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RESEARCH ARTICLE

Transplacental oxygen transfer during physiological-based cord clamping in preterm lambs

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Abstract

Physiological-based cord clamping (PBCC) involves aeration of the newborn lung before umbilical cord clamping. As the infant can continue to receive oxygen from the mother during PBCC, the dynamics of transplacental oxygen transfer are unknown, particularly following the onset of pulmonary gas exchange. We have investigated the effects of pulmonary ventilation with supplemental oxygen during PBCC on transplacental oxygen transfer in preterm lambs. Pregnant ewes and their fetuses ($n = 8$; 127 days of gestation; term 147 days) were instrumented with catheters and flow probes under general anesthesia to measure oxygen transfer across the placenta. Before umbilical cord clamping, lambs were intubated and mechanically ventilated with a fraction of inspired oxygen ($F_{I_{O_2}}$) that increased every 10 min, from 0.21 to 0.5 and then 1.0. Data were analyzed using a mixed-effects analysis with a Holm-Šídák post hoc test. During PBCC, ventilation of lambs significantly decreased oxygen uptake across the placenta from 317.7 ± 15.0 to 166.5 ± 26.9 ($P = 0.0028$) and to 73.6 ± 34.3 mL/min/kg ($P = 0.0009$) at $F_{I_{O_2}}$ levels of 0.21 and 0.5, respectively. Ventilation with a $F_{I_{O_2}}$ of 1.0 reversed oxygen uptake across the placenta (−37.2 ± 14.0 mL/min/kg; $P < 0.0001$), resulting in oxygen transfer from lamb to ewe. In contrast, oxygen delivery to the lamb, via the umbilical vein, remained unchanged with increasing $F_{I_{O_2}}$ ($P = 0.4485$). During PBCC, pulmonary oxygen uptake by the newborn reduces oxygen uptake across the placenta, and when oxygen levels are in excess, the mother acts as an "oxygen sink," reducing the risk of hyperoxemia of the newborn.

NEW & NOTEWORTHY This study investigated transplacental oxygen transfer during physiological-based cord clamping in preterm lambs. During physiological-based cord clamping, ventilating newborn lambs reduced oxygen uptake across the placenta and that ventilation with high oxygen (1.0 $F_{I_{O_2}}$) reversed the oxygen gradient, resulting in newborn to mother oxygen transfer. Our findings indicate that the mother can act as an "oxygen sink" that protects the newborn from excess oxygen during physiological-based cord clamping.

delayed cord clamping; hypoxia; oxygen gradient; ventilation

INTRODUCTION

Extremely preterm infants (<28 wk of gestation) have difficulty aerating their lungs and commencing pulmonary gas exchange, and so usually require respiratory support immediately after birth (1). Clinical care has now shifted toward initially providing noninvasive respiratory support at birth to avoid the higher risk of lung injury associated with intubation and mechanical ventilation (2–4). However, the success of noninvasive respiratory support largely depends on whether the infant is spontaneously breathing (5, 6). When apneic, newborns close their glottis, which prevents air from entering the airways and ventilating the lung, and when breathing is irregular, the glottis only opens during a breath (5, 6). As a result, when applied noninvasively via a facemask, positive pressure ventilation is ineffective unless it coincides with a breath (5, 6). Thus, it is now clear that stimulating regular, stable breathing and avoiding factors that inhibit breathing play a critical role in the success of noninvasive respiratory support at birth (7).

Hypoxia is a potent inhibitor of breathing in the fetus and newborn and is commonly experienced by infants at birth, particularly extremely preterm infants who have difficulty in aerating their lungs (8–10). This risk of hypoxia can be mitigated by increasing the oxygen concentration within the infant's inspired air (fraction of inspired oxygen; $F_{I_{O_2}}$). A

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higher $F_{I_{O_2}}$ compensates for the lung's lower gas exchange surface area when the lungs are partially liquid-filled by increasing the partial pressure gradient for oxygen diffusion. However, as the lung's gas exchange surface area increases exponentially as the lung aerates, a high $F_{I_{O_2}}$ increases the risk of hyperoxia, unless the $F_{I_{O_2}}$ is appropriately weaned. Although current clinical guidelines recommend low initial $F_{I_{O_2}}$ (0.21–0.3) levels to support preterm neonates at birth (11), recent preclinical studies, clinical trials, and meta-analyses question this recommendation and suggest that a higher $F_{I_{O_2}}$ (0.6–1.0) may better support spontaneous breathing and lower the risk of mortality (12–21).

Delaying umbilical cord clamping until after the lungs have aerated (either due to spontaneous breathing or positive pressure ventilation), termed physiological-based cord clamping (PBCC), also mitigates the risk of hypoxia at birth (22–25). This is because pulmonary gas exchange commences while the newborn is still receiving oxygen via the umbilical cord, which avoids the decrease in oxygenation associated with immediate cord clamping (23). In addition, as lung aeration stimulates an increase in pulmonary blood flow (PBF), the loss of cardiac output caused by cord clamping is also avoided. Although it is clear that the placenta can prevent the newborn from becoming hypoxic during PBCC (24), it is also conceivable that the placenta can protect the newborn from too much oxygen administered during PBCC. Indeed, although PBCC did not protect the lungs of newborn lambs from a sustained high $F_{I_{O_2}}$ of 1.0, it limited the increase in circulating oxygen levels (26).

We aimed to investigate transplacental oxygen transfer during PBCC, while newborn lambs are being mechanically ventilated with increasing $F_{I_{O_2}}$ levels. We hypothesized that during PBCC, ventilating preterm lambs, with and without supplemental oxygen, would reduce the oxygen gradient between the maternal and newborn circulations and, thereby, reduce transplacental oxygen transfer. Furthermore, we hypothesized that increasing oxygenation levels in the newborn may eventually reverse this gradient, leading to oxygen transfer into the mother, thereby protecting the newborn from over-oxygenation.

MATERIALS AND METHODS

Ethics Approval

Experimental methods were approved by Monash Medical Centre-A Animal Ethics Committee and were performed in accordance with the National Health and Medical Research Council (NHMRC) Code of Practice for the Care and Use of Animals for Scientific Purposes (27). Methodological reporting is provided per the relevant ARRIVE guidelines (28).

Surgical Preparation

At ~127 days gestation (term GA 147 days), singleton Border-Leicester ewes ($n = 8$) were anesthetized using sodium thiopentone (1 g in 20 mL; Pentothal; Jurox, New South Wales, Australia) and anesthesia was maintained, following endotracheal (ET) intubation (size 8–9 mm ET tube, cuffed, Portex Ltd., Kent, England), using inhaled isoflurane [0.5%–5% in air/oxygen blend (2:1 ratio); Isoflow, Abbott Pty. Ltd., New South Wales, Australia]. A midline incision was made in the abdomen to expose the uterus, which was incised to exteriorize the lower half of the fetus. The fetus was then instrumented by inserting indwelling catheters into a fetal femoral artery (FA) and umbilical vein (UmbV), as well as a 4-mm ultrasonic flow transducer (Transonic Systems, NY) around the common umbilical vein to measure total umbilical blood flow. As the tip of the FA catheter was located in the descending aorta above the branching point for the two external iliac arteries (which give rise to both umbilical arteries), the blood sampled from this catheter is equivalent to umbilical artery (UmbA) blood entering the fetal side of the placenta. A catheter was inserted downstream of a cotyledonary umbilical vein, and the tip was advanced toward the umbilical cord, which was validated by palpation. Catheters were also inserted into a maternal carotid artery (MA) and uterine vein (UtV), along with a 6-mm ultrasonic flow transducer around the main branch of the uterine artery (UtA) supplying the pregnant horn of the uterus (bicornate uterus) to measure uterine blood flow. Following instrumentation, the lamb was delivered, intubated (size 3.5–4.5 mm ET tube, cuffed, Portex Ltd., Kent, England), and a near-infrared spectroscopy (NIRS) sensor was placed on the skull immediately above the left cortex to measure cerebral tissue oxygen saturation (Sct_{O_2}). Blood flows and pressures, body temperature, and respiratory parameters (airway pressures and tidal volumes) were continuously recorded electronically (LabChart 8, Powerlab, ADInstruments, New South Wales, Australia) to measure the lamb's physiological parameters throughout the experiment.

Experimental Protocol

Immediately before the experiment start, lung liquid was passively drained from the endotracheal tube and paired blood samples were collected simultaneously from the MA, UtV, UmbV, and UmbA. With the umbilical cord remaining intact, lambs were then ventilated (Babylog 8000 Plus, Dräger, Germany) with a $F_{I_{O_2}}$ of 0.21, and a target tidal volume (Vt) of 7 mL/kg, while initially limiting the peak inflation pressure (PIP) to 35 cmH_2O, and an end-expiratory pressure (PEEP) of 5 cmH_2O was applied. The inspiratory time (Ti) was initially set at 0.5 s, and the expiratory time (Te) at 0.5 s, but if Vt was not reached within ~1 min, the PIP (to a max of 38 cmH_2O) and/or the Ti (to a max of 1.2 s) were increased. At 10 min after ventilation onset, which ensured that the target Vt had been reached, blood samples were collected simultaneously from all four vessels (as mentioned above), before the $F_{I_{O_2}}$ was increased to 0.5. After 10 min of ventilation with a $F_{I_{O_2}}$ of 0.5, simultaneous blood samples were again collected before the $F_{I_{O_2}}$ was increased to 1.0 for a further 10 min before more blood samples were collected. The umbilical cord was then clamped and cut while ventilation continued with a $F_{I_{O_2}}$ of 1.0. Five minutes after umbilical cord clamping, a final set of simultaneous blood samples was collected. After completion of the experiment, the fetus was humanely euthanized with sodium pentobarbitone (325 mg/mL; 0.5 mL/kg), and a postmortem analysis was performed.

Physiological and Blood Gas Analysis

For all physiological recordings, including blood pressures and flows, values were averaged over 30-s epochs at the end of each 10-min ventilation period at each $F_{I_{O_2}}$ level, to coincide with blood gas sample collections. Using the physiological measurements and blood gas values, we calculated blood

Table 1. *Equations used for the calculation of uteroplacental and neonatal oxygen transfer*

	Parameter	Equation
1	Oxygen content (To_2), mL O_2/dL	$(1.34 \times [Hb] \times So_2) + (0.0031 \times Po_2)$
2	Oxygen delivery (Do_2), mL O_2/min	$To_2 \times$ Blood Flow
3	Oxygen uptake ($\dot{V}o_2$), mL O_2/min	$(Tao_2 - Tvo_2) \times$ Blood Flow
4	Oxygen extraction, %	$\frac{\dot{V}o_2}{Do_2} \times 100$

O_2, oxygen; Hb, hemoglobin; Po_2, partial pressure of oxygen; So_2, oxygen saturation; Tao_2, total arterial oxygen content; Tvo_2, total venous oxygen content.

oxygen content in each of the four vessels, oxygen delivery, uptake, and extraction by the utero-placental unit (including the lamb), and oxygen delivery and uptake by the lamb. Uteroplacental oxygen transfer was calculated using MA (to indicate UtA oxygenation) and UtV blood gas values along with UtA blood flow. Newborn oxygen transfer was calculated using fetal UmbV and UmbA blood gas values along with UmbV blood flow. These measurements were calculated using previously described formulae (Table 1) (29, 30). A schematic of the direction of uterine blood flow and vasculature can be seen in Fig. 1. As UmbV blood flow of the preterm lamb ceased after umbilical cord clamping (CC), newborn oxygen calculations were not calculated after CC.

As it is difficult to quantify total maternal uterine blood flow perfusing the pregnant horn of the uterus, due to multiple sources of arterial blood, the percentage change from baseline (before the commencement of ventilation) [100 × (value − baseline)/baseline] was calculated. Newborn umbilical blood flow data were corrected for body weight (value/body weight). Therefore, all equations using blood flow were also corrected from baseline or body weight, as applicable.

Statistical Analysis

All data were analyzed using GraphPad Prism Version 10.4 (GraphPad Software, La Jolla, CA). Statistical significance was accepted as $P < 0.05$. Required animal numbers were calculated with a power analysis during experimental design, using α error probability of 0.05, and an effect size ($1 - \beta$ error probability) of 0.8, giving a sample size of $n = 6$–8.

All data were tested for normality using a Shapiro–Wilk test. Physiological data, including blood gas values, heart rate, blood pressure, blood flow, cerebral tissue saturation, and subsequent calculations (oxygen content, delivery, uptake, and extraction), were analyzed using a mixed-effect analysis. If the changes were significant, a Holm–Sidak post hoc test was used. All data are presented as means ± SE. As all animals were exposed to all concentrations of oxygen, we used a within-subjects experimental design.

RESULTS

Animal Characteristics

Lamb body and organ weights were not different (Table 2). Before ventilation of the lamb, pH values in the UtV were lower than after ventilation onset, followed by cord clamping (Table 3). pH was not different in the MA, UmbV, or UmbA throughout the experimental duration.

Blood Oxygenation Changes

Lamb Po_2, So_2 and $Sctо_2$ levels.

While attached to the umbilical cord, ventilation of the lamb, with increasing FI_{O_2} levels significantly increased the partial pressure of oxygen (Po_2) and oxygen saturation (So_2) in both the UmbA and UmbV, but most notably in the UmbA (Table 3). During ventilation with a FI_{O_2} of 1.0, the Po_2 in the UmbA tended to be greater than in the UmbV. At 5 min after cord clamping, when the lamb was still being ventilated with a FI_{O_2} of 1.0, arterial Po_2 levels in the lamb were significantly greater than Po_2 levels before cord clamping.

Ventilation of lambs with higher FI_{O_2} levels significantly increased cerebral tissue oxygen ($Sctо_2$; measured using

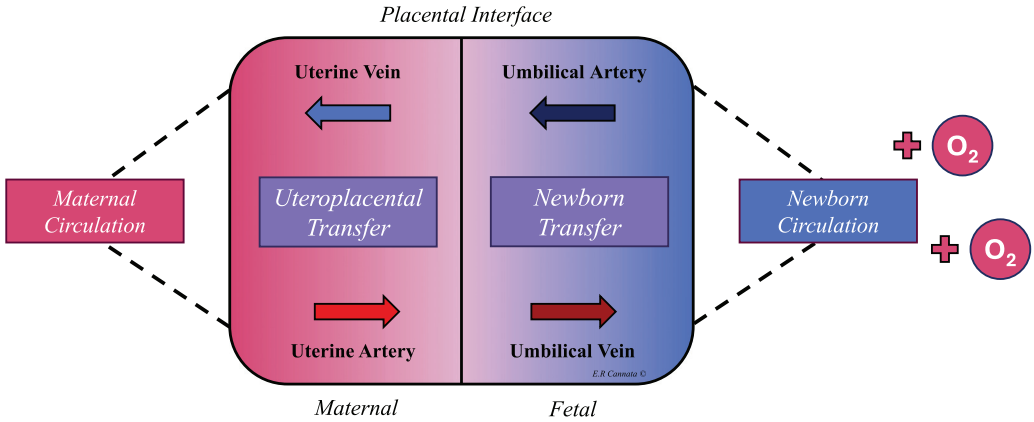

Figure 1. Schematic of uteroplacental and newborn oxygen transfer. The maternal uterine artery delivers oxygenated blood to the placenta, which supplies oxygen to the umbilical vein of the fetus. The fetus then delivers carbon dioxide back to the placenta via the umbilical artery, and through the uterine vein back into the maternal circulation.

Table 2. *Lamb characteristics*

Measure	Results	Shapiro–Wilk Test
Sex (male:female)	5:3	
Body weight, kg	3.8 ± 0.1	0.9265
Brain weight, g	47.4 ± 3.3	0.8770
Heart weight, g	27.6 ± 1.4	0.1310
Lung weight, g	142.9 ± 7.0	0.4671

Data are presented as means ± SE.

NIRS), but cord clamping had no additional effect on these levels (Table 3).

Maternal Po_2, So_2 levels, and maternal/lamb Po_2 gradients.

In the maternal circulation, the So_2 and Po_2 of blood in the UtV significantly and progressively increased as the FI_{O_2} used to ventilate the lamb increased. Despite the increase in UtV Po_2, the Po_2 gradient across the placenta (UtV − UmbA) reversed, from 33.2 ± 1.7 mmHg before ventilation of the lamb to −20.2 ± 20.4 mmHg (higher in the UmbA) following ventilation with a FI_{O_2} of 1.0 (Fig. 2). As a result, the Po_2 in arterial blood entering the fetal side of the placenta was substantially higher than the Po_2 of the venous blood leaving the maternal side of the placenta. Similarly, while Po_2 levels in the MA tended to increase during ventilation of the lamb on the cord, So_2 levels remained unchanged, likely because MA saturation levels were at or near 100% throughout the study. The increase in MA Pa_{O_2} was only significant at 0.5 FI_{O_2} (Table 3).

Maternal and lamb blood oxygen content.

The changes in blood oxygen content in lambs in response to ventilating lambs with increasing FI_{O_2} levels followed a similar pattern as the blood Po_2 and So_2 levels (Fig. 3). Before ventilation onset, the oxygen content of UmbA blood entering the fetal side of the placenta was 6.5 ± 1.2 mL O_2/dL, whereas the oxygen content of blood in the UtV leaving the maternal side of the placenta was 7.5 ± 0.5 mL O_2/dL. Following ventilation of the lamb with room air (0.21 FI_{O_2}), the blood oxygen content difference between the UmbA and UtV tended to reverse [in 5 of 8 (62.5%) lambs; mean of 9.8 ± 1.3 vs. 8.8 ± 0.4 mL O_2/dL, respectively] and was higher in UmbA, despite a large transplacental Po_2 gradient favoring the UtV (Table 3). Increasing the FI_{O_2} delivered to lambs markedly increased the blood oxygen content in the UmbA ($P < 0.0001$; Fig. 3*A*), and although it also increased in the UtV ($P < 0.0001$; Fig. 3*B*), it remained below that of the UmbA. Similarly, the blood oxygen content in the UmbV increased with increasing FI_{O_2} ($P = 0.0168$; Fig. 3*C*), but when the FI_{O_2} was 1.0, the oxygen content of blood in the UmbV was less than in the UmbA. Blood oxygen content in the MA did not change after each increase in FI_{O_2} in the lamb (Fig. 3*D*).

Uteroplacental Blood Flow and Cardiovascular Changes

UtA blood flow was variable over the experimental period and between animals, but did not change with the onset of ventilation or increasing FI_{O_2} concentrations (Fig. 4*A*). However, on the fetal side of the placenta, ventilation of lambs with a FI_{O_2} of 0.21 significantly decreased blood flow in the common UmbV from 332.2 ± 68.6 to 236.4 ± 57.9 mL/min/kg ($P = 0.0067$; Fig. 4*B*) following ventilation onset. UmbV blood flow then remained at this level with differing FI_{O_2} levels throughout the remainder of the experiment.

The lamb's heart rate did not change with increasing FI_{O_2} (Table 4), whereas the lamb's arterial blood pressure significantly decreased from 42.9 ± 3.4 mmHg to 38.7 ± 3.4 mmHg in response to ventilation onset ($P = 0.0366$; Table 4). Arterial blood pressure then increased during ventilation with 0.5 FI_{O_2} (to 42.4 ± 3.3 mmHg; $P = 0.0105$) and 1.0 FI_{O_2} (to 44.5 ± 3.5 mmHg; $P = 0.0366$). Within 5 min of clamping the umbilical cord, arterial blood pressure increased to 54.7 ± 4.7 mmHg while lambs continued to be ventilated in 1.0 FI_{O_2} ($P = 0.0105$). Maternal heart rate and maternal

Table 3. *Blood gas values (pH, Po_2, and So_2) for maternal and newborn blood vessels, and newborn NIRS in different oxygen levels (baseline, 0.21, 0.5, 1.0, and 1.0 post cord clamping)*

	Baseline	0.21 FI_{O_2}	0.5 FI_{O_2}	1.0 FI_{O_2}	1.0 FI_{O_2} Post CC	*P* Value
pH						
Uterine vein	7.36 ± 0.02^{a}	7.38 ± 0.01^{a,b}	7.38 ± 0.01^{a,b}	7.38 ± 0.01^{a,b}	7.39 ± 0.02^{b}	0.0451
Maternal artery	7.43 ± 0.01	7.42 ± 0.01	7.42 ± 0.01	7.42 ± 0.01	7.42 ± 0.01	0.0217
Umbilical vein	7.23 ± 0.03	7.25 ± 0.03	7.26 ± 0.03	7.27 ± 0.03	7.29 ± 0.04	0.0626
Umbilical artery	7.21 ± 0.03	7.23 ± 0.04	7.23 ± 0.04	7.23 ± 0.03	7.22 ± 0.04	0.1753
Po_2, mmHg						
Umbilical artery	18.6 ± 2.8^{a}	25.8 ± 3.4^{b}	42.64 ± 6.6^{c}	94.8 ± 22.1^{d}	128.6 ± 26.5^{e}	0.0038
Umbilical vein	34.2 ± 4.5^{a}	45.1 ± 5.4^{b}	51.7 ± 6.7^{b}	61.6 ± 11.5^{a,b}	N/A	0.0321
Uterine vein	51.8 ± 3.8^{a}	59.4 ± 3.6^{b}	68.3 ± 3.9^{c}	74.6 ± 3.6^{d}	74.0 ± 5.6^{b,c,d}	<0.0001
Maternal artery	139.8 ± 11.2^{a}	148.5 ± 10.3^{a,b}	155.1 ± 9.6^{b}	148.9 ± 10.9^{a,b}	149.3 ± 12.8^{a,b}	0.0496
So_2, %						
Maternal artery	100.1 ± 0.3	100.0 ± 0.1	99.9 ± 0.1	99.9 ± 0.1	99.2 ± 0.4	0.0548
Uterine vein	67.7 ± 4.4^{a}	78.8 ± 3.3^{b}	86.1 ± 2.8^{c}	90.3 ± 2.1^{d}	89.4 ± 4.0^{b,c,d}	<0.0001
Umbilical vein	71.6 ± 6.9	84.4 ± 3.5	89.0 ± 2.6	92.1 ± 2.1	N/A	0.0167
Umbilical artery	40.8 ± 8.3^{a}	59.9 ± 8.1^{b}	86.0 ± 2.7^{c}	96.2 ± 1.4^{d}	97.5 ± 1.5^{d}	0.0001
NIRS, %	46.1 ± 4.6^{a}	52.9 ± 4.8^{a}	65.3 ± 2.3^{b}	73.8 ± 2.7^{c}	76.5 ± 2.0^{c}	<0.0001

Data were analyzed with a mixed-effect analysis followed by Holm-Sidak post hoc test and presented as means ± SE. $n = 8$. Baseline, preventilation. N/A presented in 1.0 FI_{O_2} post CC (cord clamping) columns for the umbilical vein (UmbV) is due to the cutting of the umbilical cord. FI_{O_2}, fraction of inspired oxygen; NIRS, near infrared spectroscopy; Po_2, partial pressure of oxygen; So_2, oxygen saturation. Superscripted values that do not share a common letter are significantly different from each other ($P < 0.05$).

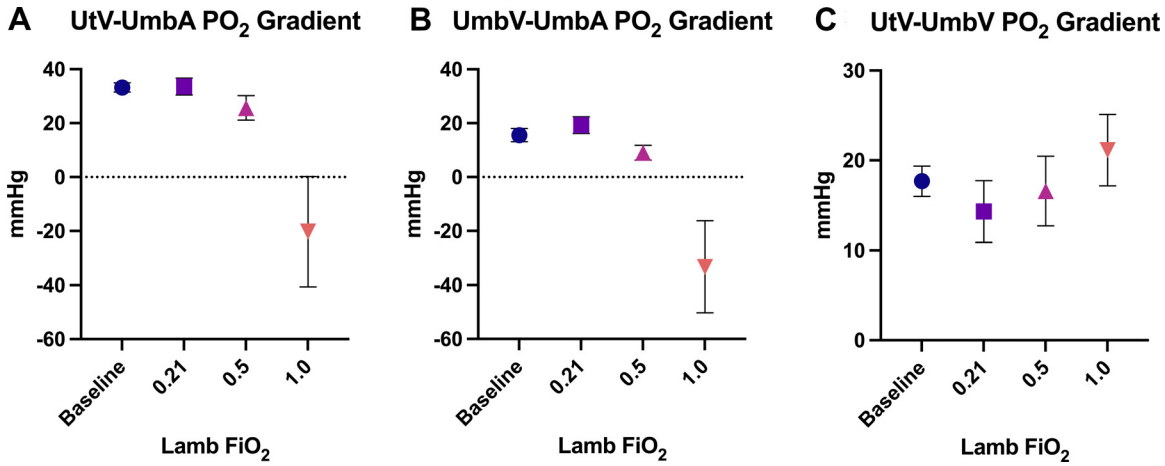

Figure 2. Oxygen gradient. Uterine vein (UtV)-umbilical artery (UmbA) (*A*), umbilical vein (UmbV)-UmbA (*B*), and UtV-UmbV (*C*) partial pressure of oxygen (P_{O_2}) gradients with increasing lamb oxygen supplementation during physiological-based cord clamping. Data were analyzed with a mixed-effect analysis and presented as means ± SE. $n = 8$. Baseline, preventilation. CC, cord clamping; $F_{I_{O_2}}$, fraction of inspired oxygen.

blood pressure were not altered in response to ventilation onset or increasing $F_{I_{O_2}}$ levels in lambs (Table 4).

Uteroplacental Oxygen Uptake from the Maternal Circulation

On the maternal side of the placenta, uteroplacental oxygen delivery was not affected by ventilation of the lamb with differing $F_{I_{O_2}}$ levels (Fig. 5*A*). However, compared with baseline, the percentage of oxygen taken up ($P < 0.0001$; Fig. 5*B*) by the placenta markedly decreased with each increasing $F_{I_{O_2}}$ level. When the lamb was ventilated with a $F_{I_{O_2}}$ of 1.0, oxygen uptake was reduced by 77.2 ± 6.0%, compared with before ventilation onset (baseline; $P < 0.0001$). The negative value in Fig. 5*B* indicates that there was a net reduction in oxygen taken up by the maternal side of the placenta as the lamb was being ventilated.

Oxygen Uptake from the Placenta by the Lamb

During ventilation of the lamb with increasing $F_{I_{O_2}}$ levels, UmbV blood oxygen content gradually increased, but this increase was offset by a reduced UmbV blood flow associated with pulmonary ventilation. As a result, the delivery of oxygen into the lamb from the placenta did not change (Fig. 6*A*). In contrast, there was a marked decrease in oxygen uptake across the placenta by the lamb with each increasing $F_{I_{O_2}}$ level ($P < 0.0001$; Fig. 6*B*). Oxygen uptake decreased from 317.7 ± 15.0 mL O_2/min/kg before ventilation onset (baseline) to −37.2 ± 14.0 mL O_2/min/kg ($P < 0.0001$) when the lamb was ventilated with a $F_{I_{O_2}}$ of 1.0. The negative value indicates that there was a net loss of oxygen from the fetal compartment, back across the placenta. As a result, the percentage of oxygen extracted (uptake/delivery) by the lamb from the placenta also significantly decreased with each increasing $F_{I_{O_2}}$ level, decreasing from 40 ± 8.0% before ventilation onset to −8.0 ± 3.0% during ventilation with a $F_{I_{O_2}}$ of 1.0 ($P = 0.0019$; Fig. 6*C*).

Changes in Blood CO_2 Levels

The partial pressure of carbon dioxide (CO_2) in the UtV tended to decrease during ventilation of the lamb in a $F_{I_{O_2}}$ of 0.5 ($P = 0.0580$; Fig. 7*A*). CO_2 also tended to increase in the

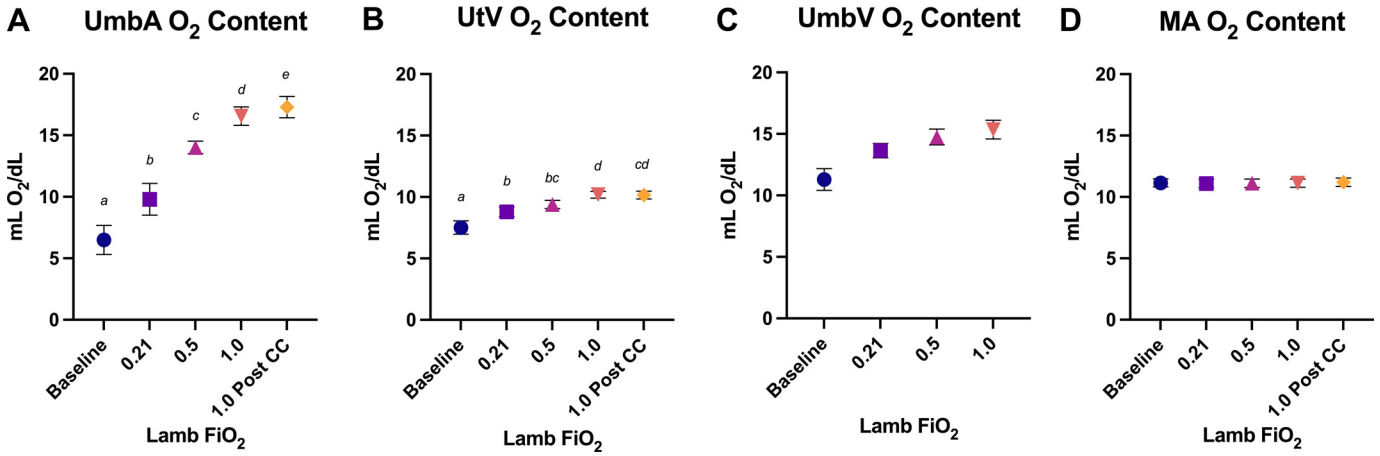

Figure 3. Oxygen content. Maternal artery (MA) (*A*), uterine vein (UtV) (*B*), umbilical vein (UmbV) (*C*), and umbilical artery (UmbA) (*D*) oxygen content with increasing lamb oxygen supplementation during physiological-based cord clamping. Data were analyzed with a mixed-effect analysis followed by Holm–Sidak post hoc test and presented as means ± SE. $n = 8$. Values that do not share a common letter are significantly different from each other ($P < 0.05$). Baseline, preventilation. CC, cord clamping; $F_{I_{O_2}}$, fraction of inspired oxygen.

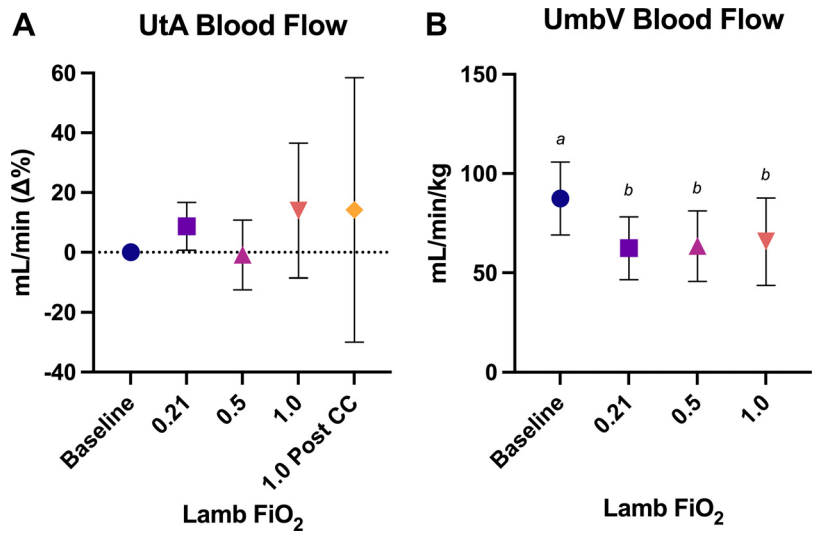

Figure 4. Blood flow. Changes in uterine artery (UtA) blood flow (*A*), and umbilical vein (UmbV) (*B*) blood flow with increasing lamb oxygen supplementation during physiological-based cord clamping. Data were analyzed with a mixed-effect analysis followed by Holm–Sidak post hoc test and presented as means ± SE. $n = 8$. Values that do not share a common letter are significantly different from each other ($P < 0.05$). Baseline, preventilation. CC, cord clamping; $F_{I_{O_2}}$, fraction of inspired oxygen.

UmbA as the lambs were ventilated ($P = 0.0570$; Fig. 7*B*). The gradient across the placenta (UmbA-UtV) was not different over the experimental period ($P = 0.4170$; Fig. 7*C*).

DISCUSSION

We have shown that during PBCC, which involves ventilating the newborn while the umbilical cord remains intact, increasing P_{O_2} levels in the newborn markedly alters the transfer of oxygen across the placenta between mother and newborn. When preterm lambs were ventilated with a $F_{I_{O_2}}$ of 1.0, the oxygen content of blood entering the fetal side of the placenta (i.e., in UmbA) increased above the oxygen content and P_{O_2} of blood leaving both the maternal and fetal sides of the placenta (i.e., in the UtV and UmbV). As a result, oxygen moved backward across the placenta (from lamb to ewe), as indicated by a reversal in oxygen uptake and extraction by the lamb from the placenta. Indeed, when ventilating the lamb with a $F_{I_{O_2}}$ of 1.0, the oxygen transfer from the lamb to ewe was large enough to increase the P_{O_2} and blood oxygen content in the UtV. However, the oxygen content in maternal arterial blood did not change, indicating that transplacental oxygen transfer was insufficient to influence maternal oxygenation. Thus, under these steady-state conditions, the ewe acted as a large "oxygen sink" that prevented the newborn lamb's blood from being over-oxygenated during PBCC.

As expected, ventilating lambs with increasing $F_{I_{O_2}}$ levels during PBCC caused a significant stepwise increase in P_{O_2} (Table 3), S_{O_2} (Table 3), and oxygen content in the UmbA (Fig. 3*A*). Throughout gestation, oxygen normally diffuses across the placenta from mother to fetus, due to the P_{O_2} gradient between mother and fetus. Oxygen enters the maternal side of the placenta via the maternal UtA, diffuses across the placenta, and leaves the fetal side of the placenta via the UmbV (Fig. 1). As this UmbV blood preferentially returns to the left ventricle (via the foramen ovale), we would normally expect to measure higher oxygen levels in pre- compared with post-ductal arteries before ventilation onset. However, this difference in pre- and post-ductal artery oxygen levels is rapidly lost following ventilation onset due to the decrease in pulmonary vascular resistance (PVR) and the associated reversal in ductal shunting from right-to-left to left-to-right (31). As a result, the oxygen levels in pre- and post-ductal vessels would be expected to be the same during the experiment.

Before ventilation onset (at baseline), despite the P_{O_2} being substantially higher in the maternal artery (MA) than in the UmbV, the blood oxygen content (mL O_2/dL) in both vessels was similar. This is not surprising as fetal sheep, like humans, have fetal hemoglobin and so a higher blood oxygen content with a lower P_{O_2} reflects a leftward shift in the oxygen dissociation curve due to the higher affinity that fetal (vs. adult) hemoglobin has for oxygen. Following ventilation onset (with a $F_{I_{O_2}}$ of 0.21), the oxygen content in UmbV increased above the MA blood, but despite the higher oxygen content in the UmbV, oxygen continued to pass from ewe to lamb, albeit at a reduced rate (Figs. 3 and 5). This is due to the higher P_{O_2} in maternal blood providing the gradient for

Table 4. *Cardiovascular outcomes (maternal and newborn) in different oxygen levels (baseline, 0.21, 0.5, 1.0, and 1.0 post cord clamping)*

	Baseline	0.21 $F_{I_{O_2}}$	0.5 $F_{I_{O_2}}$	1.0 $F_{I_{O_2}}$	1.0 $F_{I_{O_2}}$ Post CC	*P* Value
Heart rate (beats/min) (newborn)	180.1 ± 11.1	163.1 ± 8.6	168.6 ± 6.3	174.3 ± 13.0	170.8 ± 8.5	0.4995
Blood pressure (mmHg) (newborn)	42.9 ± 3.4[a]	38.7 ± 3.4[b]	42.4 ± 3.3[a]	44.5 ± 3.5[a]	54.7 ± 4.7[c]	0.0002
Heart rate (beats/min) (maternal)	93.9 ± 5.7	95.7 ± 4.3	92.8 ± 3.6	95.2 ± 5.5	100.6 ± 5.6	0.3312
Blood pressure (mmHg) (maternal)	76.6 ± 11.4	74.3 ± 10.0	73.9 ± 10.0	73.4 ± 10.1	73.7 ± 10.3	0.6025

Data were analyzed with a mixed-effect analysis followed by Holm–Sidak post hoc test and presented as means ± SE. $n = 8$. Baseline, preventilation. CC, cord clamping; $F_{I_{O_2}}$, fraction of inspired oxygen. Superscripted values that do not share a common letter are significantly different from each other ($P < 0.05$).

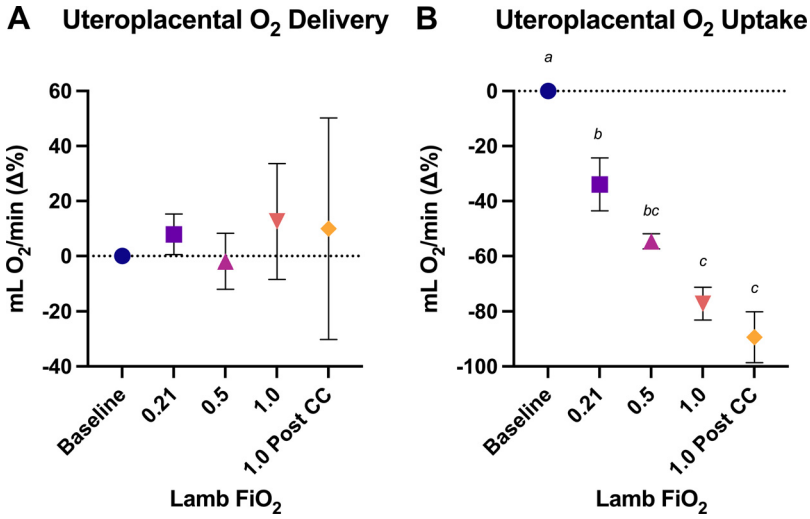

Figure 5. Uteroplacental oxygen transfer. Changes in uteroplacental oxygen delivery (*A*) and uteroplacental oxygen uptake (*B*) with increasing lamb oxygen supplementation during physiological-based cord clamping. Data were analyzed with a mixed-effect analysis followed by Holm–Sidak post hoc test and presented as means ± SE. $n = 8$. Values that do not share a common letter are significantly different from each other ($P < 0.05$). Baseline, preventilation. CC, cord clamping; $F_{I_{O_2}}$, fraction of inspired oxygen.

oxygen diffusion into the lamb's circulation, demonstrating the robustness of the maternal-to-newborn oxygen diffusion capacity.

Ventilation of lambs with a high $F_{I_{O_2}}$ gradually reduced and then reversed (at a $F_{I_{O_2}}$ of 1.0) oxygen uptake by the lamb from the placenta (indicated by negative values), demonstrating that oxygen diffusion across the placenta had reversed and was moving from lamb to ewe. This was most likely due to a reversal in the P_{O_2} gradient across the placenta (Fig. 2). Indeed, ventilation of the lamb with a $F_{I_{O_2}}$ of 1.0 increased the P_{O_2} of blood entering the fetal side of the placenta by approximately fivefold, which was greater than the P_{O_2} of blood leaving the placenta on both the fetal (UmbV) and maternal (UtV) sides. Thus, as blood passed through the fetal side of the placenta, from UmbA to UmbV, the P_{O_2} decreased by ~30 mmHg (Table 3). At the same time, the P_{O_2} and oxygen content of blood in the UtV increased, supporting the concept that oxygen was diffusing from the lamb into the ewe. It is also possible that the opening of arteriovenous (A-V) shunts between the maternal uterine arteries and veins contributed to the increase in P_{O_2} and oxygen content in the UtV with increasing $F_{I_{O_2}}$. Indeed, the presence of A-V shunts in the ovine uterus has been demonstrated previously (32), and they are thought to be dynamically regulated in response to changes in uterine blood flow (30). However, if present, the contribution of A-V shunts is likely to be small as the net loss of oxygen from the lamb's circulation was quite high (~30 mL O_2/min/kg of lamb weight; Fig. 6) and unlikely to be explained by increased uptake and utilization by placental tissue as the only alternative.

Our data indicate that maintaining placental perfusion during PBCC greatly reduces the ability to over-oxygenate the lamb, even during ventilation with a $F_{I_{O_2}}$ of 1.0, by allowing excess oxygen to pass out of the lamb into the maternal circulation. Previously, we have also shown that maintaining placental perfusion during PBCC avoids the transient hypoxia

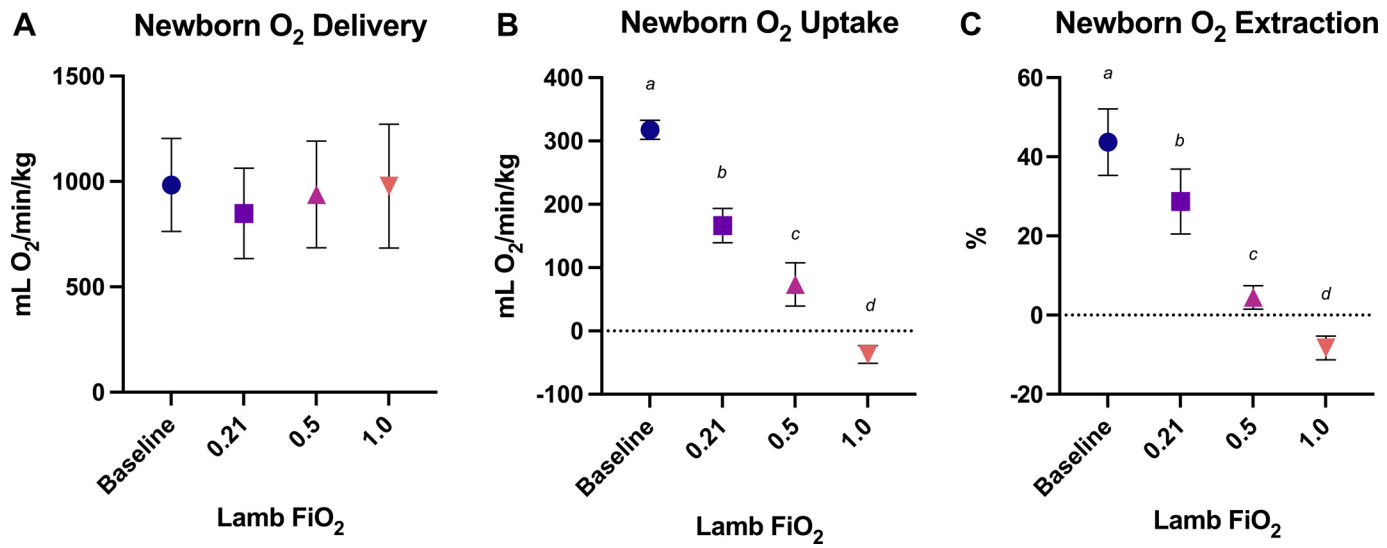

Figure 6. Newborn oxygen transfer. Changes in newborn oxygen delivery (*A*), newborn oxygen uptake (*B*), and newborn oxygen extraction (*C*) from the uteroplacental circulation with increasing lamb oxygen supplementation during physiological-based cord clamping. Data were analyzed with a mixed-effect analysis followed by Holm–Sidak post hoc test and presented as means ± SE. $n = 8$. Values that do not share a common letter are significantly different from each other ($P < 0.05$). Baseline, preventilation. $F_{I_{O_2}}$, fraction of inspired oxygen.

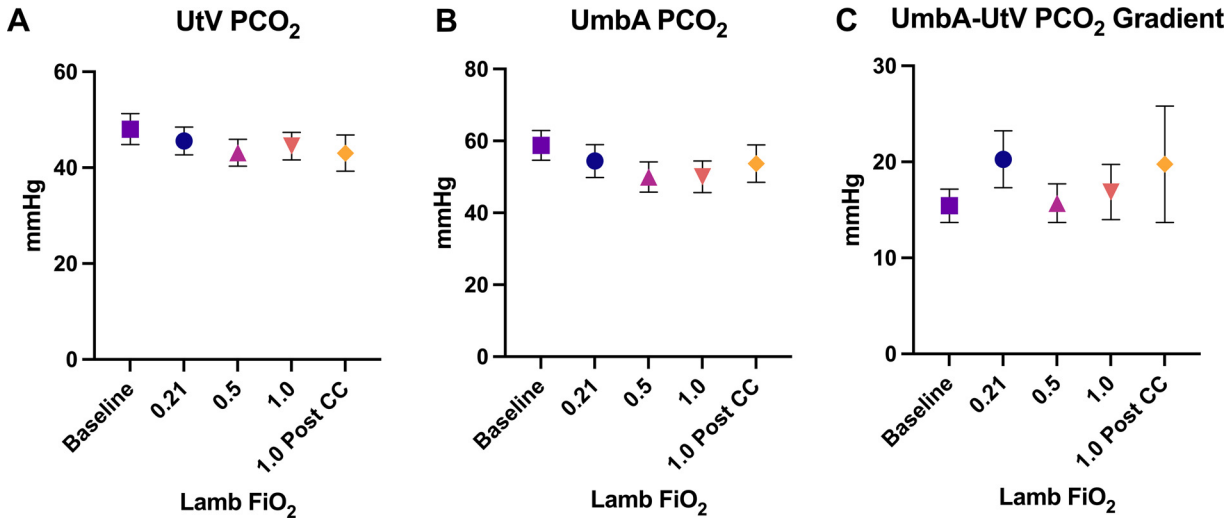

Figure 7. Carbon dioxide. Partial pressure of carbon dioxide (P_{CO_2}) in the uterine vein (UtV) (*A*), umbilical artery (UmbA) (*B*), and across the UtV-UmbA gradient (C) during lamb oxygen supplementation with increasing physiological-based cord clamping. Data were analyzed with a mixed-effect analysis and presented as means ± SE. $n = 8$. Baseline, preventilation. CC, cord clamping; $F_{I_{O_2}}$, fraction of inspired oxygen.

that occurs following immediate cord clamping (23). Similarly, a recently published study (DOXIE trial) has shown that PBCC improves oxygenation in extremely preterm infants (25). This is because PBCC allows the lungs to aerate and pulmonary gas exchange to commence while the lamb is still receiving oxygen across the placenta from the mother. Combined, these studies indicate that PBCC can protect the premature newborn against both hypoxia and hyperoxemia at birth by regulating the bidirectional movement of oxygen across the placenta between mother and newborn. This concept is consistent with the finding that, during ventilation of the lamb with a $F_{I_{O_2}}$ of 1.0, within 5 min of clamping the umbilical cord, the P_{O_2} in the UmbA blood markedly increased. That is, as clamping the umbilical cord prevented the placenta from removing excess oxygen from the lamb, circulating blood P_{O_2} levels rapidly increased in the lamb after cord clamping. Although we suggest that ventilation while attached to the umbilical cord may protect the newborn from hyperoxemia, further investigations into oxidative stress markers within tissues are warranted.

During PBCC, ventilation onset in the lamb was associated with a reduction in UmbV blood flow, which did not decrease further with increasing $F_{I_{O_2}}$, indicating that it is not related to oxygenation level. This reduction in UmbV flow has been reported previously and is due to the ventilation-induced decrease in PVR, which redirects right ventricular output (RVO) through the lungs rather than across the ductus arteriosus into the descending aorta (31, 33). As a result, blood flow to the lower body and placenta is reduced, particularly as net blood flow through the ductus arteriosus can reverse direction (from right-to-left to left-to-right), resulting in the left ventricle also contributing to PBF (31). Considering that oxygen is a well-known dilator of the pulmonary vascular bed, we would have expected PVR to decrease further with increasing oxygen, resulting in a further reduction in UmbV blood flow. However, this did not occur and likely reflects the much greater dominance of lung aeration at birth in reducing PVR compared with the increase in oxygenation (34).

It is interesting to note that we also detected a rapid increase in the lamb's blood pressure (~23%) in response to clamping the umbilical cord, despite the fact that the lungs had already aerated. We have previously demonstrated that immediate cord clamping at birth causes a similar rapid increase in blood pressure in the newborn, which can be avoided by aerating the lungs before cord clamping (24). This is because a dilated pulmonary vascular bed can act as an alternative low-resistance pathway for blood flow, thereby mitigating the loss of the low-resistance placental circulation upon cord clamping. Ventilation onset during PBCC in this study caused a significant reduction in arterial blood pressure, which reflects the overall decrease in systemic vascular resistance associated with a reduction in pulmonary vascular resistance caused by lung aeration. However, we suggest that in this study, the highly oxygenated state of the lamb before cord clamping (due to ventilation with a $F_{I_{O_2}}$ of 1.0) caused vasoconstriction in other oxygen-sensitive vascular beds, particularly the cerebral circulation, thereby increasing total systemic vascular resistance. As a result, cord clamping and the removal of the low-resistance placental vascular bed under these circumstances had a significant impact on arterial blood pressure. Nevertheless, the oxygen-induced vasoconstriction of the cerebral circulation likely protected the delicate cerebral microcirculation from this pressure surge, unlike following immediate cord clamping. This is consistent with the finding that Sct_{O_2} levels in lambs did not change following cord clamping, despite a large increase in arterial blood P_{O_2}.

Limitations

In this study, it was not possible to randomize the order of supplemental oxygen delivery due to the time delay required to stabilize the circulating oxygen levels and physiological changes associated with progressing from a high to a low oxygenation level. As a result, we cannot definitively dismiss the effect of ventilation time on the differences observed between oxygen concentrations. Nevertheless, we consider that any effect of ventilation time will be minimal as all physiological data were stable and unchanging by the end of each ventilation period (i.e., before the subsequent increase in $F_{I_{O_2}}$), when

our measurements were collected. Furthermore, as numerous previous studies have demonstrated that ventilation of a fetus with an intact umbilical cord for a prolonged period (up to 24 h) is at least as safe as ventilation for the same period following cord clamping, the time spent ventilating the lamb on the cord is believed to have little impact on our findings (35–38). Moreover, as we did not measure carotid arterial blood oxygen levels or blood flow, the effect on cerebral oxygen delivery during ventilation with PBCC remains unclear and warrants further investigation. Finally, we acknowledge the limitations of undertaking these experiments under general anesthesia. However, to minimize this limitation, we carefully monitored both the ewe and the lamb to ensure that their blood pressures and heart rates remained within normal physiological ranges.

Conclusions

Ventilating premature newborns with an intact cord (termed PBCC) not only protects them against hypoxia but also protects them from hyperoxemia resulting from ventilation with high (1.0 $F_{I_{O_2}}$) inspired oxygen concentrations. The latter is achieved by reversing the direction of oxygen diffusion across the placenta, with oxygen diffusing from newborn to mother once the newborn's blood P_{O_2} exceeds the P_{O_2} of blood on the maternal side of the placenta. This "threshold" P_{O_2} is below the maternal arterial blood P_{O_2} and above the uterine venous blood P_{O_2}. When lambs were ventilated with high inspired oxygen levels, the amount of oxygen transferred from lamb to ewe was high enough to significantly increase the oxygen content of blood in the uterine vein, but not in the maternal artery. This finding indicates that the mother can act as a large "oxygen sink" that absorbs excess oxygen from the newborn without influencing her oxygenation level during physiological-based cord clamping.

DATA AVAILABILITY

Data will be made available upon reasonable request.

GRANTS

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DISCLOSURES

Prof. S. B. Hooper acts as a part-time casual consultant for Fisher and Paykel Healthcare in developing new resuscitation equipment for use in the delivery room. However, there is no overlap or conflict of interest with this study, which focused on the physiological control of oxygen uptake by lambs during physiological-based cord clamping and did not involve the use of any F&P equipment. None of the other authors has any conflicts of interest, financial or otherwise, to disclose.

AUTHOR CONTRIBUTIONS

E.R.C., S.B.H., D.A.B., and K.J.C. conceived and designed research; E.R.C., S.B.H., A.M.T., C.D., P.J.R., V.A.Z., D.A.B., and K.J.C. performed experiments; E.R.C. analyzed data; E.R.C., S.B.H., D.A.B., and K.J.C. interpreted results of experiments; E.R.C. prepared figures; E.R.C. and S.B.H. drafted manuscript; E.R.C., J.D., S.B.H., C.D., V.A.Z., A.B.t.P., G.R.P., D.A.B., and K.J.C. edited and revised manuscript; E.R.C., J.D., S.B.H., A.M.T., C.D., P.J.R., V.A.Z., A.B.t.P., G.R.P., D.A.B., and K.J.C. approved final version of manuscript.

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