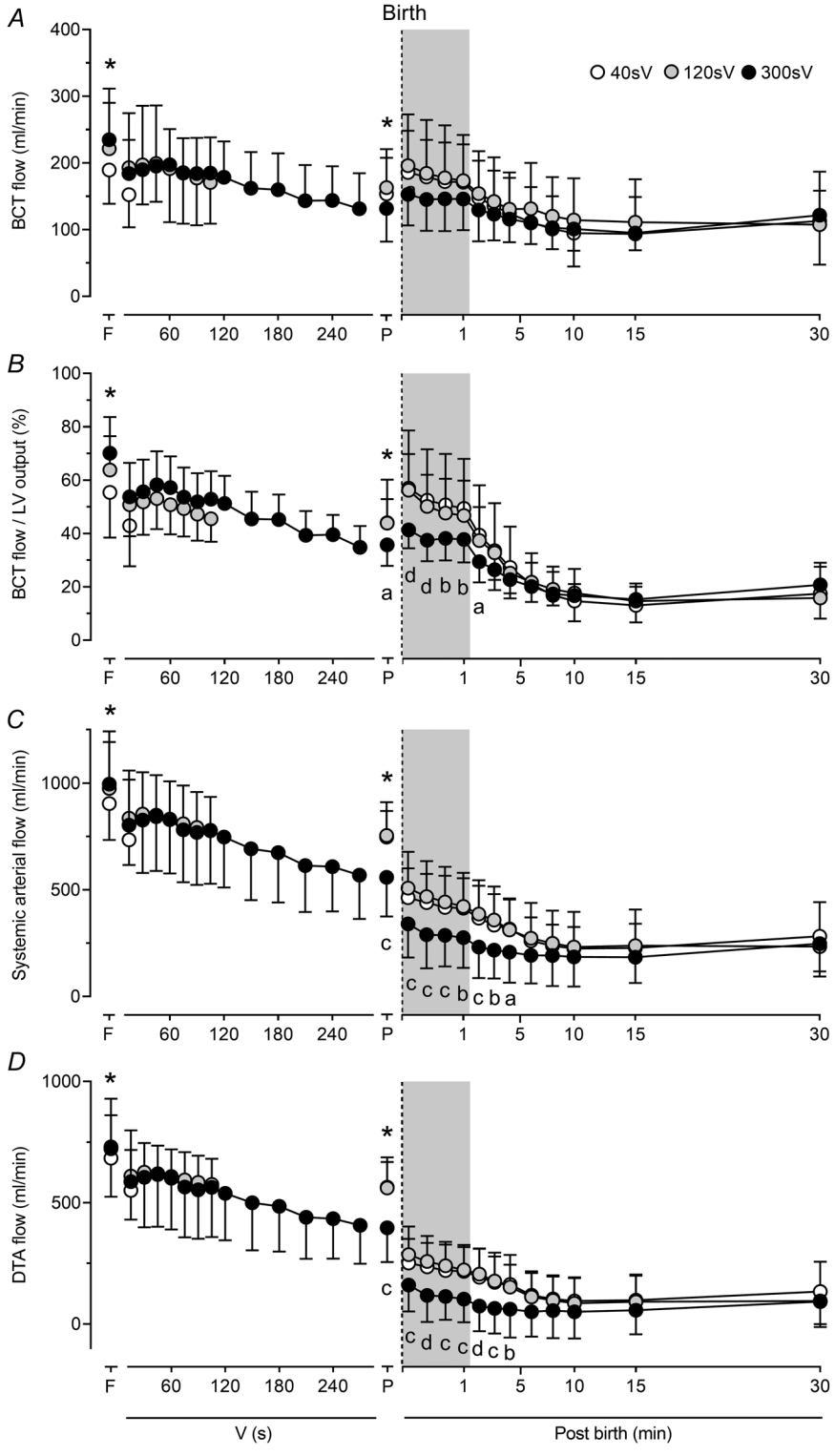

Figure 4. Blood flow in the brachiocephalic trunk (*A*), the brachiocephalic trunk flow-to-left ventricular output ratio (*B*), total systemic arterial blood flow (*C*) and blood flow in the descending thoracic aorta (*D*)
Abbreviations: BCT, brachiocephalic trunk; DTA, descending thoracic aorta; LV, left ventricular. Results are displayed using the same format and time points defined in Fig. 2 and expressed as means (SD); $n = 11$ in the 40sV and 120sV groups and $n = 12$ in the 300sV group, with only one limb of the bidirectional SD displayed to aid visualization. $^{a}P \leq 0.039$, $^{b}P \leq 0.012$, $^{c}P \leq 0.005$ and $^{d}P < 0.001$, 40sV and 120sV *vs.* 300sV; $^{*}P < 0.001$, compared with subsequent time point. Newborn data in the 40sV and 120sV groups of *A–D* were not statistically different on two-way repeated-measures ANOVA ($P \geq 0.707$).

(15)% in the 300sV group ($P < 0.001$). This contribution increased further after DCC ($P < 0.001$), with the increment in the 300sV group (13 (8)%) tending to be greater than in the 40sV and 120sV groups [average = 7 (8)%, $P = 0.068$], and was higher in the 300sV group in the first 4 min after birth ($P \leq 0.028$). This contribution rapidly plateaued at 31 (13)% between 0.5 and 10 min after birth in the 300sV group, but rose more gradually to a peak of 26 (12)% by the 10 min time point in the 40sV and 120sV groups (Fig. 9*C*).

The contribution of combined ductal and FO L→R shunting to PA blood flow in the 300sV group was substantially higher than in the 40sV and 120sV groups, both at the end of the period of initial ventilation [28 (15) *vs.* 8 (8)%, $P = 0.002$] and in the first 6 min after DCC ($P \leq 0.044$), but peaked at 79 (12)% of PA flow by 10–15 min after birth in all groups (Fig. 10*A*). Furthermore: (i) a positive relationship was evident between PVC and total L→R shunt flow passing to the lungs ($R^2 = 0.73$, $P < 0.001$; Fig. 10*B*); and (ii) a negative relationship was present between the natural logarithm of total L→R shunt flow passing to the lungs and total systemic arterial blood flow ($R^2 = 0.62$, $P < 0.001$; Fig. 10*C*).

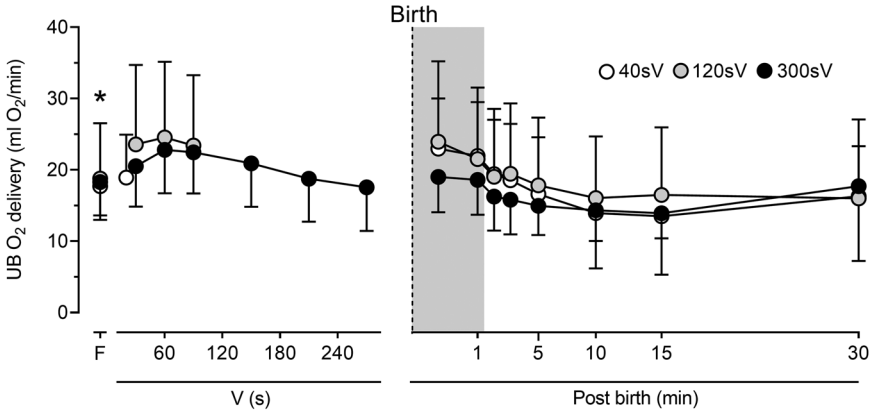

Figure 5. Upper body O_2 delivery
Abbreviation: UB, upper body. Results are displayed using the same format and time points defined in Fig. 2 and expressed as means (SD); $n = 11$ in the 40sV and 120sV groups and $n = 12$ in the 300sV group, with only one limb of the bidirectional SD displayed to aid visualization. $^*P < 0.001$, compared with subsequent time point. Newborn data in the 40sV and 120sV groups were not statistically different on two-way repeated-measures ANOVA ($P = 0.806$).

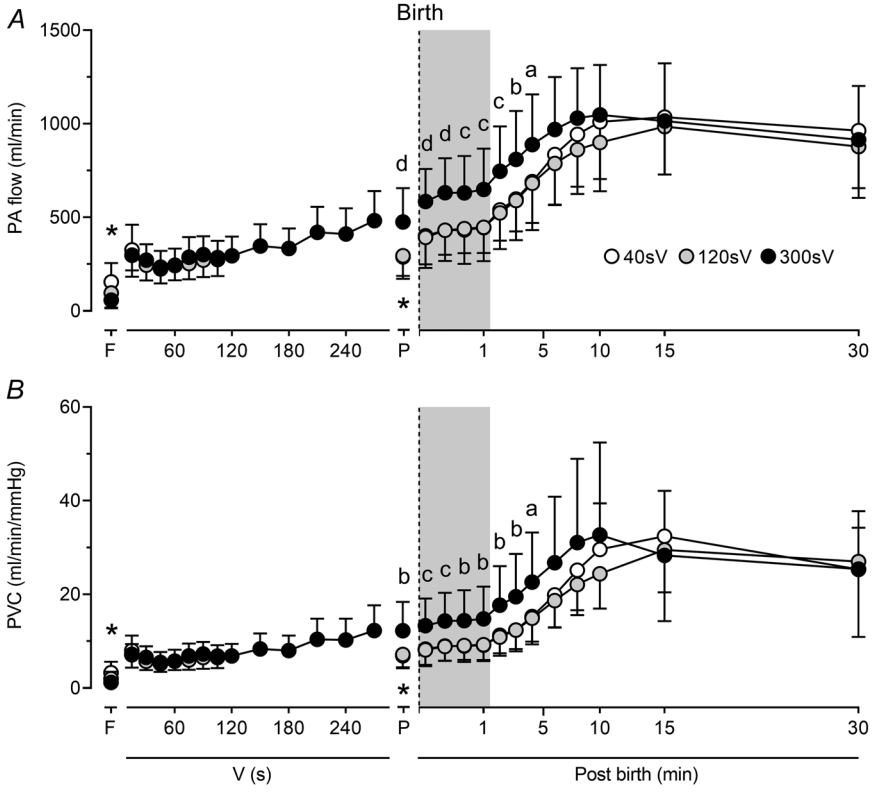

Figure 6. Mean pulmonary arterial blood flow (*A*) and pulmonary vascular conductance (*B*)
Abbreviations: PA, pulmonary arterial; PVC, pulmonary vascular conductance. Results are displayed using the same format and time points defined in Fig. 2 and expressed as means (SD); $n = 11$ in the 40sV and 120sV groups and $n = 12$ in the 300sV group, with only one limb of the bidirectional SD displayed to aid visualization. $^aP \leq 0.026$, $^bP \leq 0.016$, $^cP \leq 0.005$ and $^dP < 0.001$, 40sV and 120sV *vs.* 300sV; $^*P < 0.001$, compared with subsequent time point. Newborn data in the 40sV and 120sV groups of *A* and *B* were not statistically different on two-way repeated-measures ANOVA ($P \geq 0.663$).

Discussion

This study, which compared the effect of a brief (∼40 s), moderate (∼2 min) or extended (∼5 min) period of initial ventilation prior to DCC on central arterial blood flows during the birth transition of preterm lambs, has produced three main findings. First, central arterial blood flow patterns displayed relatively minor differences during brief and moderate periods of initial ventilation, with these patterns being very similar following birth after DCC. Second, in comparison to brief and moderate periods of ventilation, and with unchanged levels of LV and RV output, an extended period of initial ventilation was accompanied by a larger rise in PA blood flow, but a

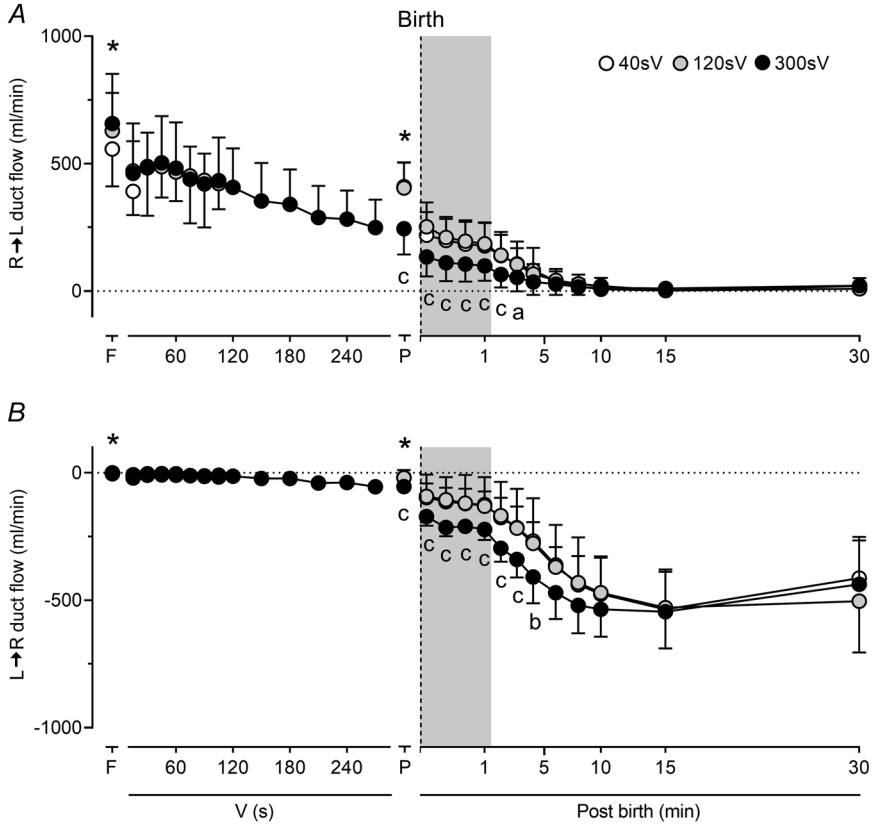

Figure 7. Right-to-left (*A*) and left-to-right (*B*) components of ductal shunt flow
Abbreviations: L→R, left to right; R→L, right to left. Results are displayed using the same format and time points defined in Fig. 2 and expressed as means (SD); $n = 11$ in the 40sV and 120sV groups and $n = 12$ in the 300sV group, with only one limb of the bidirectional SD displayed to aid visualization. $^{a}P = 0.017$, $^{b}P = 0.003$ and $^{c}P < 0.001$, 40sV and 120sV *vs.* 300sV; $^{*}P < 0.001$, compared with subsequent time point. Newborn data in the 40sV and 120sV groups of *A* and *B* were not statistically different on two-way repeated-measures ANOVA ($P \geq 0.613$).

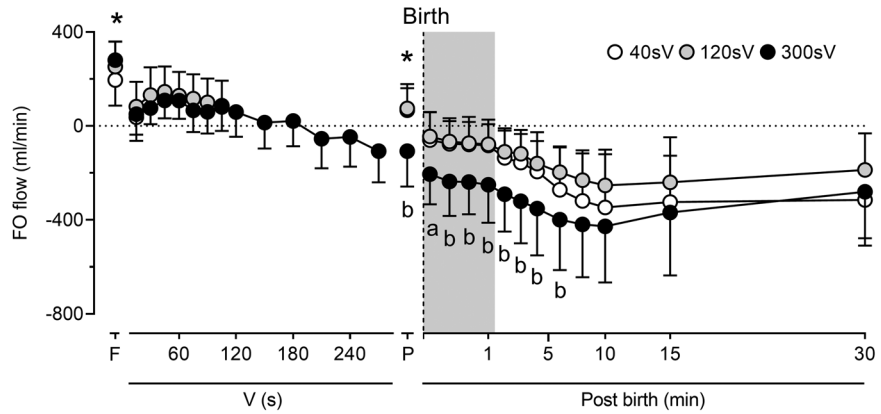

Figure 8. Net foramen ovale blood flow
Abbreviation: FO, foramen ovale. Results are displayed using the same format and time points defined in Fig. 2 and expressed as means (SD); $n = 11$ in the 40sV and 120sV groups and $n = 12$ in the 300sV group, with only one limb of the bidirectional SD displayed to aid visualization. $^{a}P = 0.009$ and $^{b}P \leq 0.005$, 40sV and 120sV *vs.* 300sV; $^{*}P < 0.001$, compared with subsequent time point. Newborn data in the 40sV and 120sV groups were not statistically different on two-way repeated-measures ANOVA ($P = 0.328$). Note that positive and negative FO flows represent net right-to-left and left-to-right FO shunting, respectively.

more pronounced fall in total systemic arterial blood flow, with these differences in flow being maintained for up to 4 min after birth. Third, this enhanced perinatal rise in PA flow was supported by a larger distribution of RV output to the lungs, in addition to an earlier onset of L→R shunting across both the ductus arteriosus and FO. These findings suggest that an extended period of ventilation prior to DCC does not confer a greater circulatory benefit than shorter periods of initial ventilation, because a larger perinatal increase in pulmonary blood flow evident with extended ventilation is not accompanied by a greater LV output, but arises mainly from augmented L→R shunting and occurs at a cost of decreased systemic arterial perfusion.

In our study, the pattern of changes in AoT blood gases, arterial blood pressures and heart rate, in addition to LV and RV outputs, showed comparatively minor differences between the three groups during initial ventilation prior to DCC and were very similar after birth (Figs 2 and 3 and Tables 1 and 2). This similarity of global parameters is in accord with the few available clinical reports, wherein little or no difference was present between PBCC (i.e. DCC preceded by an extended 4–5 min period of initial ventilation) and conventional DCC with 1 min of prior ventilation, with respect to changes in heart rate and peripheral arterial oxygenation (via pulse oximetry) in term infants (Schwaberger et al., 2022) or short-term neonatal outcomes in very preterm (<32 weeks gestation) infants (Knol et al., 2020).

In contrast, clearcut differences in PA blood flow patterns emerged with extension of the duration of initial ventilation prior to DCC. Thus, although an expected

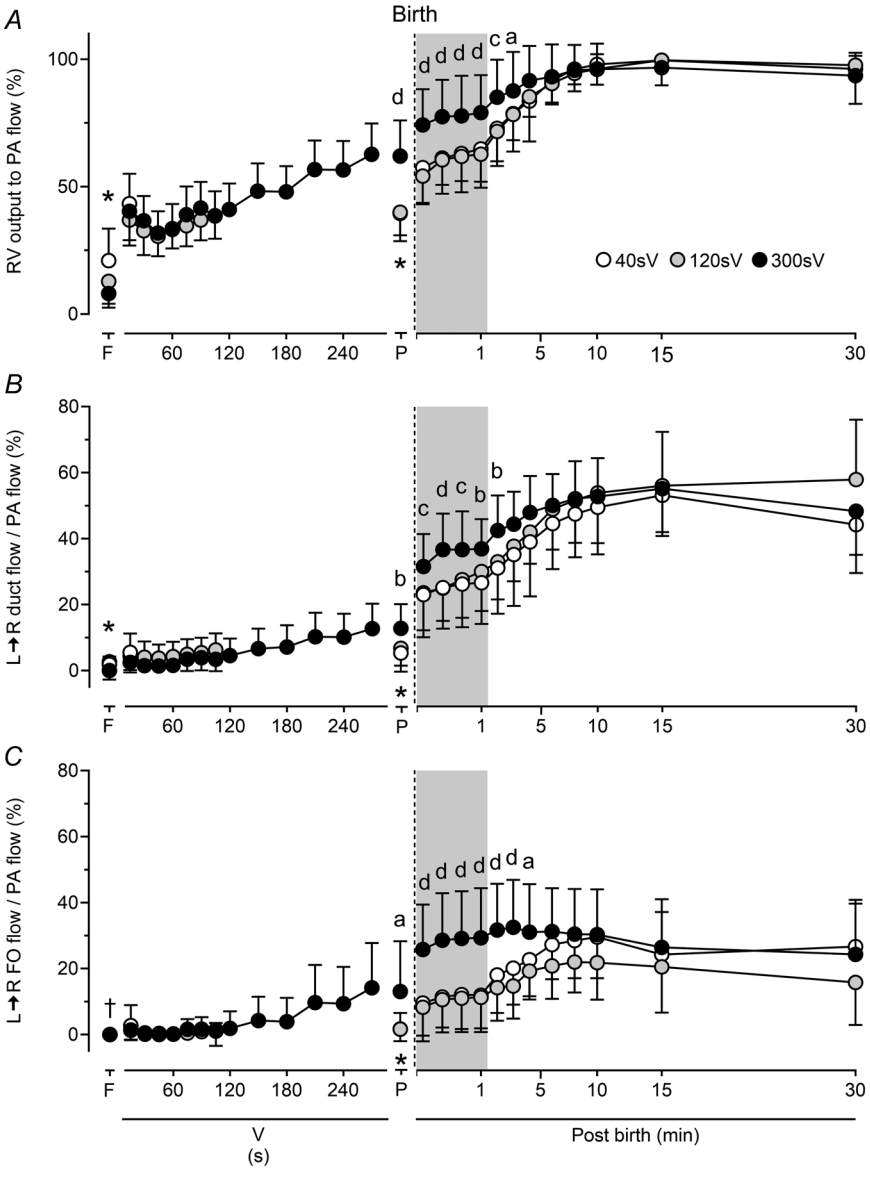

Figure 9. The percentage of right ventricular output passing to the lungs (*A*) and the percentage of pulmonary arterial blood flow arising from left-to-right shunting across the ductus (*B*) and foramen ovale (*C*)
Abbreviations: FO, foramen ovale; L→R, left to right; PA, pulmonary arterial; RV, right ventricular. Results are displayed using the same format and time points defined in Fig. 2 and expressed as means (SD); $n = 11$ in the 40sV and 120sV groups and $n = 12$ in the 300sV group, with only one limb of the bidirectional SD displayed to aid visualization. $^{a}P \leq 0.026$, $^{b}P \leq 0.013$, $^{c}P \leq 0.006$ and $^{d}P \leq 0.001$, 40sV and 120sV *vs.* 300sV; $^{\dagger}P \leq 0.019$ and $^{*}P < 0.001$, compared with subsequent time point. Newborn data in the 40sV and 120sV groups of *A*–*C* were not statistically different on two-way repeated-measures ANOVA ($P \geq 0.329$).

large increase in PA blood flow occurred with the onset of initial ventilation (Bhatt et al., 2013; Smolich & Kenna, 2022), this flow decreased as ventilation continued in the 40sV group, fell then recovered in the 120sV group, but in the 300sV group it rose progressively after 120 s of ventilation. Moreover, the rise was of such a degree that, although the increments in PA blood flow occurring with DCC were similar in all three groups, the level of PA flow in the 300sV group was higher than in the 40sV and 120sV groups for 4 min after birth (Fig. 6*A*). Moreover, these findings were mirrored in PVC (Fig. 6*B*), suggesting that higher PA flows in the 300sV group reflected greater pulmonary vasodilatation and/or vascular recruitment during the period of initial ventilation before DCC.

Our findings also suggested that a pronounced shift occurred in the mechanisms underpinning changes in PA flow during initial ventilation prior to DCC. Thus, a stepwise increase in PA flow occurring with the onset of ventilation was primarily related to a larger distribution of an unaltered RV output to the lungs, with alterations

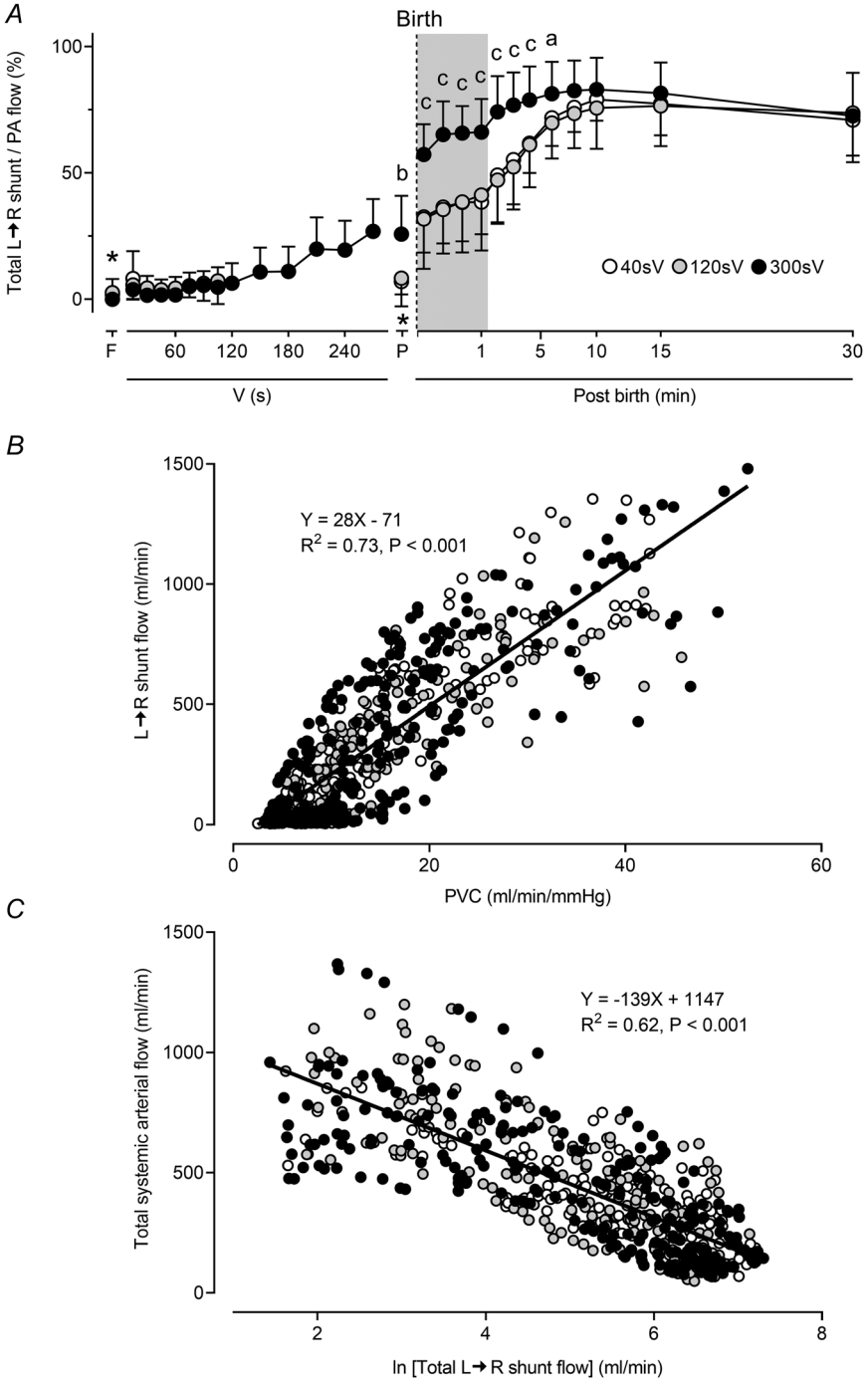

Figure 10. The percentage contribution of total left-to-right shunt flow to pulmonary arterial blood flow (*A*) and the relationships of this shunt flow to pulmonary vascular conductance (*B*) and to total systemic arterial blood flow (*C*)
Abbreviations: L→R, left to right; PA, pulmonary arterial; PVC, pulmonary vascular conductance. Results are displayed using the same format and time points defined in Fig. 2 and expressed as means (SD); $n = 11$ in the 40sV and 120sV groups and $n = 12$ in the 300sV group, with only one limb of the bidirectional SD displayed to aid visualization. $^{a}P = 0.026$, $^{b}P = 0.002$ and $^{c}P < 0.001$, 40sV and 120sV *vs.* 300sV; $^{*}P < 0.001$, compared with subsequent time point. Newborn data in the 40sV and 120sV groups of *A* were not statistically different on two-way repeated-measures ANOVA ($P = 0.909$). Relationships of total L→R shunt flow passing to the lungs with PVC (*B*) and total systemic arterial blood flow with the natural logarithm of total L→R shunt flow passing to the lungs (*C*) each contain 601 data points.

in PA flow up to the 120 s time point (i.e. in the 40sV and 120sV groups) mainly reflecting variations in this redistribution (Figs 6*A* and 9*A*). However, although a redistribution of RV output towards the lungs continued to rise during subsequent increments of PA flow in the 300sV group, it was increasingly supplemented by a progressively greater degree of L→R shunting via not only the ductus (Figs 7*B* and 9*B*), but also the FO (Figs 8 and 9*C*), with the combination of these shunts providing ∼25% of pulmonary perfusion immediately before DCC (Fig. 10*A*). Moreover, this augmented contribution of L→R ductal and FO shunting to pulmonary perfusion in the 300sV group continued into the initial 2–6 min after birth (Figs 7*B*, 8 and 9*B* and *C*). Note that: (i) a marked and rapid rise in PVC (Fig. 6*B*) appeared to be a key driver underpinning the major contribution of L→R shunting to perinatal increases in PA blood flow (Fig. 10*B*); and (ii) an augmented contribution of L→R FO shunting to PA flow was related to the combination of a rise in L→R FO shunting *per se* (Fig. 8) and a greater distribution of RV output (which contained the L→R FO shunt flow) to the lungs (Fig. 9*A*).

Emergence of substantial phasic L→R ductal shunting within minutes after birth is a characteristic feature of the birth transition (Crossley et al., 2009; Smolich & Kenna, 2022; Smolich et al., 2016, 2020). This rapid reversal of ductal shunt flow initially originates almost entirely via an abrupt onset of retrograde diastolic discharge from a descending aortic reservoir that is initiated by umbilical cord clamping (Smolich & Mynard, 2021b; Smolich et al., 2016). However, given that the umbilical cord is still patent during ventilation prior to DCC, this descending aortic reservoir then continues to discharge in an antegrade direction, thereby perfusing lower fetal body tissues and the placenta (Smolich & Mynard, 2021b). Thus, although L→R ductal shunting increased progressively during an extended period of ventilation before DCC, such shunting was relatively minor (Fig. 7*B*), made a lesser contribution to PA blood flow than after cord clamping (Fig. 9*B*) and was largely related to a redistribution of LV output from the upper to lower body region (Fig. 4*B*). However, as expected, the magnitude of L→R ductal shunting (Fig. 7*B*) and its contribution to PA blood flow (Fig. 9*B*) increased stepwise in all groups after DCC. As with immediate/early cord clamping and subsequent ventilation (Crossley et al., 2009; Smolich et al., 2020), phasic L→R ductal shunting became substantial in the early neonatal period following initial ventilation and DCC, rising to a peak of ∼55% of PA blood flow by 15 min after birth in all groups (Fig. 9*B*).

In accord with prior observations (Anderson et al., 1985; Rudolph, 2009a; Teitel et al., 1987; Smolich & Mynard, 2021), FO shunting was R→L in fetal lambs. However, net R→L FO shunting fell abruptly with the onset of initial ventilation (Fig. 8), which was consistent with the findings that *in utero* ventilation with oxygenated gas reduced R→L FO shunting to near-zero in fetal lambs (Teitel et al., 1987) and that ventilation of fetal lambs prior to DCC substantially decreased umbilical arterial and venous flows (Blank et al., 2018), given that umbilical venous return is the major source of R→L FO shunt flow (Rudolph, 2009a).

Although net L→R FO shunting emerged in all groups during the birth transition, this emergence occurred after DCC in the 40sV and 120sV groups, but before DCC in the 300sV group (Figs 8 and 9*C*). Furthermore, this difference extended into the neonatal period, with a higher level of L→R FO shunting in the 300sV group for 6 min after birth (Figs 8), in conjunction with a greater contribution of L→R FO shunting to PA blood flow for 4 min (Fig. 9*C*). However, in all three groups, the contribution of L→R FO shunting to PA blood flow peaked at ∼30% by 10 min after birth (Fig. 9*C*), compared with a peak contribution of ∼55% by phasic L→R ductal shunting at 15 min (Fig. 9*B*). Our findings are thus in accord with the conclusion of clinical studies that L→R ductal shunting exceeds L→R FO shunting in the newborn period (Evans & Iyer, 1994a, b).

Somewhat surprisingly, no previous experimental study has specifically assessed changes in FO shunting patterns during the birth transition, with this omission likely to be related to two main factors. First, a longstanding view of prominent developmental physiologists has been that a rapid functional closure of the flap-like valve of the FO occurs at birth, secondary to an increase in LA blood pressure that accompanies a surge in pulmonary venous return (Dawes, 1968; Rudolph, 2009b; Teitel et al., 1987). However, incompetence of the FO valvular apparatus is a recognized phenomenon in the newborn period (Evans & Iyer, 1994b; Markhorst et al., 1995), with the presence of L→R FO shunting demonstrable via Doppler echocardiography within an hour of birth in most normal human neonates (Baik et al., 2016; Hiraishi et al., 1991; Noori et al., 2012). Furthermore, complete closure of the FO in humans after birth generally takes 3–4 months (Ozcelik et al., 2006), and incomplete closure is relatively common, with the incidence of a patent FO in adult humans being 26–27% in echocardiographic or post-mortem studies (Hansen & Oxhoj, 1997; Stendel et al., 2000). Second, the level of FO flow is difficult to measure directly, even via MRI (Prsa et al., 2014), because the FO is a windsock-like channel located in the atrial septum (Dawes, 1968), rather than a discrete vessel. However, as in the present study, net FO flow can be obtained indirectly as the difference between LV output and pulmonary blood flow (Anderson et al., 1981; Prsa et al., 2014; Smolich & Mynard, 2021), although this approach can be technically challenging during an experimental birth transition study, because it requires accurate and concurrent measurement of multiple central

arterial blood flows during rapid and often large changes in haemodynamics.

As in the present study (Fig. 6*A*), previous birth transition studies in lambs have observed that PA blood flow rises rapidly to a peak at 10–15 min after birth (Crossley et al., 2009; Smolich & Kenna, 2022; Smolich et al., 2015, 2016, 2020; Sobotka et al., 2011). Furthermore, our data suggest that this rapid postnatal rise in PA flow is largely supported by the combination of L→R ductal and FO shunting. Thus, by 15 s after birth, L→R ductal and FO shunting together accounted for ∼25% of PA blood flow in the 40sV and 120sV groups and for ∼50% of PA blood flow in the 300sV group, but this contribution rose to ∼80% in all groups by the time peak PA blood flow was attained 10–15 min after birth (Fig. 10*A*). Correspondingly, and consistent with a progressive fall in systemic arterial blood flow (Fig. 4*C*), the latter pattern was indicative of an ongoing decline in the contribution of systemic venous return to PA blood flow, with this contribution being only ∼20% by 10–15 min after birth. Indeed, our finding of a negative relationship between total L→R shunt flow passing to the lungs and total systemic arterial blood flow (Fig. 10*C*) provides direct experimental support for a close linkage between the degree of L→R shunting and the level of systemic arterial perfusion in the perinatal period.

One potential interpretation of a larger increase in PA blood flow and PVC, in addition to associated changes in systemic arterial flows, during extended ventilation prior to DCC (Figs 4 and 6–8) was that they simply reflected a normal neonatal circulatory pattern being reached sooner. However, close scrutiny of our data suggested that changes in blood flow in the 300sV group instead represented a large shift in perinatal circulatory dynamics that directly predisposed to reduced perfusion of fetal organs. Specifically, an under-appreciated but pivotal consequence of extended ventilation before DCC is that the interconnected systemic and pulmonary arterial circuit then simultaneously contains two major organs with a low vascular resistance/high vascular conductance (i.e. the placenta and ventilated lungs), which compete for available arterial flow not only with each other, but also with fetal systemic organs. With LV and RV outputs (and thus the combined ventricular output) essentially being unchanged during extended ventilation before DCC (Fig. 3), adequate perfusion of fetal systemic organs is therefore precarious.

Moreover, a greater PA blood flow during extended ventilation prior to DCC was not supported by two key mechanisms that underpinned a rapid rise of PA blood flow in the 40sV and 120sV groups after birth. The first was emergence of substantial L→R ductal shunting, which arises mainly via retrograde diastolic discharge from a descending aortic reservoir and is not initiated until umbilical cord clamping (Smolich & Mynard, 2021b; Smolich et al., 2016). The second was a large increase in LV output beyond 1 min of cord clamping (Fig. 3*A*) that accompanied this L→R ductal shunting. Instead, an elevated PA blood flow during extended ventilation before DCC was derived from pre-birth emergence of L→R FO shunting (Figs 8 and 9*C*) and an increased distribution of an unchanged RV output towards the lungs (Fig. 9*A*), supplemented by a relatively minor degree of L→R ductal shunting (Fig. 7*B*) that largely originated from a greater upper-to-lower body redistribution of LV output (Fig. 4*B*). Subsequent increases or decreases in most central arterial blood flows after DCC were similar in all groups, implying that: (i) the mechanisms supporting a rapid rise of PA blood flow after birth in the 40sV and 120sV groups also came into play in the 300sV group; and (ii) for a short period after birth in the 300sV group, the blood flow effects of these mechanisms were superimposed on blood flow changes activated by the prior period of extended ventilation.

Not unexpectedly, changes in systemic arterial blood flows during extended ventilation before DCC were associated with alterations in systemic O_2 delivery. Thus, despite a fall in BCT blood flow (Fig. 4*A*), upper body O_2 delivery increased after the onset of ventilation in all groups owing to a large rise in AoT O_2 content (Table 1), with this increase being maintained in the 40sV and 120sV groups (Fig. 5). However, given that a continued fall in BCT flow, which occurred in conjunction with an upper-to-lower body redistribution of LV output (Fig. 4*A* and *B*), exceeded a further minor rise in AoT O_2 content during extended ventilation (Table 1), upper body O_2 delivery then fell progressively in the 300sV group to a level not different from the post-delivery fetal baseline and tended to be lower than in the 40sV and 120sV groups at the 30 s time point after birth (Fig. 5). Whether upper body O_2 consumption is maintained via greater O_2 extraction during extended ventilation before DCC or whether falls in upper body O_2 delivery are associated with a reduction in upper body O_2 consumption is an important issue that remains to be resolved.

Our study has four main potential limitations. First, experiments were performed acutely under general anaesthesia and open-chest conditions, an approach necessary because of the extent of instrumentation required to measure multiple central arterial blood flows concurrently. However, baseline blood gas and haemodynamic data in our preparation were similar to those of unanaesthetized, chronically instrumented preterm fetal lambs (Bhatt et al., 2013; Crossley et al., 2009; Galinsky et al., 2014; Hunter et al., 2003; Wassink et al., 2007). In addition, key features of the birth transition, such as a large and rapid rise in pulmonary blood flow and a rapid reversal of ductal shunting, were similar to those previously reported in chronically instrumented fetal lambs (Crossley et al., 2009; Fineman et al., 1995; Rudolph,

1979; Sobotka et al., 2011). Furthermore, we used a multidrug anaesthetic regimen, in which the low doses of drugs used have been shown to have relatively minor or no significant effects on fetal circulatory dynamics (Conklin et al., 1980; Craft et al., 1983; Strumper et al., 2004). Nonetheless, we cannot exclude the possibility that acute dissection around major arteries and alterations in thoracic compliance related to the open-chest state altered central blood flows during initial ventilation and after DCC. However, blood flow patterns in our study resembled those in the closed-chest, chronic fetal lamb preparation of Bhatt et al. (2013), suggesting that any effect was likely to be minor.

Second, the study was undertaken in preterm lambs; therefore, perinatal changes in central blood flow patterns might differ in term lambs. Specifically, premature birth in both lambs and humans is associated with delayed closure of the ductus arteriosus, and thus potentially enhanced L→R ductal shunting in the neonatal period (Clyman, 2022). Although L→R FO shunting has been observed with Doppler echocardiography in both preterm (Evans & Archer, 1990; Evans & Iyer, 1994a, b) and term neonates (Evans & Archer, 1990; Hannu et al., 1989; Hiraishi et al., 1991; Markhorst et al., 1995), as far as we are aware, no comparative data are available for these developmental time points on the extent of this shunting. Whether the degree of ductal, FO or total L→R shunting associated with an extended period of ventilation prior to DCC in near-term lambs is less than in preterm lambs thus remains to be determined.

Third, our method for estimating FO flow as the difference between LV output and PA blood flow yielded net FO flow. However, FO shunting in preterm infants is often bidirectional (Evans & Iyer, 1994a), and thus calculation of net L→R FO flow is likely to have underestimated the real level of phasic L→R FO flow.

Finally, a number of unavoidable methodological differences exist between our experimental studies in lambs and human births, as follows: (i) our studies related solely to birth by Caesarean section; (ii) the lambs required mechanical lung ventilation, with spontaneous ventilation being suppressed by general anaesthesia of the lambs, and not possible in any event given the need for an open chest in the experimental preparation; (iii) a fall in lung liquid volume normally occurring during the birth process via a combination of absorption and physical expulsion of lung liquid from the trachea (Berger et al., 1998; Pfister et al., 2001; Stockx et al., 2007) was simulated entirely by physical drainage of lung liquid, as is routine in experimental birth transition studies (e.g. Crossley et al., 2009; Polglase et al., 2015; Smolich & Kenna, 2022; Smolich et al., 1996); and (iv) pressures used during mechanical ventilation of the lambs were higher than would be used for human infants, with use of a relatively high PEEP (8 cmH_2O) physiologically appropriate to optimize lung dynamic compliance in preterm lambs not treated with antenatal steroids (Tingay et al., 2014). Despite these methodological differences, however, emergence of L→R ductal shunting after birth, for example, followed a similar time course in our experimental studies (Fig. 7*B*) and during human birth (van Vonderen et al., 2014).

Our study has three main implications for the birth transition. First, our findings do not support a key assumption underpinning use of an extended period of ventilation prior to DCC, namely that an augmented increase in PA blood flow, via greater pulmonary venous return to the left atrium and ventricle, results in a higher LV output and systemic arterial perfusion (Bhatt et al., 2014; Hooper et al., 2015, 2019; Kluckow & Hooper, 2015; Te Pas et al., 2019). Instead, our experimental findings suggest that perinatal levels of LV output are similar with brief, moderate or extended durations of initial ventilation prior to DCC (Fig. 3*A*), but that an augmented rise in PA flow that follows an extended period of ventilation prior to DCC (Fig. 6*A*) is accompanied by a progressive reduction in systemic arterial perfusion (Fig. 4*C*) that is primarily related to enhanced L→R ductal and FO shunting (Figs 9*B* and *C* and 10*C*). A corollary of these findings is that, because of diversion of pulmonary venous return from the left to the right atrium, changes in PA blood flow in the presence of substantial L→R FO shunting do not accurately reflect alterations in LV preload, LV output or systemic arterial perfusion.

Second, a lack of consensus presently exists in the clinical arena about the duration of the interval that should be used between delivery or onset of ventilation and subsequent cord clamping, with this interval ranging from ∼30 s to ∼2 min in most published randomized controlled studies of conventional DCC (Fogarty et al., 2018; Jasani et al., 2021; McDonald et al., 2013; Seidler et al., 2021). However, our finding that haemodynamic and arterial blood flow patterns, in addition to upper body O_2 delivery, after birth following a brief (∼40 s) or moderate period (∼2 min) of initial ventilation were similar (Figs 2–9) accords with the conclusion of subgroup analysis of randomized controlled trials (Fogarty et al., 2018) or more recent studies that specifically compared different delivery-to-DCC intervals (Chaudhary et al., 2023) that neonatal outcomes do not differ with the range of intervals used in conventional DCC.

Finally, L→R shunting is considered to be 'ineffective' blood flow, because it increases pulmonary blood flow but 'steals' nutritive perfusion from systemic tissues, predisposing to neonatal complications, such as pulmonary haemorrhage, necrotizing enterocolitis and chronic lung disease (Clyman, 2022; Kluckow & Evans, 2001; Kulik & Levy, 2022). However, it has been suggested that L→R shunting is also an integral part of the normal birth transition (Baik et al., 2016). This apparent duality,

in conjunction with our finding that L→R shunting provided ∼80% of PA blood flow in the early neonatal period of normal preterm lambs (Fig. 10*A*), raises the possibility that adverse effects of L→R shunting after preterm birth might not be attributable simply to development of an abnormally high level of such shunting, but might instead reflect a failure of an adequate reduction in substantial L→R shunting usually present after birth.

In summary, our data suggest that a large rise in PA blood flow during an extended period of ventilation prior to DCC during preterm birth is not accompanied by a corresponding increase in LV output but is instead associated with a reduction in systemic arterial blood flow that occurs via a combination of three distinct mechanisms. The first is an increased distribution of RV output to the lungs, which results in a lesser RV contribution to DTA blood flow via phasic R→L ductal shunting. The second is earlier emergence of L→R shunting across the FO, which limits LV output. The third is earlier appearance of phasic L→R ductal shunting that is initially related largely to a more pronounced upper-to-lower body redistribution of LV output, which also contributes to a fall in upper body O_2 delivery. These findings suggest that, because of a lack of an increase in LV output, an augmentation of L→R shunting and an accompanying greater reduction in systemic arterial perfusion that is associated with a fall in upper body O_2 delivery, a more pronounced rise in PA blood flow occurring during an extended period of initial ventilation prior to DCC does not confer any perinatal circulatory benefits over conventional DCC.

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Additional information

Data availability statement

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

Competing interests

None.

Author contributions

Experimental studies were performed within the Translational Research Unit of the Murdoch Children's Research Institute. J.J.S. conceptualized and designed the study and drafted the manuscript. J.J.S. and K.R.K. performed the physiological studies and analysed physiological data. J.J.S. and J.P.M. interpreted physiological data. J.J.S., K.R.K. and J.P.M. critically reviewed and revised the manuscript and approved the final version.

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Keywords

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