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Pediatric RESEARCH

BASIC SCIENCE ARTICLE

Blunted sympathoadrenal activation accompanies hemodynamic stability after early ventilation and delayed cord clamping at birth in preterm lambs

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BACKGROUND: As surges in circulating norepinephrine and epinephrine have chronotropic, pressor, and inotropic effects, we tested the hypothesis that blunted rises in these catecholamines during preterm birth accompanied hemodynamic stability observed after early ventilation and delayed cord clamping (DCC), with findings compared to immediate cord clamping (ICC) and a non-asphyxial cord clamp-to-ventilation interval.

METHODS: Anesthetized preterm fetal lambs were instrumented with arterial micromanometers to obtain pressure and the maximal rate of pressure rise (dP/dt_{max}) as a surrogate of ventricular contractility and an aortic catheter to obtain blood samples for catecholamine assay. Fetuses were delivered and mechanically ventilated before cord clamping ~1.5 min later (DCC, $n = 9$) or subjected to ICC with ventilation started ~40 s later ($n = 8$).

RESULTS: Perinatal hemodynamics were stable after DCC, with greater fluctuations evident following birth after ICC ($P \leq 0.05$). With DCC, circulating norepinephrine and epinephrine were unchanged after early ventilation but rose following cord clamping ($P \leq 0.01$), with concentrations below the threshold for hemodynamic effects. Norepinephrine was higher in the ICC group after cord clamping and immediately after ventilation ($P < 0.025$), but catecholamine levels were otherwise similar between groups.

CONCLUSION: Hemodynamic stability at birth after DCC is accompanied by sub-threshold rises in circulating norepinephrine and epinephrine and thus blunted sympathoadrenal activation.

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INTRODUCTION

Experimental studies in preterm lambs have indicated that striking differences in cardiovascular stability may exist during the birth transition between use of early ventilation with delayed cord clamping (DCC) or immediate cord clamping (ICC) with subsequent ventilation. Thus early ventilation with DCC 2.5–4 min later was accompanied by minor perinatal changes in heart rate and arterial blood pressure.[1–3] By contrast, ICC with ventilation 1.5–2 min later resulted in a pronounced bradycardia and arterial blood pressure swings before ventilation, followed by substantial surges in heart rate and arterial blood pressures after onset of ventilation.[1,4] The physiological basis of hemodynamic stability evident during birth with early ventilation and DCC has yet to be defined with experimental data, although one proposal is that a rise in pulmonary arterial (PA) blood flow (and thus pulmonary venous return passing to the left ventricle) occurring with early ventilation mitigates the effects of an abrupt fall in venous return to the heart resulting from cord clamping.[1,3,5–7] However, this proposal is not in accord with the observation that PA blood flow also initially rises after ICC and before ventilation.[4]

An alternative potential explanation for the hemodynamic stability of DCC has, however, emerged from recent studies in preterm lambs where ICC was followed by discrete cord clamp-to-ventilation intervals ranging from ~30 s to 2 min.[4,8] These studies suggested that bradycardia and blood pressure swings in this interval were related to rapid development of an asphyxial state by ~60 s after ICC and that surges in heart rate, blood pressure, and ventricular contractility accompanied relief of this asphyxia after onset of ventilation. Indeed, reducing the length of the cord clamp-to-ventilation interval to ~30 s to avoid development of an asphyxial state markedly dampened perinatal hemodynamic fluctuations.[4] Furthermore, measurement of circulating concentrations of norepinephrine and epinephrine after ICC revealed that (1) these catecholamines rose exponentially with reductions in arterial oxygenation during development of asphyxia in the cord clamp-to-ventilation interval, reflecting a pronounced sympathoadrenal activation, and (2) highly significant relationships existed after birth between catecholamine concentrations and rises in heart rate, blood pressures, and ventricular contractility.[8] As arterial blood oxygenation, measured with pulse-oximetry, does not fall but instead rises with early ventilation and DCC,[2] these data imply that the hemodynamic stability occurring with DCC[1–3] reflects a marked dampening of rises in circulating norepinephrine and epinephrine during birth and thus a blunting of sympathoadrenal activation.

The primary aim of this study, in which aortic blood gas status, hemodynamics, and circulating concentrations of norepinephrine

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and epinephrine were measured during the birth transition in preterm lambs, was therefore to test the hypothesis that a stability of heart rate, blood pressures, and ventricular contractility after early ventilation and DCC was accompanied by only minor changes in circulating norepinephrine and epinephrine concentrations. A secondary aim was to compare hemodynamics and circulating catecholamine concentrations in DCC with those from a concurrent series of preterm lambs undergoing ICC followed by a brief (~40 s) non-asphyxial period of cord clamping prior to ventilation, where hemodynamics are also relatively stable at birth.[4,8]

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.

Surgical preparation

The general features of anesthetic and monitoring procedures have been previously described.[4,8] Briefly, 17 Border-Leicester cross ewes were anesthetized at a gestation of 127 ± 1 days (mean ± SD, term = 147 days) with an intramuscular injection of ketamine (5 mg/kg) and xylazine (0.1 mg/kg), followed by 4% isoflurane given by mask. After tracheal intubation, anesthesia was maintained with isoflurane (2–3%) and nitrous oxide (10–20%) delivered by a ventilator in O_2-enriched air, combined with an intravenous (i.v.) infusion of ketamine (1–1.5 mg/kg/h), midazolam (0.1–0.15 mg/kg/h), and fentanyl (2–2.5 µg/kg/h). Transcutaneous oxygen saturation (S_pO_2) was monitored continuously with a pulse-oximetry sensor applied to the ear. The right common carotid artery was cannulated for monitoring of blood pressure and blood gas status (ABL800, Radiometer, Copenhagen, Denmark), with the ewe's ventilation adjusted to maintain arterial O_2 tension (P_ao_2) at 100–120 mm Hg and CO_2 tension (P_aco_2) at 35–40 mm Hg.

The fetal head was exteriorized via a midline laparotomy and placed in a saline-filled glove to prevent loss of lung liquid. The neck was incised and two fluid-filled catheters passed into the superior vena cava through the left external jugular vein for fluid and drug administration and for blood infusion during arterial blood sampling. A 6-Fr sheath was introduced via the left common carotid artery into the brachiocephalic trunk for blood sampling, pressure measurement, and passage of a 3.5-Fr high-fidelity micromanometer catheter (SPR-524, Millar Instruments, Houston, TX) into the aortic trunk (AoT). After delivery of the left forelimb and adjacent thorax, a thoracotomy was performed in the third interspace, with a fluid-filled catheter and another 3.5-Fr micromanometer inserted via purse-string sutures into the pulmonary trunk (PT) to measure pressure. Finally, a clamped 4.5 mm endotracheal tube with a side-port to measure pressure was introduced into the trachea through a proximal intercartilaginous space and tied into place.

Experimental protocol

After removal of the glove from the fetal head at the end of surgery, the endotracheal tube was unclamped to allow lung liquid to drain passively via gravity for ~20 s and then re-clamped to prevent lung aeration prior to ventilation. Baseline fetal hemodynamics were subsequently recorded onto computer, and aortic samples were collected for blood gas and catecholamine determinations. While hemodynamics were recorded continuously, the fetus was then completely delivered from the uterus, placed on the ewe's abdomen, and covered with warmed towels, without tension on the umbilical cord. After collection of aortic samples ~30 s later for blood gas and catecholamine analyses, lambs underwent one of the two protocols allocated prior to surgery: (1) early ventilation and DCC ($n = 9$; 5 males/4 females; 3 singletons/6 twins), where positive-pressure mechanical ventilation was commenced via the endotracheal tube using warmed and humidified gases, with aortic samples collected for blood gas and catecholamine analyses 30 and 60 s later, and cord clamping performed 96 ± 7 s after the start of ventilation; (2) ICC with a non-asphyxial cord clamp-to-ventilation interval ($n = 8$; 3 males/5 females; 2 singletons/6 twins), where umbilical cord clamping was followed by aortic sampling for blood gas and catecholamine analyses at 20 s, and ventilation started at 44 ± 2 s.

Birth was defined as the point of cord clamping in the DCC group or onset of ventilation in the ICC group (i.e., the point when both ventilation and cord clamping had occurred). After birth, aortic samples were collected at 0.5, 1, 2, and 5 min for catecholamine assay and at 0.5, 1, 2, 3, 5 and 10 min for gas analysis, with blood gas samples taken just prior to and during the initial 3 min after birth stored on ice before analysis. Following birth, anesthesia in lambs was continued with an i.v. infusion of ketamine (4 mg/kg/h) and midazolam (0.05 mg/kg/h).

Lambs were ventilated using an infant ventilator (SLE5000, SLE Ltd, Croydon, UK), with initial settings comprising a positive end-expiratory pressure of 8 cm H_2O, a respiratory rate of 60 inflations/min, an inspiratory time of 0.4 s, a tidal volume of 7 ml/kg estimated body weight, and an inspired fractional O_2 concentration (Fio_2) of 0.3. After birth, ventilation was adjusted to progressively increase preductal S_pO_2, measured with a cheek or left forelimb sensor, to 85–95% by the 10 min time point.[9]

The recording of physiological data commenced with delivery of the fetus was continued for 10 min after birth. After completion of this recording, the lamb was carefully transferred onto a heated resuscitation bed. Hemodynamic data were then recorded at 15 and 30 min after onset of ventilation, with each recording preceded by withdrawal of an aortic sample for blood gas and catecholamine determination. After the 5-min time point, ventilator settings were adjusted on the basis of blood gas results, with a target hemoglobin O_2 saturation (S_aO_2) of 90–95% and P_aco_2 of 45–55 mmHg. Ewes were euthanized with an i.v. overdose of sodium pentobarbitone (100 mg/kg) after cord clamping and lambs after completion of the study protocol.

Physiological data

AoT, PT, and tracheal fluid-filled catheter pressures were measured with transducers referenced to atmospheric pressure at the left atrial level. All physiological signals were digitized at a sampling rate of 1 kHz using programmable acquisition and analysis software (Spike2, Cambridge Electronic Design, Cambridge, UK).

As hemodynamics can change rapidly during the birth transition, data were analyzed in subfiles extracted as: (1) 10–20 s blocks before complete delivery of the fetus and then just before ventilation in the DCC group or cord clamping in the ICC group, (2) 5 s blocks at 15 s intervals after onset of ventilation in the DCC group or cord clamping in the ICC group, as well as immediately before and at 15 s intervals in the first minute after birth, and (3) 10–20 s blocks at subsequent time points to 30 min. The overall experimental protocol, with timing of hemodynamic measurements and aortic sampling for blood gas and catecholamine analyses, is depicted schematically in Fig. 1.

During data analysis, 50 Hz mains electrical interference was removed with a 48 Hz low-pass filter, with measurements performed on ensemble-averaged signals typically generated from 10–15 beats in 5 s subfiles and > 20 beats in 10–20 s subfiles. Mean micromanometer pressures were matched to corresponding fluid-filled catheter pressures, with the maximal rates of rise of high-fidelity AoT and PT blood pressure (i.e., dP/dt_{max}) used as surrogate measures of left and right ventricular contractility, respectively.[8,10]

Catecholamine assay

Blood samples for catecholamine assay were immediately transferred into ice-chilled tubes containing an anticoagulant

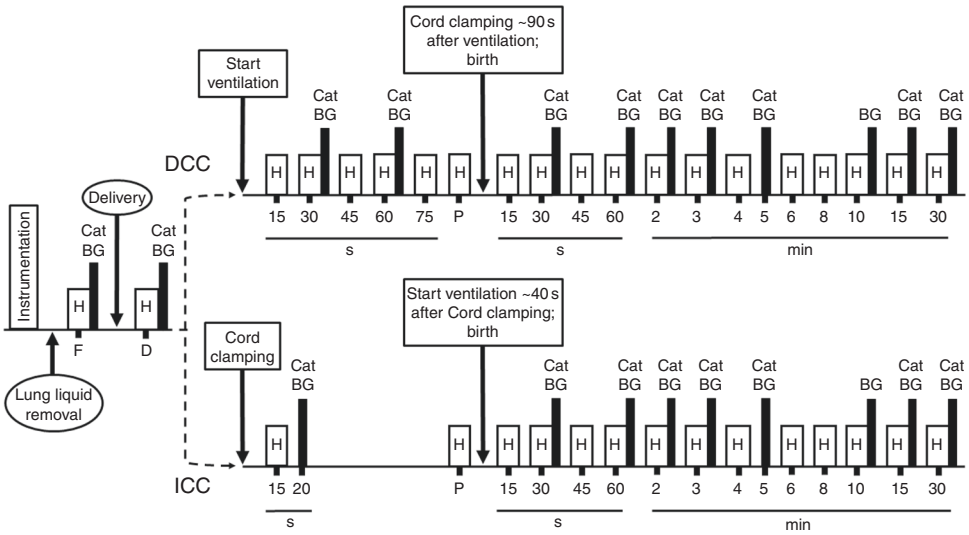

Fig. 1 Schematic depiction of experimental protocol after early ventilation with delayed umbilical cord clamping (DCC) ~90 s later or immediate cord clamping (ICC) with ventilation ~40 s later. BG blood gas sample, Cat catecholamine sample, H hemodynamic measurement. Time points: F fetus just before delivery, D fetus ~30 s after delivery, P just prior to cord clamping (DCC group) or ventilation (ICC group)

and antioxidant mixture (EGTA plus reduced glutathione). Withdrawn blood was replaced with an equal volume of heparinized fetal blood delivered via an infusion pump during the 10 min birth recording and manually at 15 and 30 min after birth. Tubes were kept on ice until centrifugation at 4 °C, with plasma then stored at −80 °C until assay. Endogenous norepinephrine and epinephrine were extracted from 1 ml plasma samples using alumina adsorption and separated by high-performance liquid chromatography, with quantitation of peaks using coulometric detection.[8,11]

Statistical analysis

Results were analyzed using GraphPad Prism 6 (GraphPad Software Inc., La Jolla, CA), preceded by logarithmic transformation of data with a non-normal distribution. As treatment up to and including fetal delivery at birth was not different between groups, data at these time points were pooled before analysis. Post-delivery longitudinal data in each group were analyzed using repeated-measures one-way analysis of variance (ANOVA) and specific comparisons evaluated by partitioning the within-animal sums of squares into individual degrees of freedom, with a Bonferroni correction applied as required for multiple comparisons. Post-delivery data between groups were analyzed with two-way repeated-measures ANOVA, and between-group differences at time points compared with post hoc Fisher least significant difference tests. Data are expressed as mean ± SD and significance was accepted at $P < 0.05$.

RESULTS

Blood gas variables

Minor falls in pH, S_aO_2, and P_ao_2 and a rise in P_aco_2 occurred with delivery ($P \leq 0.001$). After onset of ventilation in the DCC group, pH, S_aO_2, and P_ao_2 rose ($P < 0.01$), while P_aco_2 fell ($P < 0.005$). Subsequently, S_aO_2 and P_ao_2 were unchanged in the initial 3 min after cord clamping but then rose ($P < 0.001$) to a plateau at ≥10 min (Fig. 2). By contrast, pH, S_aO_2, and P_ao_2 fell ($P < 0.025$) and P_aco_2 rose ($P < 0.001$) after cord clamping in the ICC group but recovered within 1–2 min after onset of ventilation (Fig. 2).

Blood pressures and heart rate

Fetal mean AoT and PT blood pressures rose from an average of 42.6 ± 5.6 to 45.2 ± 4.9 mm Hg with delivery ($P < 0.025$). In the DCC group, AoT and PT pressures subsequently fell from 45.0 ± 4.9 to 40.3 ± 3.6 mm Hg with ventilation ($P < 0.005$) and increased from 41.2 ± 4.1 to 47.5 ± 5.6 mm Hg after cord clamping ($P < 0.001$), with no change in AoT pressure thereafter, but a fall in PT pressure at ≥6 min ($P < 0.005$; Fig. 3a, b). In the ICC group, AoT and PT pressures increased from 45.4 ± 4.4 to 52.4 ± 6.8 mm Hg after cord clamping ($P < 0.005$) and then fell from 51.3 ± 7.8 to 41.2 ± 10.0 mm Hg after ventilation ($P < 0.001$), with recovery to similar levels as the DCC group within 1 min after birth (Fig. 3a, b). Comparing groups, cord clamping increased AoT and PT pressures similarly in the DCC and ICC groups (6.2 ± 2.0 vs. 6.9 ± 3.8 mm Hg, $P > 0.6$), but ventilation produced a lesser pressure fall in the DCC group (−4.7 ± 2.5 vs. −10.1 ± 4.2 mm Hg, $P < 0.01$).

Heart rate rose from 149 ± 15 to 164 ± 11 beats/min with delivery ($P < 0.002$). In the DCC group, heart rate then decreased from 165 ± 10 to 148 ± 20 beats/min with onset of ventilation ($P < 0.05$) and was unchanged after cord clamping (Fig. 3c). By contrast, heart rate was unchanged in the ICC group by cord clamping and in the initial minute after ventilation but then fell ($P < 0.001$) to be similar between groups at ≥2 min after birth (Fig. 3c).

Arterial dP/dt_{max}

AoT dP/dt_{max} was unchanged with delivery ($P > 0.8$), but PT dP/dt_{max} increased 14% ($P < 0.025$). In the DCC group, AoT and PT dP/dt_{max} were unchanged during early ventilation, but while AoT dP/dt_{max} was unaffected by cord clamping and then rose progressively to 15 min ($P < 0.001$), PT dP/dt_{max} fell 11% with cord clamping ($P < 0.001$) and declined a further 24% by 15 min ($P < 0.001$; Fig. 4a, b). In the ICC group, AoT dP/dt_{max} was unchanged with cord clamping and in the first minute after ventilation but then increased ($P < 0.001$), albeit by a lesser degree than in the DCC group ($P < 0.01$). However, PT dP/dt_{max} fell 7% after cord clamping ($P < 0.025$), rose transiently within the first minute after ventilation ($P < 0.025$), and then declined ($P < 0.001$), with no difference between groups after 1 min (Fig. 4a, b). Consequently, the AoT-to-PT dP/dt_{max} ratio increased in both groups over the initial 15 min after birth ($P < 0.001$) but was higher in the DCC group at ≥ 8 min ($P < 0.01$, Fig. 4c).

Plasma catecholamines

With delivery, fetal norepinephrine rose from 1612 ± 833 to 2072 ± 802 pmol/l ($P < 0.001$) and epinephrine from 120 ± 86 to

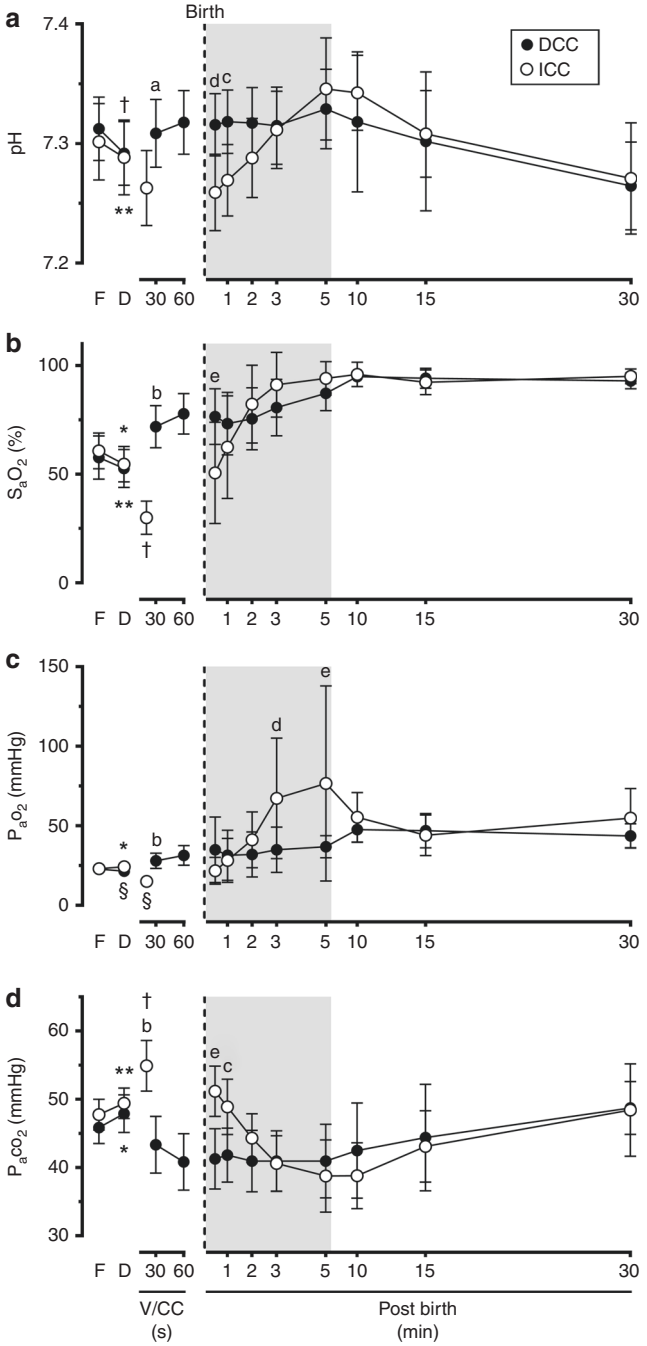

Fig. 2 Ascending aortic pH (**a**), hemoglobin O_2 saturation (**b**), Po_2 (**c**), and Pco_2 (**d**) in fetus before (F) and after delivery (D), after ventilation (V) in delayed cord clamp group (DCC) or cord clamping (CC) in immediate cord clamp group (ICC), and in initial 30 min after birth. Note: (1) dashed vertical line represents the point of birth, (2) timescale of initial 5 min after birth (shaded area) is magnified. Data are expressed as mean ± SD; $n = 9$ for DCC and $n = 8$ for ICC groups. $^aP < 0.01$, $^bP < 0.001$, 20 s after cord clamping in ICC group vs. 30 s after ventilation in DCC group; $^cP < 0.01$, $^dP < 0.005$, $^eP < 0.001$, DCC vs. ICC; $^§P < 0.025$, $^†P < 0.01$, $^*P < 0.005$, $^{**}P < 0.001$, compared to subsequent time point in each group

360 ± 87 pmol/l ($P < 0.001$). In the DCC group, neither catecholamine concentration changed with ventilation ($P \geq 0.2$), but after cord clamping, norepinephrine increased from 1892 ± 886 to 2315 ± 1018 pmol/l ($P < 0.005$) and then remained unchanged to 30 min, while epinephrine rose from 470 ± 260 to 595 ± 329 pmol/l

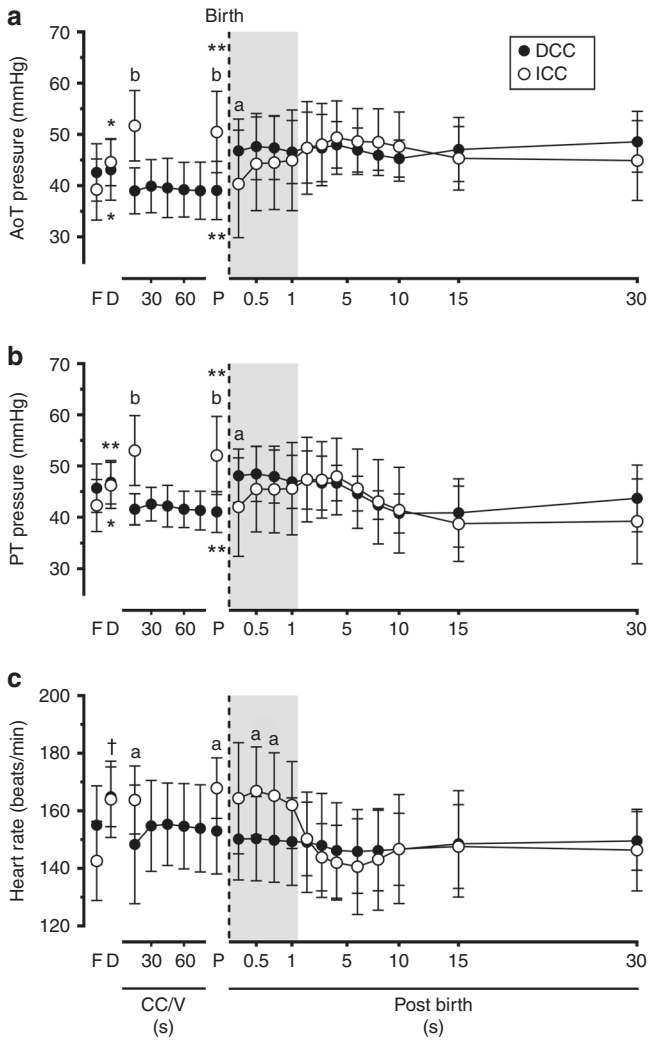

Fig. 3 Aortic trunk (AoT, **a**) and pulmonary trunk mean blood pressure (PT, **b**) and heart rate (**c**) in fetus before (F) and after delivery (D), after ventilation (V) in delayed cord clamp group (DCC) or cord clamping (CC) in immediate cord clamp group (ICC), just prior (P) to cord clamping in the DCC group or ventilation in the ICC group, and in initial 30 min after birth. Data are expressed as mean ± SD; $n = 9$ for DCC and $n = 8$ for ICC groups. $^aP \leq 0.05$, $^bP < 0.001$, DCC vs. ICC; $^†P < 0.05$, $^*P < 0.005$, $^{**}P < 0.001$, compared to subsequent time-point in each group

($P = 0.01$) and then declined to a plateau at ≥5 min after birth ($P < 0.001$; Fig. 5). After cord clamping in the ICC group, norepinephrine rose to 2797 ± 505 pmol/l ($P < 0.005$) but epinephrine was unchanged ($P = 0.4$). However, concentrations of both norepinephrine (3530 ± 985 pmol/l) and epinephrine (802 ± 551 pmol/l) were then higher at 30 s after onset of ventilation ($P \leq 0.01$) but subsequently declined to a plateau at ≥5 min after birth ($P < 0.002$). Apart from a slightly higher norepinephrine in the ICC group after cord clamping and at 30 s after birth, catecholamine concentrations did not differ between groups (Fig. 5).

DISCUSSION

The main finding of this study, which primarily evaluated the effects of early ventilation and DCC on perinatal arterial blood gases, hemodynamics, and circulating catecholamine concentrations, was that circulating norepinephrine and epinephrine displayed only minor changes during the birth transition, in conjunction with (1) an improved arterial blood gas status after onset of ventilation and

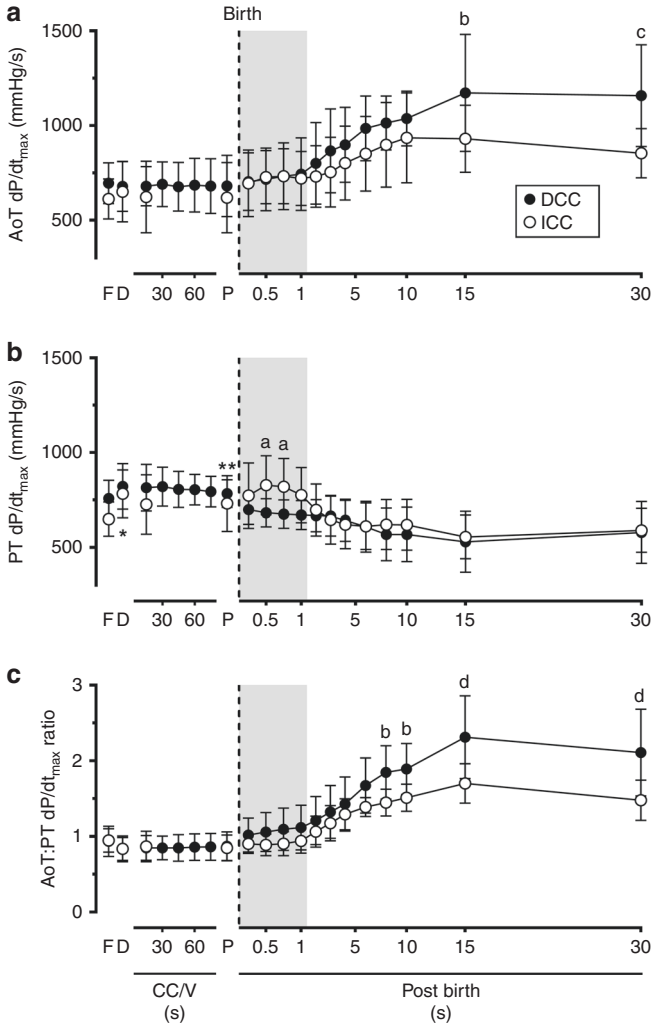

Fig. 4 Aortic trunk dP/dt$_{max}$ (**a**), pulmonary trunk dP/dt$_{max}$ (**b**), and the aorta-to-pulmonary dP/dt$_{max}$ ratio (**c**), using the same format/time points defined in Fig. 3. Data are expressed as mean ± SD; $n = 9$ for delayed cord clamping (DCC) and $n = 8$ for ICC groups. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.005$, $^{d}P < 0.001$, DCC vs. ICC; $^{*}P < 0.025$, $^{**}P < 0.001$, compared to subsequent time point in each group

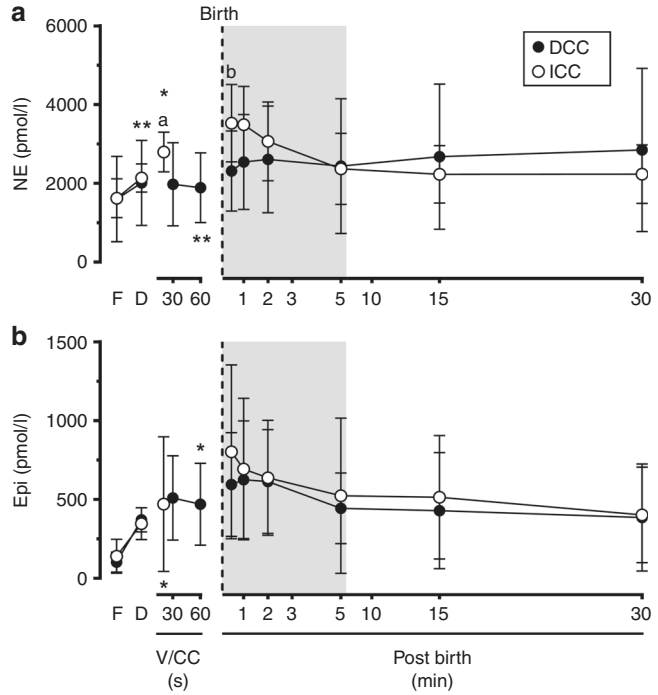

Fig. 5 Changes in circulating norepinephrine (NE, **a**) and epinephrine concentrations (Epi, **b**) using the same format/time points defined in Fig. 2. Data are expressed as mean ± SD; $n = 9$ for delayed cord clamping (DCC) and $n = 8$ for ICC groups. $^{a}P < 0.01$, 20 s after cord clamping in ICC group vs. 30 s after initial ventilation in DCC group; $^{b}P < 0.025$, DCC vs. ICC; $^{*}P \leq 0.01$, $^{**}P < 0.001$, compared to subsequent time point in each group

maintenance of this improvement after cord clamping, and (2) relatively small fluctuations in heart rate, arterial blood pressure, and arterial dP/dt$_{max}$. As birth-related surges in circulating norepinephrine principally arise from sympathetic nerves[12] and circulating epinephrine mainly from the adrenal medulla,[13] these findings suggest that a marked blunting of sympathoadrenal activation accompanied a hemodynamic stability evident with use of DCC at birth.

As observed in prior studies,[8,14,15] small rises in circulating norepinephrine and epinephrine occurred with fetal delivery. As these rises were associated with increases in heart rate, blood pressure, and PT dP/dt$_{max}$, they likely reflected a minor degree of sympathoadrenal activation related to a combination of factors, including (1) manual handling of the fetus during delivery, as squeezing of fetal body parts can augment sympathetic discharge,[16] (2) exposure of the fetus to a cooler ambient temperature,[17,18] and (3) a slight deterioration of aortic blood oxygenation.[8]

Not unexpectedly, increases in blood oxygenation and pH, and a fall in $P_a co_2$, rapidly occurred after onset of ventilation in the DCC group with, as noted with pulse-oximetric measurements,[2] improvements in oxygenation maintained after cord clamping. However, ventilation was not accompanied by increased circulating catecholamines in the DCC group, implying that, while the lungs can be a source of circulating norepinephrine and epinephrine in newborn lambs,[19] this contribution was not instigated by lung ventilation per se.

Conversely, cord clamping in the DCC group was followed by a rise in circulating concentrations of both catecholamines. However, it is unlikely that this rise was due to increased release of catecholamines into the circulation from sympathoadrenal activation as (1) heart rate was unchanged (Fig. 3c), while arterial dP/dt$_{max}$ was unaltered or reduced (Fig. 4a, b), and (2) a fall in arterial oxygenation, the usual stimulus for markedly increased sympathoadrenal activation during birth,[8] was lacking. On the other hand, as circulating catecholamine concentrations are determined by the net balance of their release into and clearance from the circulation,[20] a more likely explanation was that these rises were related to two direct consequences of cord clamping,[8,21] namely, (1) release of fetal catecholamines into a smaller vascular compartment following removal of the placenta from the systemic circulation, and (2) reduced clearance of catecholamines from the fetal circulation, as the placenta is a major site of catecholamine uptake.[22]

Via their actions on cardiovascular α- and β-adrenoceptors, norepinephrine and epinephrine have rapid-onset and very potent circulatory effects[23,24] that are a major component of large but transient increases in heart rate, blood pressure, and ventricular contractility after birth.[8] However, while a rise in circulating catecholamines did occur after cord clamping in the DCC group, this rise was of minor degree and not associated with any significant stimulatory effect on heart rate or arterial dP/dt$_{max}$. A plausible explanation for this lack of effect was that mean circulating concentrations of both norepinephrine (~2300 pmol/l)

and epinephrine (~600 pmol/l) in the DCC group after birth were well below the respective thresholds of ~3600–4700 pmol/l (600–800 pg/ml) and ~2700–4400 pmol/l (500–800 pg/ml) required to produce hemodynamic effects in preterm lambs.[24]

Given that catecholamine concentrations after both ventilation and cord clamping in the DCC group were below the thresholds for hemodynamic effects,[24] it is likely that other factors contributed to observed hemodynamic changes. Thus a neural reflex arising from afferents located within the airways and stimulated by gaseous inflation of fetal lungs[25] can produce falls in heart rate and blood pressure similar to that observed in the DCC group with onset of ventilation. A decrease in arterial blood pressure with ventilation, which has been noted previously with DCC,[1,2] may have also resulted from a marked fall in pulmonary vascular resistance (PVR) in the presence of an open ductus.[26] It is likely that the rise in arterial blood pressure after cord clamping in the DCC group also had a mechanical basis, related to sudden loss of the low resistance placental circulation and a consequent increase in total circulatory resistance.[4,27]

Although perinatal hemodynamics were also relatively stable after ICC followed by a non-asphyxial cord clamp-to-ventilation interval, greater fluctuations in arterial blood pressures, heart rate, and PT dP/dt_{max}, as well as a higher circulating norepinephrine concentration, were evident in the initial minute after birth compared with DCC. The most likely basis of this difference was that falls in oxygenation and pH, as well as a rise in P_aco_2, detectable in the blood sample taken ~20 s after ICC represented the early stage of an emerging asphyxial state.[4,8] However, mechanical factors may have also played a role, as the greater fall in arterial blood pressure evident with ventilation in the ICC group (~10 mmHg), compared to the DCC group (~5 mmHg), presumably occurred because the blood pressure effect of a reduction in PVR with ventilation after ICC was not buffered by the presence of the low-resistance and high-compliance placental circulation.

Taken together, the DCC and ICC findings of our study challenge two widely held beliefs about the birth transition. The first is that cord clamping/cutting is the stimulus for large surges in circulating catecholamines during the birth transition,[14,28] with the proposed mechanism comprising a reflex mediated via a massive generalized increase in central sympathoadrenal outflow and initiated by rises in total peripheral resistance and arterial baroreceptor activation occurring secondary to increased systemic arterial blood pressure after cord clamping.[14,28] However, as no catecholamine surge was evident with cord clamping/cutting either after DCC or after ICC followed by a brief, non-asphyxial cord clamp-to-ventilation interval, the present study provides further evidence for our previous conclusion that, rather than cord clamping/cutting per se, it is a rapid-onset asphyxial state developing by ~60 s in the interval between cord clamping/cutting and onset of ventilation that leads to exponential rises in circulating norepinephrine and epinephrine levels during the birth transition.[8]

Second, a robust sympathoadrenal activation is considered to be an integral component of the birth process, as it stimulates processes such as heat generation via non-shivering thermogenesis, clearance of lung liquid and release of surfactant in the lungs, and mobilization of the key metabolic substrates glucose and free fatty acids.[28,29] However, the results of the present study suggest that, despite a lack of substantial sympathoadrenal activation, postnatal changes in arterial blood gas status and hemodynamics were not impaired, and indeed left ventricular contractility was improved, after DCC. This conclusion is consistent with evidence from clinical studies of preterm infants pointing to the beneficial hemodynamic effects of DCC in reducing (1) the requirement for inotropic therapy, and (2) the risk of intraventricular hemorrhage and necrotizing enterocolitis.[30,31]

Our study had two main potential limitations. The first was the use of an acutely-instrumented fetal/newborn preparation studied under general anesthesia. However, baseline fetal concentrations of norepinephrine and epinephrine before delivery were, respectively, within or below the ranges of 1200–2640 pmol/l (203–446 pg/ml) and 125–275 pmol/l (23–50 pg/ml) reported in chronically instrumented fetal lambs of similar gestation,[24,32–35] suggesting that, despite acute surgical preparation under general anesthesia, fetuses in the current study were not in a stressed state. The latter is also consistent with the observations that (1) baseline arterial blood gas, blood pressure, and heart rate data in our study were similar to those of unanesthetized, chronically instrumented preterm fetal lambs,[1,35–37] and (2) ketamine, which may increase sympathetic activation if given as a relatively high dose (3 mg/kg) i.v. bolus,[38] had no effect on fetal hemodynamics when infused i.v. into chronically instrumented pregnant ewes at a more than five-fold higher rate than used in the present study.[39] The second limitation was that our experimental preparation modeled a cesarean section delivery without labor. Labor may itself lead to an increase in the baseline level of sympathoadrenal activity, as uterine contractions in labor are commonly accompanied by transient interruptions to placental perfusion that are associated with repeated episodes of fetal hypoxemia/asphyxia,[40] which can result in persistent elevations of norepinephrine and epinephrine concentrations.[35]

In conclusion, the finding of only minor perinatal changes in heart rate, arterial blood pressures, arterial dP/dt_{max}, and circulating catecholamines suggest that a marked blunting of sympathoadrenal activation accompanied a hemodynamic stability evident with the use of DCC at birth.

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AUTHOR CONTRIBUTIONS

J.J.S.: (1) substantial contributions to study conception and design and data acquisition, analysis and interpretation, (2) drafted and critically revised article for important intellectual content, (3) gave final approval of the published version. K.R.K. and S.E.P.: (1) substantial contributions to data acquisition and analysis, (2) revised article critically for important intellectual content, (3) gave final approval of the published version. J.P.M.: (1) substantial contributions to data interpretation, (2) revised article critically for important intellectual content, (3) gave final approval of the published version. G.W.L.: (1) substantial contributions to study conception and data interpretation, (2) revised article critically for important intellectual content, (3) gave final approval of published version.

ADDITIONAL INFORMATION

Competing interests: G.W.L.'s laboratory receives/has received research funding from Medtronic, Abbott Pharmaceuticals, Servier Australia, and Allergan. G.W.L. has acted as a consultant for Medtronic and has received honoraria or travel support for presentations from Pfizer, Wyeth Pharmaceuticals, Servier, and Medtronic. J.P.M. is a consultant for the Brain Protection Company. These companies provided no input into this study. The other authors declare no competing interests.

REFERENCES

1. Bhatt, S. et al. Delaying cord clamping until ventilation onset improves cardiovascular function at birth in preterm lambs. *J. Physiol.* **591**, 2113–2126 (2013).
2. Polglase, G. R. et al. Ventilation onset prior to umbilical cord clamping (physiological-based cord clamping) improves systemic and cerebral oxygenation in preterm lambs. *PLoS ONE* **10**, e0117504 (2015).

3. Blank, D. A. et al. Haemodynamic effects of umbilical cord milking in premature sheep during the neonatal transition. *Arch. Dis. Child Fetal Neonatal Ed.* **103**, F539–F546 (2018).
4. Smolich, J. J., Kenna, K. R. & Cheung, M. M. Onset of asphyxial state in non-respiring interval between cord clamping and ventilation increases hemodynamic lability of birth transition in preterm lambs. *J. Appl. Physiol. (1985)* **118**, 675–683 (2015).
5. Hooper, S. B., Polglase, G. R. & te Pas, A. B. A physiological approach to the timing of umbilical cord clamping at birth. *Arch. Dis. Child Fetal Neonatal Ed.* **100**, F355–F360 (2015).
6. Hooper, S. B. et al. Cardiovascular transition at birth: a physiological sequence. *Pediatr. Res.* **77**, 608–614 (2015).
7. Kluckow, M. & Hooper, S. B. Using physiology to guide time to cord clamping. *Semin. Fetal Neonatal Med.* **20**, 225–231 (2015).
8. Smolich, J. J., Kenna, K. R., Esler, M. D., Phillips, S. E. & Lambert, G. W. Greater sympathoadrenal activation with longer pre-ventilation intervals after immediate cord clamping increases hemodynamic lability at birth in preterm lambs. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **312**, R903–R911 (2017).
9. Wyckoff, M. H. et al. Part 13: Neonatal Resuscitation: 2015 American Heart Association Guidelines Update for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. *Circulation* **132**, S543–S560 (2015).
10. Masutani, S., Iwamoto, Y., Ishido, H. & Senzaki, H. Relationship of maximum rate of pressure rise between aorta and left ventricle in pediatric patients. Implication for ventricular-vascular interaction with the potential for noninvasive determination of left ventricular contractility. *Circ. J.* **73**, 1698–1704 (2009).
11. Lambert, G. W. & Jonsdottir, I. H. Influence of voluntary exercise on hypothalamic norepinephrine. *J. Appl. Physiol. (1985)* **85**, 962–966 (1998).
12. Agata, Y., Padbury, J. F., Ludlow, J. K., Polk, D. H. & Humme, J. A. The effect of chemical sympathectomy on catecholamine release at birth. *Pediatr. Res.* **20**, 1338–1344 (1986).
13. Padbury, J. et al. Effect of fetal adrenalectomy on catecholamine release and physiologic adaptation at birth in sheep. *J. Clin. Invest.* **80**, 1096–1103 (1987).
14. Padbury, J. F., Diakomanolis, E. S., Hobel, C. J., Perelman, A. & Fisher, D. A. Neonatal adaptation: sympatho-adrenal response to umbilical cord cutting. *Pediatr. Res.* **15**, 1483–1487 (1981).
15. Padbury, J. F., Agata, Y., Polk, D. H., Wang, D. L. & Callegari, C. C. Neonatal adaptation: naloxone increases the catecholamine surge at birth. *Pediatr. Res.* **21**, 590–593 (1987).
16. Biscoe, T. J., Purves, M. J. & Sampson, S. R. Types of nervous activity which may be recorded from the carotid sinus nerve in the sheep foetus. *J. Physiol.* **202**, 1–23 (1969).
17. van Bel, F., Roman, C., Iwamoto, H. S. & Rudolph, A. M. Sympathoadrenal, metabolic, and regional blood flow responses to cold in fetal sheep. *Pediatr. Res.* **34**, 47–50 (1993).
18. Gunn, T. R. et al. Haemodynamic and catecholamine responses to hypothermia in the fetal sheep in utero. *J. Dev. Physiol.* **7**, 241–249 (1985).
19. Smolich, J. J., Cox, H. S., Eisenhofer, G. & Esler, M. D. Pulmonary clearance and release of norepinephrine and epinephrine in newborn lambs. *Am. J. Physiol. Lung Cell Mol. Physiol.* **273**, L264–L274 (1997).
20. Esler, M. et al. Overflow of catecholamine neurotransmitters to the circulation: source, fate, and functions. *Physiol. Rev.* **70**, 963–985 (1990).
21. Smolich, J. J., Cox, H. S., Eisenhofer, G. & Esler, M. D. Increased spillover and reduced clearance both contribute to rise in plasma catecholamines after birth in lambs. *Am. J. Physiol. Heart Circ. Physiol.* **270**, H668–H677 (1996).
22. Jones, C. T. in *Biogenic Amines in Development* (eds Parvez, H. & Parvez, S.) 63–86 (Elsevier/North-Holland Biomedical Press, Amsterdam, 1980).
23. Seri, I. Circulatory support of the sick preterm infant. *Semin. Neonatol.* **6**, 85–95 (2001).
24. Padbury, J. F., Ludlow, J. K., Ervin, M. G., Jacobs, H. C. & Humme, J. A. Thresholds for physiological effects of plasma catecholamines in fetal sheep. *Am. J. Physiol. Endocrinol. Metab.* **252**, E530–E537 (1987).
25. Nail, B. S., Lumbers, E. R. & Stevens, A. D. The effect of fetal lung inflation on fetal heart rate. *Am. J. Physiol. Heart Circ. Physiol.* **266**, H1395–H1400 (1994).
26. Teitel, D. F., Iwamoto, H. S. & Rudolph, A. M. Changes in the pulmonary circulation during birth-related events. *Pediatr. Res.* **27**, 372–378 (1990).
27. Smolich, J. J., Kenna, K. R. & Mynard, J. P. Retrograde lower body arterial reservoir discharge underlies rapid reversal of ductus arteriosus shunting after early cord clamping at birth in preterm lambs. *J. Appl. Physiol. (1985)* **120**, 399–407 (2016).
28. Padbury, J. F. & Martinez, A. M. Sympathoadrenal system activity at birth: integration of postnatal adaptation. *Semin. Perinatol.* **12**, 163–172 (1988).
29. Lagercrantz, H. & Slotkin, T. A. The "stress" of being born. *Sci. Am.* **254**, 100–107 (1986).
30. Duley, L. & Batey, N. Optimal timing of umbilical cord clamping for term and preterm babies. *Early Hum. Dev.* **89**, 905–908 (2013).
31. Rabe, H., Diaz-Rossello, J. L., Duley, L. & Dowswell, T. Effect of timing of umbilical cord clamping and other strategies to influence placental transfusion at preterm birth on maternal and infant outcomes. *Cochrane Database Syst. Rev.* **8**, CD003248 (2012).
32. Cheung, C. Y. & Brace, R. A. Norepinephrine effects on fetal cardiovascular and endocrine systems. *Am. J. Physiol. Heart Circ. Physiol.* **254**, H734–H741 (1988).
33. Palmer, S. M. et al. Catecholamine physiology in the ovine fetus. I. Gestational age variation in basal plasma concentrations. *Am. J. Obstet. Gynecol.* **149**, 420–425 (1984).
34. Jensen, A., Hohmann, M. & Kunzel, W. Redistribution of fetal circulation during repeated asphyxia in sheep: effects on skin blood flow, transcutaneous PO_2, and plasma catecholamines. *J. Dev. Physiol.* **9**, 41–55 (1987).
35. Galinsky, R. et al. Sustained sympathetic nervous system support of arterial blood pressure during repeated brief umbilical cord occlusions in near-term fetal sheep. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **306**, R787–R795 (2014).
36. Crossley, K. J. et al. Dynamic changes in the direction of blood flow through the ductus arteriosus at birth. *J. Physiol.* **587**, 4695–4704 (2009).
37. Hunter, C. J., Blood, A. B. & Power, G. G. Cerebral metabolism during cord occlusion and hypoxia in the fetal sheep: a novel method of continuous measurement based on heat production. *J. Physiol.* **552**, 241–251 (2003).
38. Appel, E., Dudziak, R., Palm, D. & Wnuk, A. Sympathoneuronal and sympathoadrenal activation during ketamine anesthesia. *Eur. J. Clin. Pharm.* **16**, 91–95 (1979).
39. Strumper, D. et al. The effects of S+-ketamine and racemic ketamine on uterine blood flow in chronically instrumented pregnant sheep. *Anesth. Analg.* **98**, 497–502 (2004).
40. Lear, C. A. et al. The myths and physiology surrounding intrapartum decelerations: the critical role of the peripheral chemoreflex. *J. Physiol.* **594**, 4711–4725 (2016).