Onset of asphyxial state in nonrespiring interval between cord clamping and ventilation increases hemodynamic lability of birth transition in preterm lambs

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Smolich JJ, Kenna KR, Cheung MM. Onset of asphyxial state in nonrespiring interval between cord clamping and ventilation increases hemodynamic lability of birth transition in preterm lambs. J Appl Physiol 118: 675–683, 2015. First published January 22, 2015; doi:10.1152/japplphysiol.01147.2014.—Experimentally, a typical ~2-min cord clamp-to-ventilation interval in preterm lambs is accompanied by increased hemodynamic lability of the birth transition. However, whether this lability is related to development of asphyxia after cord clamping, or can be avoided with a shorter clamp-to-ventilation interval, is unknown. To address these questions, anesthetized preterm fetal lambs (gestation 127 ± 2 days) were instrumented with ductus arteriosus and left pulmonary artery flow probes to obtain right ventricular (RV) output, brachiocephalic trunk and aortic isthmus flow probes to measure left ventricular (LV) output, and aortic trunk catheters for pressure measurement and blood gas analysis. With hemodynamics recorded continuously, fetuses were delivered onto the ewe’s abdomen and the cord clamped for 1.5 min before ventilation (n = 8), with aortic sampling at 15, 30, 45, and 60 s, or for 0.5 min, with sampling at 15 s (n = 4). With 1.5-min cord clamping, an asphyxial state (P<sub>O2</sub> < 10 mmHg) was evident at ≥45 s, with bradycardia and marked falls in LV and RV outputs (by 60% and 50%, P < 0.001), followed after ventilation onset by tachycardia and LV and RV output surges (4- and 3-fold, P < 0.001). By contrast, heart rate and outputs remained stable after 0.5-min cord clamping, with no postventilation change in heart rate or RV output, and a lesser rise in LV output (22%, P < 0.005). In preterm lambs, rapid development of an asphyxial state within 45 s in the cord clamp-to-ventilation interval increased hemodynamic lability of the birth transition, which was reduced with a shorter (~0.5 min) cord clamp-to-ventilation interval.

preterm birth; perinatal hemodynamics; cardiovascular stability; ventricular outputs; umbilical cord clamping

THE BIRTH TRANSITION involves a striking circulatory reorganization. Thus in the fetus, only a small portion of the right ventricular (RV) output passes to the fluid-filled lungs, with most of this output shunted right-to-left across the ductus arteriosus into the descending thoracic aorta, from where it perfuses lower body tissues and passes to the placenta for O2 and CO2 exchange. On the other hand, fetal left ventricular (LV) output is mainly distributed to upper body tissues, with only a minor portion crossing the aortic isthmus into the descending thoracic aorta. With ventilation at birth, the bulk of RV outflow is redirected toward the lungs, with pulmonary blood flow further enhanced by a reversal of ductal shunting, while LV output increases to provide not only the entire systemic output, but also left-to-right ductal shunt flow (10, 14, 29, 32). In the preterm newborn, this transition may be ac- companied by an increased lability of blood pressures and organ blood flows (2), due to factors such as a structural and functional immaturity of the heart and vasculature (17, 29). As cardiovascular lability increases the risk of adverse events like cerebral intraventricular hemorrhage in preterm infants (7, 13), minimization of blood pressure and flow fluctuations is thus an important component of treatment strategies in the neonatal period.

As their lungs are structurally immature and surfactant-deficient (18), preterm newborns often require respiratory support, with a common practice comprising rapid clamping of the umbilical cord followed by the start of mechanical ventilation (8). Although the time between these events is not usually provided in publications, available data suggest that a typical interval of 1.5–2 min in chronically instrumented fetal lambs (3, 28) is accompanied by 1) bradycardia, arterial blood pressure fluctuations and falls in RV output attributed to alterations in cardiac loading conditions arising from loss of the placental circulation, and 2) substantial rises in heart rate, blood pressure, and RV output after birth (3). However, cord clamping prior to ventilation also gives rise to a “nonrespiring” interval within the fetal-to-newborn transition. This is of particular relevance as induction of an analogous interval in utero by either complete occlusion of the uterine arteries (22, 23) or umbilical cord (16, 20, 39, 40) results in rapid development of an asphyxial state that is evident by 1 min and marked at 2 min. Importantly, this asphyxial state is accompanied by bradycardia and changes in arterial blood pressure, as well as falls in cardiac output and peripheral blood flows (2, 16, 22, 23), that constitute an integrated and coordinated response with an initial vagal stimulation and subsequent sympathoadrenal activation (2). Moreover, relief of this asphyxial state may be associated with transient overshoots of heart rate, blood pressure and regional blood flows (2, 22). However, the question of whether perinatal hemodynamic fluctuations seen with a typical nonrespiring interval between cord occlusion and onset of ventilation (3) are related to development of an asphyxial state has never been specifically addressed. Furthermore, it is unknown whether these fluctuations can be ameliorated by shortening the duration of the cord clamping-to-ventilation interval.

This study, where aortic trunk blood was sampled frequently for gas analysis while arterial blood pressures, ventricular outputs, and associated major blood flows were measured continuously at birth of preterm fetal lambs, therefore tested two main hypotheses. The first was that a ~1.5-min cord clamping-to-ventilation interval would be accompanied by rapid emergence of an asphyxial state underpinning substantial fluctuations in hemodynamics during the ensuing birth transition. On the basis of results obtained in this initial study, the second hypothesis was that reducing this interval to ~0.5 min

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O2 content, mM 90sCC 3.53
PaO2, mmHg 90sCC 20.1
arterial O2 tension; PaCO2, arterial CO2 tension. *
SaO2,% 90sCC 55.0
pH 90sCC 7.302
(90sCC) or approximately 30 s (30sCC)
Hb, g/dl 90sCC 10.6
METHODS

would avoid development of an established asphyxial state and thus lessen hemodynamic lability of the birth transition.

METHODS

Studies were approved by the Murdoch Childrens Research Institute Animal Ethics Committee and conformed to National Health and Medical Council of Australia guidelines.

Surgical preparation. The general features of the anesthetic and monitoring procedures were as previously described (34–36). Briefly, 12 Border-Leicester cross ewes were anesthetized at a gestation of 127 ± 2 days (mean ± SD, term = 147 days) with intramuscular 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 ventilator in O2-enriched air, supplemented by intravenous infusion of midazolam (0.1–0.15 mg·kg⁻¹·h⁻¹) and fentanyl (2–2.5 mg·kg⁻¹·h⁻¹). Transcutaneous oxygen saturation (Sao2) was monitored continuously with a pulse oximeter sensor applied to the ear. The right common carotid artery was cannulated for monitoring of blood pressure and regular blood gas analysis (ABL800, Radiometer, Copenhagen, Denmark), with ventilation of the ewe adjusted to maintain arterial O2 tension (PaO2) at 100–120 mmHg and CO2 tension (PaCO2) at 35–40 mmHg.

Following a midline laparotomy and hysterotomy, the fetal head was exteriorized and placed in a saline-filled glove to prevent loss of lung liquid. The left and right common carotid arteries were exposed through a midline neck incision and encircled with 3-mm transit-time flow probes (Transonic Systems, Ithaca, NY). After delivery of the left forelimb and thorax, a fluid-filled catheter was passed into the superior vena cava via the left axillary vein, for fluid and drug administration. The aortic trunk was cannulated via the left axillary artery with a short fluid-filled catheter for pressure measurement and blood sampling, and a 3.5-Fr micromanometer (SPR-524, Millar Instruments, Houston, TX) to measure high-fidelity pressure. A thoracotomy was performed in the third interspace and major vessels carefully dissected for nonconstrictive placement of flow probes (Transonic Systems) around the brachiocephalic trunk (4–6 mm), aortic isthmus (6 mm), ductus arteriosus (8–10 mm), and left pulmonary artery (4–6 mm). A fluid-filled catheter and 3.5-Fr micromanometer were inserted via purse-string sutures into the pulmonary trunk close to its junction with the ductus and common pulmonary artery to measure pressures (Fig. 1). Following completion of the thoracic procedure, a clamped 4.5-mm endotracheal tube was inserted via a tracheostomy in a proximal intercartilaginous space and tied into place. The external part of the endotracheal tube contained a side port for measurement of tracheal pressure via a fluid-filled catheter.

Experimental protocol. Just prior to the birth delivery, the endotracheal tube was unclamped to allow lung liquid to drain passively via gravity for ~20 s and then reclamped to prevent lung aeration prior to ventilation. While hemodynamics were recorded continuously onto computer, the fetus was completely delivered from the uterus, placed on the ewe’s abdomen without any tension on the umbilical cord, and covered with warmed towels. After an aortic sample was withdrawn anaerobically 30 s later for blood gas analysis, the umbilical cord was occluded with a clamp 1–2 cm from its abdominal insertion site, and animals were then subjected to one of two protocols. In the main protocol (90sCC, n = 8), aortic blood gas samples were collected ~30 and ~60 s after cord clamping, and in five of these lambs, also at ~15 and ~45 s after cord clamping. In the second

Table 1. Aortic trunk blood gas variables before and after clamping of umbilical cord for either approximately 90 s (90sCC) or approximately 30 s (30sCC)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Baseline</th>
<th>15 s CC</th>
<th>30 s CC</th>
<th>45 s CC</th>
<th>60 s CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb, g/dl</td>
<td>90sCC</td>
<td>10.6 ± 1.1</td>
<td>10.6 ± 1.1</td>
<td>10.4 ± 1.1</td>
<td>10.4 ± 1.1</td>
<td>10.5 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>30sCC</td>
<td>11.1 ± 0.6</td>
<td>11.3 ± 0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>90sCC</td>
<td>7.302 ± 0.023†</td>
<td>7.278 ± 0.024</td>
<td>7.271 ± 0.021</td>
<td>7.266 ± 0.021</td>
<td>7.254 ± 0.021</td>
</tr>
<tr>
<td></td>
<td>30sCC</td>
<td>7.329 ± 0.016*</td>
<td>7.304 ± 0.014</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>SaO2, %</td>
<td>90sCC</td>
<td>55.0 ± 6.1†</td>
<td>29.1 ± 3.7</td>
<td>15.5 ± 5.5</td>
<td>9.4 ± 2.3</td>
<td>6.5 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>30sCC</td>
<td>62.0 ± 4.6†</td>
<td>35.1 ± 2.4</td>
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<td></td>
</tr>
<tr>
<td>PaO2, mmHg</td>
<td>90sCC</td>
<td>20.1 ± 2.1†</td>
<td>14.4 ± 1.0</td>
<td>10.7 ± 1.6</td>
<td>8.3 ± 1.0</td>
<td>6.9 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>30sCC</td>
<td>22.0 ± 2.2*</td>
<td>15.3 ± 1.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PaCO2, mmHg</td>
<td>90sCC</td>
<td>48.6 ± 2.5†</td>
<td>54.6 ± 2.2</td>
<td>55.4 ± 2.7</td>
<td>55.7 ± 2.7</td>
<td>57.1 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>30sCC</td>
<td>46.0 ± 3.5†</td>
<td>51.6 ± 3.5</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>O2 content, mM</td>
<td>90sCC</td>
<td>3.53 ± 0.42†</td>
<td>1.86 ± 0.22</td>
<td>0.96 ± 0.31</td>
<td>0.59 ± 0.13</td>
<td>0.41 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>30sCC</td>
<td>4.16 ± 0.23†</td>
<td>2.40 ± 0.06</td>
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<td></td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD; n = 8 for 90sCC and 4 for 30sCC groups. Hb, hemoglobin concentration; SaO2, hemoglobin oxygen saturation; PaO2, arterial O2 tension; PaCO2, arterial CO2 tension. *P < 0.005 and †P < 0.001 compared with values after cord clamping.

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protocol, an aortic blood gas sample was collected ~15 s after cord clamping (30sCC, n = 4). After the last aortic blood sample was taken in each protocol, the endotracheal tube was connected to an infant ventilator (SLE 5000) and positive-pressure mechanical ventilation commenced with a warmed and humidified O2/air mixture. The interval between umbilical cord clamping and the start of ventilation, obtained from subsequent analysis of hemodynamic and tracheal pressure profiles, was 86 ± 5 and 25 ± 1 s for the 90sCC and 30sCC groups, respectively.

Initial ventilator settings comprised a peak inspiratory pressure of 50 cm H2O, a positive end-expiratory pressure of 8 cm H2O, a respiratory rate of 60 breaths/min, an inspiratory time of 0.4 s, a tidal volume of 6 ml/kg estimated body weight, and an inspired O2 concentration of 30%. Ventilator settings were not changed in the initial 2 min after the start of ventilation, but were subsequently adjusted as required to maintain a SaO2 of >90% measured with a pulse oximetry sensor applied to the cheek pouch. After delivery, anesthesia in newborn lambs was continued with an intravenous infusion of ketamine (4–8 mg·kg−1·h−1) and midazolam (0.05–0.1 mg·kg−1·h−1), with aortic blood gas samples obtained at 0.5, 1, 2, 3, 5, and 10 min after the start of ventilation in both groups.

The recording of physiological data onto computer commenced just prior to delivery was continued for a further 10 min after the start of ventilation. After completion of this recording and cutting of the umbilical cord, the lamb was carefully transferred onto a heated neonatal resuscitation table. Hemodynamic data was subsequently collected at 15 and 30 min after onset of ventilation, with each recording preceded by withdrawal of an aortic sample for blood gas analysis. Beyond the 10-min time point, ventilator settings, tracheal catheter pressures were measured with transducers referenced to atmospheric pressure at left atrial level and calibrated against a water manometer before each study. Signals from catheters, micromanometers and flow probes were digitized at a sampling rate of 1 kHz with programmable acquisition and analysis software (Spike2, Cambridge Electronic Design, Cambridge, UK).

As hemodynamics can change very rapidly in the perinatal period, 5-s data blocks were analyzed from the delivery data file at 1) 15, 30, 45, and 60 s after cord clamping in the 90sCC group, and at 15 s after cord clamping in the 30sCC group; 2) immediately before the start of ventilation; and 3) at 15 s intervals in the first minute after the start of ventilation. In addition, 10-s data blocks were analyzed 1) just before cord clamping and 2) at 2, 3, 4, 6, 8, 10, 15, and 30 min after ventilation onset. Apart from a 48-Hz low-pass filter to remove electrical interference from signals, no filtering was employed during analysis, with analyses undertaken on ensemble-averaged signals typically generated from 12–25 beats.

Mean aortic and pulmonary micromanometer pressures were matched to corresponding catheter pressures. LV output (minus coronary blood flow) was calculated as the sum of the brachiocephalic trunk and aortic isthmus flows, noting that the brachiocephalic trunk is the only major cephalic branch of the aorta in sheep. RV output was derived as the sum of ductal flow and the combined left and right pulmonary arterial flow, calculated as the product of the measured left pulmonary arterial flow and the total-to-left lung weight ratio (31, 35). Total carotid arterial flow, which is closely related to cerebral perfusion in the perinatal period (37), equaled the summed left and right carotid arterial flows. Aortic O2 content was computed as (1.36·SaO2·Hb/100) + 0.003·P50·O2, where Hb = hemoglobin concentration (g/dl).

Statistical analysis. Results were analyzed with GraphPad Prism (v6.02, La Jolla, CA). Time-related changes in fetal blood oxygenation variables after cord clamping were analyzed with least squares linear regression. Longitudinal hemodynamic and blood gas data in the 30sCC and 90sCC groups were analyzed with one way repeated measures analysis of variance, with separate analyses performed for fetal and newborn data. Specific comparisons in each group 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. Data at given time points between groups were compared with unpaired Student’s t-tests. Data are expressed as means ± SD, and significance was taken at P < 0.05.

RESULTS

Blood gases. Cord clamping reduced SaO2, PaO2, O2 content, and pH and increased PaCO2 within 15 s (P < 0.005), with an asphyxial state evident by 45 s in the 90sCC group (Table 1). Indeed, oxygenation decreased linearly over 30–35 s after clamping, with SaO2, PaO2, and O2 content falling at rates of...
1.3% /s, 0.3 mmHg /s, and 0.1 mM /s, respectively, before plateauiing (Fig. 2). SaO2, PaO2, and aortic O2 content rose rapidly in both groups after ventilation (P < 0.001), while PaCO2 fell (P = 0.01). However, although unaltered in the 30sCC group, pH initially declined further in the 90sCC group after birth (P = 0.01), but recovered by 10 min (Table 2).

Blood pressures and heart rate. Cord clamping increased mean aortic and pulmonary blood pressures stepwise by 8.2 mmHg (P < 0.001; Fig. 3 and 4, A and B). These pressures then remained unchanged in the 30sCC group, but fell by 15.1 mmHg after 60 s in the 90sCC group (P < 0.001), before partially recovering just before ventilation. In the 30sCC group, aortic and pulmonary blood pressures fell transiently by 11.5 mmHg in the initial 2 min after ventilation (P < 0.001), before partly recovering. By contrast, these pressures surged by 23.6 mmHg to a peak at 60 s in the 90sCC group (P < 0.001), and then declined to a plateau that was 7.7 mmHg higher than in the 30sCC group at ≥15 min (P < 0.02; Fig. 4, A and B).

Heart rate (158 beats/min) was unchanged in the initial 30 s after cord clamping, but then fell to a nadir of 113 beats/min by 60 s in the 90sCC group (P = 0.002). With ventilation, heart rate was unaltered in the 30sCC group, but almost doubled within 15 s in the 90sCC group (P < 0.001), and then declined to a similar plateau in both groups (Fig. 4C).

Ventricular outputs. After cord clamping, LV output fell 24% and RV output by 18% (P < 0.001; Fig. 3 and 5). LV and RV outputs then remained unchanged in the 30sCC group, but in the 90sCC group fell at ≥45 s to be 39% and 49% of their immediate postclamp value just prior to ventilation (P < 0.001). With ventilation in the 30sCC group, LV output rose 22% (P < 0.005), but RV output was unaltered (P > 0.5). LV output then increased a further 85% over the ensuing 30 min (P = 0.001), while RV output remained unchanged (P = 0.5). By contrast, in the 90sCC group, LV output rose 4-fold and RV output 2.9-fold within 15 s of ventilation (both P < 0.001). LV output then increased 73% in the next 30 min (P < 0.001), while RV output fell 37% by 4 min (P < 0.001) and then partly recovered by 30 min (Fig. 5).

Central blood flows. Representative examples of perinatal changes in central blood flows are shown in Fig. 3. With cord clamping, brachiocephalic trunk flow rose 26% (P < 0.001) and carotid arterial flow 29% (P < 0.001). These flows remained stable in the 30sCC group, but fell at ≥60 s in the 90sCC group (P < 0.05), with an increase in the carotid arterial-to-brachiocephalic trunk flow ratio (from 57 ± 18 to 69 ± 19%, P = 0.005). With ventilation in the 30sCC group, brachiocephalic trunk flow fell 24% (P < 0.001) and carotid arterial flow 22% (P = 0.01). However, within 1 min of ventilation in the 90sCC group, a stepwise increase in brachiocephalic trunk flow exceeded that of carotid artery flow (32 ± 30 vs. 12 ± 16%, P < 0.025), so that the carotid arterial-to-brachiocephalic trunk flow ratio fell (P < 0.025), with a ~50% decline in both flows after 10 min (P < 0.001; Fig. 6, A and B).

Aortic ishmus flow fell abruptly to near zero with cord clamping (P < 0.001), and remained at this level in the 30sCC group, but became negative at ≥60 s in the 90sCC group (P = 0.001). Ishmus flow quickly rose after ventilation, with a greater increment in the 90sCC group (57 ± 27 vs. 34 ± 5 ml·kg⁻¹·min⁻¹, P < 0.05), and then increased more than threefold over the next 30 min in both groups (P < 0.001; Fig. 6C).

Pulmonary arterial flow rose 43% with cord clamping (P < 0.001) and remained unaltered in the 30sCC group, but fell to near zero at ≥60 s in the 90sCC group (P < 0.001). This flow increased with ventilation (P ≤ 0.01) and attained a similar peak by 15 min in the 90sCC and 30sCC groups (Fig. 6D). However, as pulmonary arterial flow was near zero just prior to ventilation in the 90sCC group, the ensuing increment after 15 s of ventilation (136 ± 36 ml·min⁻¹·kg⁻¹) was greater than in the 30sCC group (45 ± 17 mg·min⁻¹·kg⁻¹, P < 0.001).

Right-to-left ductal flow fell 36% after cord clamping (P < 0.001) and remained stable in the 30sCC group, but in the 90sCC group, fell further to be 60% of the initial postclamp value just prior to ventilation (P < 0.005). A fall in right-to-left ductal flow with ventilation tended to be greater in the 30sCC group (40 ± 17 vs. 11 ± 35 ml·kg⁻¹·min⁻¹, P < 0.08), with rapid emergence of left-to-right ductal shunting by 2–4 min in both groups (Fig. 6E).

DISCUSSION

With frequent sampling of aortic blood in the cord clamping-to-ventilation interval during the birth transition in preterm lambs of this study, a highly linear deoxygenation was evident in the initial half minute after cord clamping (Fig. 2), such that an asphyxial state with SaO2 ≤ 10%, PaO2 < 10 mmHg, and

Table 2. Aortic trunk blood gas variables with start of ventilation after clamping of umbilical cord for either approximately 90 s (90sCC) or approximately 30 s (30sCC)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>0.5 min</th>
<th>1 min</th>
<th>2 min</th>
<th>3 min</th>
<th>5 min</th>
<th>10 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb, g/dl</td>
<td>90sCC</td>
<td>11.3 ± 0.9</td>
<td>11.0 ± 0.9</td>
<td>10.9 ± 1.0</td>
<td>10.7 ± 1.1</td>
<td>10.6 ± 1.1</td>
<td>10.4 ± 1.1</td>
</tr>
<tr>
<td>30sCC</td>
<td>11.2 ± 0.5</td>
<td>11.2 ± 0.5</td>
<td>11.1 ± 0.6</td>
<td>10.9 ± 0.5</td>
<td>10.7 ± 0.4</td>
<td>10.9 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>90sCC</td>
<td>7.236 ± 0.018*</td>
<td>7.241 ± 0.021†</td>
<td>7.268 ± 0.043*</td>
<td>7.279 ± 0.054</td>
<td>7.289 ± 0.073</td>
<td>7.298 ± 0.071</td>
</tr>
<tr>
<td>30sCC</td>
<td>7.301 ± 0.008</td>
<td>7.304 ± 0.013</td>
<td>7.307 ± 0.018</td>
<td>7.297 ± 0.034</td>
<td>7.289 ± 0.064</td>
<td>7.308 ± 0.074</td>
<td></td>
</tr>
<tr>
<td>SaO2, %</td>
<td>90sCC</td>
<td>74.0 ± 19.4</td>
<td>84.6 ± 13.4</td>
<td>94.7 ± 8.3</td>
<td>95.6 ± 7.5</td>
<td>96.3 ± 6.7</td>
<td>98.0 ± 4.2</td>
</tr>
<tr>
<td>30sCC</td>
<td>65.4 ± 27.6</td>
<td>68.1 ± 26.1</td>
<td>74.1 ± 33.0</td>
<td>81.7 ± 30.6</td>
<td>95.9 ± 7.9</td>
<td>99.6 ± 0.5</td>
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</tr>
<tr>
<td>PaO2, mmHg</td>
<td>90sCC</td>
<td>34.2 ± 16.6</td>
<td>42.2 ± 17.8</td>
<td>75.2 ± 37.7</td>
<td>78.0 ± 36.6</td>
<td>83.1 ± 36.3</td>
<td>71.8 ± 16.2</td>
</tr>
<tr>
<td>30sCC</td>
<td>26.9 ± 11.8</td>
<td>27.7 ± 11.4</td>
<td>35.3 ± 18.2</td>
<td>65.0 ± 54.9</td>
<td>80.0 ± 32.8</td>
<td>91.0 ± 35.0</td>
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<tr>
<td>PacO2, mmHg</td>
<td>90sCC</td>
<td>54.7 ± 2.8</td>
<td>53.0 ± 3.3</td>
<td>47.6 ± 7.1</td>
<td>45.3 ± 8.8</td>
<td>44.3 ± 9.9</td>
<td>43.9 ± 9.6</td>
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<tr>
<td>30sCC</td>
<td>47.3 ± 2.0</td>
<td>47.0 ± 1.3</td>
<td>45.5 ± 2.0</td>
<td>46.1 ± 3.6</td>
<td>46.6 ± 6.6</td>
<td>45.4 ± 7.1</td>
<td></td>
</tr>
<tr>
<td>O2 content, mM</td>
<td>90sCC</td>
<td>5.06 ± 1.41</td>
<td>5.69 ± 1.20</td>
<td>6.30 ± 0.85</td>
<td>6.21 ± 0.79</td>
<td>6.18 ± 0.80</td>
<td>6.19 ± 0.68</td>
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<tr>
<td>30sCC</td>
<td>4.37 ± 1.75</td>
<td>4.56 ± 1.66</td>
<td>4.92 ± 2.09</td>
<td>5.32 ± 1.87</td>
<td>6.24 ± 0.36</td>
<td>6.56 ± 0.21</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD; n = 8 for 90sCC and 4 for 30sCC groups. Blood gas variables were not significantly different between the 90sCC and 30sCC groups at 15 and 30 min time points (data not shown). *P = 0.05 and †P < 0.001 90sCC vs. 30sCC groups.
aortic O₂ content < 0.6 mM, accompanied by a substantial fall in pH and rise in PaCO₂, was present at ≈45 s (Table 1). Although new, these findings are not surprising given prior reports in chronically instrumented preterm fetal lambs that a marked reduction in SaO₂ (from 54 to 14%) occurred by 1 min after arrest of uterine blood flow (22), whereas striking falls in PaO₂ from 20–23 to 5 mmHg (20, 39) and arterial O₂ content from 4.3 to 0.6 mM (20) were evident by 2 min of umbilical cord occlusion.

Despite a rapid deoxygenation after cord clamping, heart rate, blood pressures, ventricular outputs, and major flows remained stable in the initial 30 s, suggestive of a time lag between blood gas changes and the hemodynamic manifestations of hypoxemia progressing to anoxemia. However, hemodynamic changes were evident at 45 s and clearly established by 60 s after cord clamping, with bradycardia, marked falls in ventricular outputs and central blood flows and, consistent with preferential passage of systemic flow toward the brain, a rise in the carotid arterial-to-brachiocephalic trunk flow ratio. This pattern closely resembles that of in utero asphyxia, which is characterized by bradycardia, a pronounced drop in the combined ventricular output within 2 min, and a redistribution of blood flow away from peripheral tissues toward central organs such as the brain (2, 21–23).

The basis of hemodynamic alterations seen with in utero asphyxia is well delineated, with bradycardia due to an initial chemoreceptor-mediated vagal stimulation and blood flow changes related to subsequent sympathoadrenal activation (21, 39). This activation produces >20-fold rises in plasma norepinephrine and epinephrine levels by 1 min (22), which increase even higher after 2 min (16, 22). A plausible interpretation of hemodynamic fluctuations in our study was therefore that cord clamping prior to ventilation at delivery resulted in an activation of similar neurohumoral mechanisms after 45 s, secondary to development of an asphyxial state. As a corollary, absence of these hemodynamic fluctuations strongly implied that acti-

![Fig. 3. Illustrative examples of changes in aortic trunk (AoT), pulmonary trunk (PT), and tracheal (Tr) pressures, left (LV) and right (RV) ventricular outputs, and total carotid arterial (CA), brachiocephalic trunk (BCT), aortic isthmus (AI), total pulmonary arterial (PA), and ductus arteriosus (DA) blood flows after clamping of the umbilical cord (shaded areas) for −30 s (30sCC) or −90 s (90sCC), followed by the first 90 s after the start of ventilation. In the 90sCC group, note slight delay (~10 s) before marked changes in hemodynamics occur after the start of ventilation.](image-url)
vation of these mechanisms was substantially attenuated with a shorter cord clamp-to-ventilation interval of ~30 s. Definitive confirmation of these conclusions will, however, require measurement of plasma norepinephrine and epinephrine levels in future studies.

Three hemodynamic changes occurring after cord clamping in the present study are of particular interest. First, the stepwise rise in systemic blood pressure (Fig. 3 and 4) implied that this change directly resulted from the loss of vascular connection to the low-resistance placental circulation. With subsequent emergence of an asphyxial state in the 90sCC group, systemic blood pressure then transiently decreased, presumably reflecting a short-lived dominant effect of vagal stimulation (26), before rising again just before the onset of ventilation due to a pressor effect of sympathoadrenal activation (21).

Second, mean pulmonary arterial blood flow initially rose, despite falls in RV output (Fig. 5B) and right-to-left ducal blood flow (Fig. 6E), indicative of a redistribution of RV output away from the ducus and toward the lungs that was presumably related to an increase in postdural vascular resistance, relative to pulmonary vascular resistance. The subsequent fall in pulmonary arterial blood flow to near zero in the 90sCC group was most likely due to an increased pulmonary vasoconstriction known to occur with low blood O2 levels in the fetus (1, 23, 25), particularly when the latter is associated with acidemia (9).

Third, net aortic isthmus flow fell abruptly to a value not significantly different from zero (Fig. 6C), in part due to an increased retrograde (i.e., negative) flow component (Fig. 3). This change, which is reminiscent of the pattern seen with a large rise in placental vascular resistance (5), effectively separated the circulation into an upper body compartment perfused by the LV, and a lung and lower body compartment receiving blood from the RV. The presence of a negative net isthmus flow after development of an asphyxial state in the 90sCC group indicated that retrograde isthmus flow then exceeded forward flow (15), i.e., that blood of RV origin passing across the ducus contributed to perfusion of upper body tissues.

The findings of the present study run counter to the view that the hemodynamic impact of a 2-min period of umbilical cord clamping at the time of delivery relates primarily to its diminution of cardiac venous return, with a large reduction in RV output attributed solely to falls in cardiac filling (3). Instead, our findings strongly suggested that hemodynamic changes after cord clamping prior to ventilation had two distinct components. The first, manifest for ~30 s, was directly due to loss of the low-resistance placental circulation and a reduction in venous return, with ventricular outputs reduced by 20–25%.
Fig. 6. Total carotid arterial (A), brachiocephalic trunk (B), aortic isthmus (C), total pulmonary arterial (D), and ductus arteriosus (E) blood flows in the baseline fetal state (F), after umbilical cord clamping (Post CC), immediately before ventilation, and for 30 min after ventilation in study groups undergoing cord clamping prior to delivery for either 30 s (30sCC) or 90 s (90sCC). Note that the time scale of the initial minute after ventilation (shaded area) has been magnified to aid visualization.

net isthmus flow falling to zero, and systemic arterial blood pressure rising without any change in heart rate. In the second component, evident by 45 s and marked at ≥60 s, hemodynamics reflected a supervening asphyxial state, with bradycardia, further marked falls in ventricular outputs, a net retrograde isthmus flow, and a redistribution of systemic flow toward the brain.

Importantly, the asphyxial state which developed after cord clamping in the 90sCC group rapidly reversed after ventilation, with SaO₂ and PaO₂ quite similar to those of the 30sCC group by 30 s after birth (Table 2). On the other hand, the progressive fall in pH evident in the 90sCC group during cord clamping was followed with the onset of ventilation by a further transient fall that was most likely due to tissue washout of the byproducts of anaerobic metabolism (e.g., lactate) following a surge in systemic perfusion. This interpretation is consistent with the finding that a significant rise in plasma lactate concentration after 2 min of in utero asphyxia did not occur until the postasphyxial recovery phase (22).

The combination of a tachycardia with transient surges in blood pressures, ventricular outputs, and systemic flows observed with ventilation in the 90sCC group in many respects resembles the overshoot in heart rate, pressures, and flows seen after relief of fetal asphyxia (2, 16, 20, 22). This resemblance is thus also consistent with our conclusion that sympathoadrenal activation occurred in the latter stages of cord clamping in the 90sCC group. Likewise, the lack of this overshoot response with a shorter cord clamp interval suggests an attenuation of the sympathoadrenal activation which normally occurs with the birth transition (24, 33). However, it is noteworthy that hemodynamic lability after birth in the 90sCC group was mainly confined to the initial 2–4 min after the start of ventilation, with no persisting differences in heart rate, ventricular outputs, or major regional flows between study groups, apart from slightly higher arterial pressures in the 90sCC group.

As autoregulatory mechanisms in the preterm cerebral vasculature are immature (7), fluctuations in carotid blood flow seen when cord clamping precedes ventilation have been attributed to a pressure dependency of flow (3). However, while aortic pressure and carotid arterial flow both increased in the first minute after ventilation in the 90sCC group, these increases did not parallel one another, with the former rising more than carotid flow after cord clamping in the 90sCC group, then plateauing after an initial abrupt increment (Fig. 6A) and the latter plateauing after an initial abrupt increment (Fig. 6A). This divergent pattern suggests an alternate explanation for the brief and very rapid rise in carotid arterial (and brachiocephalic trunk) flow seen with the onset of ventilation in the 90sCC group, namely that it represented a reactive hyperemia accompanying postschematic reperfusion, akin to that of the recovery phase after in utero asphyxia (12). This explanation is also in accord with the observation that brachiocephalic trunk flow, which fell relatively further than carotid flow after cord clamping in the 90sCC group, then increased more than carotid flow with ventilation.

While this is the first study to have continuously monitored both LV and RV outputs and their associated central blood flows in lambs before and during the birth transition via flow probes placed on all major arteries, a potential limitation was that the extent of this instrumentation necessitated an open-chest study performed under general anesthesia. However, baseline blood gas and hemodynamic data in our preparation were similar to those of unanesthetized, chronically instrumented, hypoxic and asphyxial lambs and these data are consistent with those previously reported from other studies.
mented preterm fetal lambs (3, 10, 16, 20, 39). Moreover, key features of the birth transition, such as a large and rapid rise in pulmonary arterial flow and a rapid reversal of ductal shunting, were similar to those previously reported in chronically instrumental fetal lambs (3, 10, 14, 30). Nonetheless, we cannot exclude the possibility that dissection around major central arteries for placement of flow probes altered changes in central flow patterns during the birth transition, although any such effect is likely to be minor.

Our study results have significant implications for the interpretation of a recent study in preterm lambs which concluded that early ventilation with delayed cord clamping was substantially better for cardiovascular stability at birth than early cord clamping followed by ventilation 2 min later (3). Importantly, this early cord clamping-to-ventilation interval was accompanied by bradycardia and a marked reduction in RV output before birth, followed by large fluctuations in heart rate, blood pressures, and blood flows after birth (3). As these findings are very similar to those observed in the 90sCC group (Fig. 3–6), our data therefore suggest that the conclusion of Bhatt et al. (3) primarily reflected the detrimental effects of an asphyxial state resulting from an extended period of cord clamping prior to ventilation, rather than a beneficial effect per se of early ventilation with delayed cord clamping. Furthermore, on the basis of Bhatt et al. (3), ventilation prior to delayed cord clamping has been advocated as the paradigm for a smoother physiological transition from the fetal-to-newborn state (4, 19, 27). However, our data indicate that this type of transition can also be achieved with early cord clamping and a short (~30 s) clamp-to-ventilation interval, implying that the primary consideration for cardiovascular stability during the birth transition is avoidance of an asphyxial state with its attendant hemodynamic accompaniments and sequelae.

Our study results also have implications for heart rate changes seen clinically at delivery. Bradycardia commonly occurs in human infants after cord clamping, especially in those in whom respiration is delayed (6), and is considered to be reflex in nature because of its rapidity of onset (6, 11). However, although vagally mediated bradycardia is a known accompaniment of fetal hypoxemia and asphyxia (9, 16, 21–23), that the latter are also the usual stimulus for birth-related bradycardia has been questioned in a recent pulse oximetry study on the basis of clinical assessment and the APGAR score of infants after birth, with an alternate proposed mechanism being reflex bradycardia resulting from a reduction in venous return that follows cord occlusion (11). Our study results do not support this proposed mechanism, as heart rate was unchanged in the initial 30 s after cord clamping, despite a substantial decrease in cardiac output, with bradycardia only evident after the advent of an asphyxial state. Indeed, very recent data suggest that use of pulse oximetry to measure heart rate may overestimate the incidence of birth-related bradycardia, as this technique underestimates heart rate derived with gold standard electrocardiography, particularly in the initial 2 min after birth (38).

In summary, this study has shown that the duration of the nonrespiring interval between umbilical cord clamping and mechanical ventilation exerts a major effect on perinatal hemodynamics in preterm lambs. Although hemodynamics were initially stable after cord clamping, an asphyxial state emerged by 45 s, accompanied by bradycardia, changes in blood pressure, and pronounced falls in ventricular outputs and central blood flows. Relief of this asphyxial state with the onset of ventilation was followed by a transient tachycardia with pressure and flow surges. Reducing the duration of cord clamping to ~30 s to avoid establishment of an asphyxial state markedly attenuated hemodynamic alterations before and after ventilation. Given these findings, we strongly endorse the need to record the times of cord clamping and start of respiration during the birth transition (27), so that the duration of the intervening interval can be evaluated in relation to hemodynamic stability after birth.

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No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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