Effect of spontaneous breathing on umbilical venous blood flow and placental transfusion during delayed cord clamping in preterm lambs

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ABSTRACT

Introduction During delayed umbilical cord clamping, the factors underpinning placental transfusion remain unknown. We hypothesised that reductions in thoracic pressure during inspiration would enhance placental transfusion in spontaneously breathing preterm lambs.

Objective Investigate the effect of spontaneous breathing on umbilical venous flow and body weight in preterm lambs.

Methods Pregnant sheep were instrumented at 132–133 days gestational age to measure fetal common umbilical venous, pulmonary and cerebral blood flows as well as arterial and intrapleural (IP) pressures. At delivery, doxapram and caffeine were administered to promote breathing. Lamb body weights were measured continuously and breathing was assessed by IP pressure changes.

Results In 6 lambs, 491 out of 1117 breaths were analysed for change in body weight. Weight increased in 46.6% and decreased in 47.5% of breaths. An overall mean increase of 0.02±2.5 g per breath was calculated, and no net placental transfusion was observed prior to cord clamping (median difference in body weight 52.3 [−54.9–166.1] g, p=0.418). Umbilical venous (UV) flow transiently decreased with each inspiration, and in some cases ceased, before UV flow normalised during expiration. The reduction in UV flow was positively correlated with the standardised reduction in (IP) pressure, increasing by 109 mL/min for every SD reduction in IP pressure. Thus, the reduction in UV flow was closely related to inspiratory depth.

Conclusions Spontaneous breathing had no net effect on body weight in preterm lambs at birth. UV blood flow decreased as inspiratory effort increased, possibly due to constriction of the inferior vena cava caused by diaphragmatic contraction, as previously observed in human fetuses.

INTRODUCTION

Placental to infant blood transfusion during delayed umbilical cord clamping (DCC) after birth is evidenced by an increase in birth weight and a decrease in placental blood volume and is thought to occur at 2–3 mL/kg/min over the first 3 min after delivery. Animal studies have also shown that DCC until after ventilation onset prevents the reduction in cardiac output caused by immediate cord clamping (ICC). This finding has led to the concept that cord clamping should be based on the infant’s physiology (ie, whether it is breathing) rather than on ‘time after birth’, which can be unrelated to the infant’s physiological state. This concept is termed ‘physiological-based cord clamping (PBCC)’ and highlights the importance of pulmonary ventilation in mediating the benefits of DCC.

In term newborns, DCC increases haemoglobin and ferritin levels, reduces rates of iron deficiency and anaemia in the first year of life. In preterm infants, small randomised trials have shown that DCC is associated with less intraventricular haemorrhage, necrotising enterocolitis, sepsis and need for blood transfusions, which are common complications associated with preterm birth. As a result, WHO now recommends DCC for >60 s in infants not requiring resuscitation.

While placental transfusion is an accepted feature of DCC, little is known about the physiology causing the net movement of blood from the placenta into the infant during DCC. Most commentaries suggest that placental transfusion results from gravity (ie, when the infant is placed below the mother) or from uterine contractions squeezing blood out of the placenta into the infant. However, recent...
clinical trials in humans and animal experiments have failed to
demonstrate an effect of gravity or uterine contractions on
placental transfusion. 4,19 In a unique observational study in
normal term infants, Doppler ultrasound was used to investigate
blood flow patterns in both the umbilical artery and vein during
DCC. 20 This study showed that umbilical venous (UV) flow was
heavily influenced by breathing and crying. During inspiration,
umbilical venous flow appeared to increase, whereas between
breaths it appeared to cease. Similarly, umbilical artery flow was
influenced by breathing and crying and at times was bidirec-
tional, which was thought to result from uterine contractions. 20

Animal experiments investigating the mechanisms of placental
transfusion have consistently failed to detect a shift in blood
volume from the placenta into the newborn. 9,19 Possible expla-
nations for this relate to the animal models used, which have
mostly involved lambs delivered by caesarean section, followed
by mechanical ventilation. 9,19 In this situation, intermittent
increases in intrathoracic pressure combined with end-expiratory
pressures may restrict net placenta-to-infant blood transfusion.
In contrast, all human studies showing placental transfusion have
been in vaginally delivered spontaneously breathing infants. 21 In
this study, we hypothesised that the subatmospheric (negative)
intrathoracic pressure created during inspiration would enhance
UV flow into the newborn, leading to a net increase in weight.

METHODS
All experimental procedures were conducted in accordance with
the National Health and Medical Research Council code of prac-
tice for the care and use of animals for scientific purposes.

Fetal surgery
Sterile fetal surgery was performed on six pregnant ewes
(Merino X Border Leicester) at 131–132 days (term is 147 days;
131–134 days is considered late preterm) of gestational age, 3
days prior to delivery. 22 Briefly, anaesthesia was induced using an
intravenous bolus of sodium thiopentone (20 mL at 50 mg/mL)
and maintained following intubation by inhalation of isoflurane
(1%–3% Isoflow, Abbott, Australia) in air/oxygen. Antibiotics
(cefazoline, 1 g/5 mL) were administered before surgery. The
fetal head, neck and abdomen were exteriorised via hysterotomy
and jugular vein and carotid artery catheters inserted. Ultra-
sonic flow probes (Transonic Systems, Ithaca, New York, USA)
were placed around the left pulmonary artery, common umbilical
vein and right carotid artery. In lambs, the two umbilical
veins within the cord join at the umbilicus to form the intra-
abdominal common umbilical vein before it enters the fetal liver
and divides into the ductus venous or joins the hepatic portal
system. A sterile, saline-filled intrapleural (IP) balloon catheter
was also inserted into the IP space to measure changes in IP pres-
sure during breathing. Following instrumentation, the fetus was
returned to the amniotic sac and both catheters and flow-probe
leads were exteriorised via the ewe’s right flank for continuous
monitoring until delivery, 3 days later. During the postoperative
period, ewes received analgesia via a fentanyl patch (75 µg/hour)
and daily antibiotics (2.5 mL cefazoline 1 g/5 mL).

Experimental protocol
Prior to delivery, electronic recordings of blood flows in the
left pulmonary artery, umbilical vein and right carotid artery as
well as pressures in the carotid artery and IP space commenced
using a data acquisition system (Powerlab ADI, Sydney,
Australia). As general anaesthesia is known to inhibit sponta-
neous breathing activity, lambs were delivered under spinal
anaesthesia at 133–134 days of gestations. Ewes were sedated
with 10 mL propofol 1% (10 mg/mL) to administer spinal
anaesthesia (lignocain 2%, 0.1 mL/kg) via the sacral lumbar
space. Following successful induction of spinal anaesthesia, mild
sedation of the ewe continued using midazolam (1.0 mg/kg/hour
at 20–25 mL/hour). The fetal head and neck were exteriorised
via caesarean section and the lambs were intubated using an
endotracheal tube (4.5 mm cuffed endotracheal tube). Lambs
were intubated as the application of facemask without leak is
problematic and, in addition intubation ensured opening of the
larynx. A transcutanous oxygen saturation probe (Masimo,
Radical 7, California, USA) was placed around the right foreleg,
a near-infrared spectroscopy (NIRS) optode (Casmed Foresight,
CAS Medical Systems, Branford, Connecticut, USA) was placed
over the left frontal cortex, and a rectal temperature probe
was inserted and used to correct blood gas measurements.
The lamb was dried and placed on electronic scales (Bilancia
d70, Campogalliano, Italy) immediately beside the ewe, taking
care to avoid stretching or mechanically interfering with the
umbilical cord; the lamb’s weight was recorded continuously.
Lambs were given naloxone (400 µg intravenous) and Anexate
(0.04 mg bolus intravenous) to counter the inhibitory effects of
fentanyl (postoperative analgesia of the ewe) and midazolam
(maternal sedation), respectively, on respiration. They were also
given caffeine (20 mg/kg loading dose and 10 mg/kg/hour) and
doxapram (5 mg/kg loading dose and 2.5 mg/kg/hour) to stimu-
late breathing.

If the lamb was breathing spontaneously, it was given contin-
uous positive airway pressure (5 cmH2O) via endotracheal
tube (4.5 mm cuffed endotracheal tube) and disturbed as little
as possible to avoid affecting the body weight recordings and
other physiological measurements. If the lamb did not initiate
spontaneous breathing following delivery and physical stimu-
lation, a sustained inflation (SI) was administered (peak inspira-
atory pressure [PIP] of 35 cmH2O for 30 s). Following the SI,
if spontaneous breathing did not commence or if the lambs
became apnoeic, brief periods of intermittent positive pressure
ventilation (PIP of 35 cmH2O with a positive end expiratory
pressure [PEEP] of 5 cmH2O) was applied to avoid hypoxia.
During the experiment, no supplemental oxygen was adminis-
tered to the lamb as it was still receiving oxygenation from the
ewe via the umbilical cord. All physiological parameters were
recorded continuously throughout the experiment. Umbilical
cord clamping occurred once the lamb was assessed to have
established a stable breathing pattern and the pulmonary blood
flow (PBF) waveform had adopted the neonatal phenotype
(figure 1); this took up to 19:15 (15:55–20:25) min postde-

delivery to achieve in our experiments.

Following cord clamping, the lamb was placed under a radiant
heater and was ventilated using a Babylog 8000+ ventilator
(Dräger, Lübeck, Germany) with a PEEP of 5 cmH2O for an
additional 30 min. All physiological recordings commenced prior
to, or at delivery (NIRS, oxygen saturation [SpO2], weight) and
arterial blood gases were taken at regular intervals following
delivery to assess lamb well-being. All blood samples and liquid
volumes taken or given to the lamb while they were being
weighed were recorded to adjust the weight measurements.

The ewe was euthanised following cord clamping, using an
overdose of pentobarbitone (100 mg/kg intravenous). Lambs
were euthanised after completion of the ventilation period using
an overdose of pentobarbitone administered intravenously.
Lamb body and organ weights were recorded postmortem.
Figure 1 Pulmonary blood flow (PBF) waveform before and after lung aeration. Physiological recordings of instantaneous PBF measured throughout four consecutive cardiac cycles before and after lung aeration. Before lung aeration, the PBF waveform is characterised by a positive, narrow systolic peak followed by retrograde (negative value) flow throughout most of diastole. This blood flows retrogradely out of the pulmonary circulation and across the ductus arteriosus (DA) into the dorsal aorta, resulting in continuous right-to-left shunting in the DA throughout the cardiac cycle. After transition, the systolic peak is broader and flow continues into the lung throughout the cardiac cycle. The continuous forward flow into the lungs during diastole results from a reversal in the pressure gradient between the pulmonary and systemic circulations resulting in left-to-right shunting through the DA.

Table 1  Fetal characteristics

| n=6 | Fetal | 5 min prior to cord clamping | 5 min after cord clamping | 10 min after cord clamping |
|-----|-------|-------------------------------|---------------------------|---------------------------|
| Gestational age (days) | 134 (134 – 134) | – | – | – |
| pH | 7.39 (7.37–7.42) | 7.27 (7.27 –) | 7.13 (7.12–7.22) | 7.12 (7.07–7.24) |
| pCO₂ | 45.6 (44.6–49) | 54.2 (44.9 –) | 70.2 (64.4–73.3) | 72.1 (52.5–85.8) |
| pO₂ | 23.6 (21.2–27.1) | 35.1 (32.9 –) | 42.4 (22.7–51.7) | 74.0 (47.0–96.1) |
| Saturation (%) | 69.4 (58.9–72.3) | 82 (74.9 –) | 84.4 (50.5–90.9) | 96.8 (74.5–98.1) |

Characteristics displayed as median (IQR).

Effect of spontaneous breaths on lamb weight

The breath-by-breath analysis evaluated 1117 breaths, which resulted in an average reduction of IP pressure of 8.6±0.3 mm Hg.
(range 2.2–72.2 mm Hg); this equates to a mean reduction in pressure of 11.2±0.6 cmH2O (range 2.9–93.8 cmH2O). Of the 1117 breaths analysed, 626 breaths were excluded from weight analysis due to major weight changes caused by artefacts such as body movement. Of the 491 breaths analysed for weight, 46.6% of breaths were associated with an increase in weight, 5.9% were associated with no change in weight and 47.5% of breaths were associated with a decrease in weight. Overall, the mean increase in lamb weight per breath was 0.02±2.5 g (figure 2A). The increase in weight was positively correlated with the standardised reduction in IP pressure. The regression coefficient was 0.839 meaning that for an IP pressure that is 1 SD lower than the mean basal value, weight would be reduced by 0.839 g. Median (IQR) lamb body weight was 3969 (3635–4131) g at delivery, 3924 (3668–4080) g at 10 min before cord clamping and 4038 (3610–4201) g at time of cord clamping. No net placental transfusion was measured or observed during spontaneous breathing prior to cord clamping as measured by body weight change (p=0.418).

Common umbilical venous flow
In 44% of the breaths analysed, blood flow in the common UV transiently (0.31±0.19 s in duration) decreased with inspiration (figures 3 and 4); the delay between peak reduction in IP pressure and peak reduction in UV flow varied between 120 and 300 ms. The mean reduction in common UV flow per breath was 183.4±4.5 mL/min (range 79.4–627.8 mL/min). Reductions in UV flow that decreased below zero were also commonly observed, indicating that flow in the common UV had reversed (indicated by negative blood flow value) and transiently flowed back towards the placenta (figure 4). These reversals in flows were most often associated with large inspiratory efforts that were immediately followed by transient increases in IP pressure. Following these reversals in flow, common UV flow transiently increased above baseline values following the restoration in IP pressure. Reductions in UV flow that often resulted in a reversal of common UV flow, also occurred during transient increases in IP pressure that were not preceded by inspiration (figure 4); this was relatively common in all lambs.

The reduction in common UV flow was found to be positively correlated with the standardised reduction in IP pressure. The regression coefficient was 109.0 and as such, a 1 SD reduction in IP pressure below the mean basal value will reduce UV flow by 109.0 mL/min (figure 2B). Thus, as the depth of the inspiration increased, the associated reduction in common UV flow increased, causing flow to cease or even reverse with some breaths (figures 3 and 4).

Haemodynamics
Carotid arterial pressure and flow
Following delivery and ventilation onset, mean CA pressure and blood flow remained relatively constant for the 10 min leading up to cord clamping. Immediately following cord clamping, CA pressure (by −0.5 mm Hg) slightly increased. While CA pressure remained relatively constant, CA flow gradually increased, reaching a maximum of 86 mL/min at 8 min following cord clamping (table 2).

Pulmonary artery blood flow
At 10 min prior to cord clamping, PBF was quite variable (table 2) between lambs, with some lambs having already aerated their lungs and transitioned their pulmonary circulation (as indicated by PBF wave form; figure 1), whereas others still had a predominantly fetal PBF waveform phenotype. Immediately following cord clamping, PBF increased and tended to increase further over the subsequent 10 min following umbilical cord clamping.

DISCUSSION
International resuscitation guidelines currently recommend DCC for 60s in infants who do not need resuscitation, largely based on the concept of placental to infant blood transfusion.23 24 While previous studies have shown a beneficial effect of DCC on neonatal outcomes,14 25 the physiological mechanisms underpinning the net shift of blood from the placenta into the infant during DCC remains unknown. We consider that a better understanding of this physiology and the factors influencing placental transfusion could help optimise the practice of DCC in the delivery room. We hypothesised that the reduction in

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**Figure 3** Physiological recordings of intrapleural (IP) pressure, body weight and common umbilical venous (UV) blood flow. Changes in IP pressure, body weight and UV blood flow before umbilical cord clamping. Transient reductions in IP pressure occur in response to inspiration which are closely associated with transient reductions in, or cessations of, UV blood flow. No changes in lamb weight were associated with individual breaths or an entire period of spontaneous breathing.

**Figure 4** Physiological recordings of intrapleural (IP) pressure and common umbilical venous (UV) blood flow. Changes in IP pressure and UV blood flow in a spontaneously breathing lamb displaying a forced expiration (transient increase in IP pressure) following each inspiration (transient decrease in IP pressure). While transient reductions in IP pressure associated with inspiration caused reduction/cessation of UV blood flow, increases in IP pressure associated with abdominal contractions also caused a reduction in UV blood flow. Indeed, increases in IP pressure were closely associated with a reversal in UV blood flow, causing blood to flow away from the lung and pool up stream in either the cord or placenta. As a result, following restoration of IP pressure, UV blood flow transiently increased above baseline (indicate by black bars).
intrathoracic pressure associated with inspiration would provide a pressure gradient favouring blood flow from the placenta into the infant and restrict flow in the opposite direction. However, contrary to our hypothesis, we found that UV flow into newborn lambs transiently reduced, ceased or even reversed during individual breaths, depending on the respiratory pattern, and that the reduction in flow was positively correlated with the depth of the inspiration. It is not surprising, therefore, we also did not observe a relationship between spontaneous breathing and net weight gain of the lamb.

Evidence for placental transfusion in humans is well established and includes an increase in infant weight during DCC, an increase in neonatal blood volume and a decrease in blood volume remaining within the placenta after DCC. However, our previous animal studies have been unable to provide any evidence of placental transfusion, which we considered was due to the absence of spontaneous breathing; instead all lambs were mechanically ventilated. As such, in this study we aimed to investigate whether subatmospheric pressures generated during inspiration resulted in placental transfusion. To measure placental transfusion, lamb body weights and UV flows were measured continuously throughout a period of DCC. However, the body weight measurements proved to be challenging as any movement or touching of the lamb caused large rapid changes in measured weight that were artefactual and unrelated to body weight changes; these sections of physiological recordings were manually detected and excluded from analysis. To counter the inhibitory effects of maternal sedation on spontaneous breathing, all lambs received drugs to reverse these effects and therefore body movements were common during the experiment, resulting in some unreliable weight measurements (figure 3). Nevertheless, even when the lambs were not moving and the weights were stable, we could not identify a relationship between breathing (IP) and weight change over an entire breathing episode (figure 2A). Nevertheless, the linear regression analysis detected a relationship between the standardised IP reduction and a very small increase in weight. Considering that the UV flow was markedly reduced with each breath (see below), it is difficult to explain how this might occur. However, the effect of individual breaths on weight change are transient, reversed at the end of the breath and may involve a transient discrepancy between UV flow into and UA flow out of the lamb.

Although we found that UV flow was influenced by spontaneous breathing and the associated decrease in IP pressure, the relationship was opposite to what we hypothesised. We hypothesised that the decrease in intrathoracic pressure associated with inspiration would provide a pressure gradient to increase UV flow into the lamb. However, we found that UV flow transiently decreased during inspiration, commonly decreasing to zero at end-inspiration. Furthermore, we found a strong positive relationship between the degree of decrease in UV flow and the size of the reduction in intrathoracic pressure generated by inspiration (figure 2B). While we were initially surprised by this finding, it is consistent with previously described findings measured by ultrasound in human fetuses during periods of fetal breathing movements (FBM). This study showed that during inspiration, contraction of the diaphragm causes narrowing and closure of the inferior vena cava (IVC) as it passes through the diaphragm, causing IVC flow to cease. Blood returning from the UV, either via the ductus venosus or hepatic veins, also has to pass through the diaphragm via the same orifice as the IVC. As such, it is likely that the ductus venosus constricts along with the IVC as the diaphragm contracts, causing blood flow in the UV to cease during inspiration. Due to the large compliance of the low pressure venous system, it is highly likely that these reductions in flow are slightly delayed in relation to the timing of inspiration. We found the delay to be 120–300 ms in the intra-abdominal common UV, but this delay is likely to be larger when measured further upstream, closer to the placenta. As a result, when measured upstream in the cord, depending on the breathing rate, the reductions in UV flow may be completely out of synchrony with breathing. This finding explains why increases in UV flow appeared to be related to inspiration, whereas cessations in UV flow appeared to be related to expiration, in our previous study in human infants; in that study UV flows were measured upstream in the cord. Nevertheless, the findings convincingly demonstrate that large subatmospheric reductions in intrathoracic pressure cannot account for placental transfusion.

As noted in figure 3, both increases in IP pressure by itself, or following a large inspiratory effort, can result in a transient

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**Table 2** Averaged measurements in the 10 min prior to and 10 min after cord clamping

| Time (min) | CA pressure (mm Hg) | CA flow (mL/min) | PA flow (mL/min) | Body temperature |
|------------|---------------------|------------------|------------------|-----------------|
| −10        | 48.73 (40.37–56.11) | 60.35 (43.10–83.63) | 137.82 (62.22–254.00) | 39.5 (39.0–39.8) |
| −5         | 50.32 (46.94–58.39) | 43.32 (27.25–61.75) | 240.53 (138.00–298.67) | 38.6 (37.7–39.5) |
| −2         | 48.02 (41.16–55.40) | 42.33 (32.94–57.20) | 312.64 (179.14–403.90) | 38.2 (37.4–38.9) |
| Cord clamped | 54.45 (49.03–61.00) | 49.27 (40.37–62.33) | 354.40 (224.28–457.53) | 38.2 (37.8–38.8) |
| 1          | 54.98 (50.06–62.14) | 52.15 (46.46–73.45) | 255.87 (218.88–422.99) | 38.2 (37.8–38.7) |
| 2          | 58.96 (47.83–65.98) | 58.14 (50.32–86.52) | 314.78 (218.62–460.20) | 38.0 (37.2–38.4) |
| 3          | 55.51 (50.16–64.59) | 62.65 (47.64–92.08) | 281.72 (244.34–464.64) | 38.1 (37.8–38.4) |
| 4          | 58.83 (49.43–70.26) | 68.41 (61.64–105.60) | 313.45 (239.18–541.80) | 38.0 (37.2–38.3) |
| 5          | 59.97 (48.41–69.04) | 73.61 (61.91–92.85) | 319.15 (217.48–449.91) | 38.0 (37.5–38.6) |
| 6          | 55.28 (48.74–70.17) | 74.36 (53.45–93.81) | 260.98 (200.38–320.13) | 37.9 (37.0–38.6) |
| 7          | 58.27 (49.60–74.29) | 82.93 (69.51–126.51) | 389.07 (268.02–462.93) | 36.7 (35.7–38.5) |
| 8          | 55.30 (47.03–79.75) | 85.66 (64.12–106.34) | 343.40 (280.24–437.31) | 37.8 (37.4–38.6) |
| 9          | 71.65 (48.77–75.96) | 71.93 (59.60–102.05) | 356.79 (286.36–600.84) | 38.1 (37.2–38.7) |
| 10         | 64.26 (46.97–71.41) | 78.96 (48.34–97.13) | 355.93 (275.70–567.71) | 38.0 (37.3–38.7) |

CA pressure, CA flow and PA flow are displayed as median (IQR), 10 min before and 10 min after cord clamping. CA pressure increases after cord clamping with −4 mm Hg at 4 min, after which it remains stable. CA flow increases up to 8 min with −36 mL/min. PA flow increases with a maximum of 35 mL/min after cord clamping. Temperature decreases with 0.2°C.

CA, carotid arterial; PA, pulmonary artery.
reversal of blood flow in the common UV, pushing blood back out of the lamb towards the placenta. These increases in IP pressure were undoubtedly due to abdominal muscle contractions that must also have resulted in large increases in intra-abdominal pressure. In the physiological recording displayed in figure 3, each inspiratory effort is immediately followed by a forced expiratory effort, as indicated by a large increase in IP pressure (up to 40 mm Hg), which must have been driven by abdominal muscle contractions. In addition, a reversal in UV flow was also observed when transient increases in IP pressure were not preceded by inspiration. The precise reasons for these are unclear, but abdominal muscle contractions associated with grunting or body movements are possible explanations. Nevertheless, whether or not the increases in intrathoracic (and abdominal) pressure are associated with breathing, they are associated with a transient reversal in UV flow. This finding is not surprising given that the IP pressure increase (up to 40 mm Hg; figure 3) is considerably larger than UV pressure in the cord. However, once the IP pressures begin to stabilise, UV blood flow transiently increases above baseline, which likely reflects a rebound increase in flow caused by blood pooling upstream in the cord and placenta (figure 3).

Ventilating the lung before clamping the umbilical cord, also known as PBCC, is known to promote haemodynamic stability immediately after birth.\textsuperscript{6,28} In particular, it avoids the large rapid increase in blood pressure caused by increasing systemic vascular resistance when the cord is clamped and the reduction in cardiac output caused by the loss of umbilical venous return and ventricular preload.\textsuperscript{10} Avoiding these significant cardiovascular events is likely to be a major contributing factor to the reduction in neonatal morbidities, such as intraventricular haemorrhage, associated with DCC.\textsuperscript{6} We found that CA pressure increased slightly following umbilical cord clamping (by \(-0.5\) mm Hg), but this increase is substantially less than the increase that occurs with cord clamping before ventilation/breathing onset (\(-20\) mm Hg).\textsuperscript{5} While the CA pressure tended to increase over the subsequent 9 min (by \(-15\) mm Hg), this increase appeared more to do with the lamb’s activity levels than to cord clamping. Similarly, CA blood flow increased over the same time frame (by 30 mL/min), which indicates that cardiac output was sustained throughout the cord clamping period. PBF doubled in the minutes leading up to cord clamping, which was expected as it is well established that the increase in PBF after birth is triggered by lung aeration resulting from breathing onset.\textsuperscript{29,30} Although PBF tended to increase in response to cord clamping, before cord clamping the majority of right ventricular output was mainly directed through the lungs rather than across the ductus arteriosus. This is clearly indicated by the PBF wave form (figure 1), whereby after transit, forward flow into the lungs occurs throughout the cardiac cycle. Forward flow into the lung during diastole results from a reversal of flow through the ductus arteriosus due to the reduction in PVR causing a reversal in the pressure gradient between systemic and pulmonary circulations.\textsuperscript{25} As clamping of the umbilical cord increases systemic arterial pressure, the increase in PBF following cord clamping likely results from increased left-to-right shunting of blood through the ductus arteriosus during diastole. These results are consistent with our previous findings in ventilated lambs.\textsuperscript{6} Taken together, these results clearly demonstrate a haemodynamically stable transition when lung aeration precedes cord clamping, irrespective of whether the lambs were ventilated or breathed spontaneously.

Temperature measurements stayed stable throughout the experiments, with the median temperature varying between 36.7°C and 39.5°C (39.0°C is the normal core body temperature in fetal sheep). Before cord clamping, the external application of heat required to maintain lamb body temperature was minimal and restricted to one hot water bottle and a wrap. However, after cord clamping an overhead radiant heater, two hot water bottles and a wrap were needed to maintain a stable temperature. While anecdotal, this indicates that when the cord is intact, umbilical blood flow may provide an effective source of heat that helps to maintain body temperature. While some heat loss may occur over the length of the umbilical cord, presumably this was compensated for by the continuous flow of blood flowing through the umbilical vessels at the ewe’s body temperature.

CONCLUSION

In summary, contrary to our hypothesis, we found that during inspiration, UV flow was either reduced or ceased depending on the size of the inspiration. This finding was likely caused by narrowing or closure of the IVC during contraction of the diaphragm during inspiration as previously described in human fetuses during FBM in utero.\textsuperscript{27} The reduction in UV flow was positively correlated with the depth of the inspiration. Increases in IP pressure, likely caused by abdominal contractions, also reduced UV flow and caused it to transiently reverse, resulting in blood flowing out of the lamb and pooling in the cord or placenta. This study confirms the previous results of Bhatt et al., showing haemodynamic stability when ventilation onset precedes cord clamping and demonstrates that the mode of ventilation (spontaneous breathing vs mechanical ventilation) does not impact on this stability or on placental transfusion.\textsuperscript{3} Instead, the haemodynamic stability is reliant on the increase in PBF. Clearly, further research is required to understand the mechanisms responsible for placental transfusion.

Correction notice This paper has been updated since it was published Online First. Figures 3 and 4 were the same in the original manuscript and so figure 3 has now been updated.

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