# Placental Transfusion during Neonatal Resuscitation in an Asphyxiated Preterm Model

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## Abstract

**Background:** Neonatal Resuscitation Program does not recommend placental transfusion in depressed preterm neonates.

**Methods:** Our objectives were to study the effect of delayed cord clamping (DCC) with ventilation for 5 minutes (DCCV, n-5), umbilical cord milking (UCM) without ventilation (n-6), UCM with ventilation (UCMV, n-6), early cord clamping followed by ventilation (ECCV, n-6) on red cell volume (RCV), and hemodynamic changes in asphyxiated preterm lambs. Twenty-three preterm lambs at 127–128d gestation were randomized to DCCV, UCM, UCMV, and ECCV. We defined asphyxia as heart rate < 100/minute.

**Results:** The UCMV had the highest neonatal RCV as a percentage of fetoplacental volume compared to the other groups (UCMV 85.5±10%, UCM 72±10%, ECCV 65±14%, DCCV 61±10%, p<0.01). The DCCV led to better ventilation (66±1 mmHg) and higher pulmonary blood flow (75±24 ml/kg/min). The carotid flow was significantly higher in UCM without ventilation. The fluctuations in carotid flow with milking were 25±6% higher from baseline during UCM, compared to 6±3% in UCMV (p<0.01).

**Conclusion:** Cord milking with ventilation led to higher RCV than other interventions. Ventilation during cord milking reduced fluctuation in carotid flow compared to UCM alone. DCCV led to better ventilation and pulmonary blood flow but did not increase RCV.

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**Category of the study:** Translational

## Introduction

International Liaison Committee on Resuscitation (ILCOR) suggests at least a 30-second deferral in cord clamping during preterm birth not needing immediate resuscitation [1]. This recommendation is based on the potential benefits of fewer blood transfusions, and lower risk of intraventricular hemorrhage (IVH), and a trend towards lower mortality in premature infants [2, 3]. Recently, a large randomized control trial (RCT) reported mortality data in preterm infants <30 weeks gestation between immediate and delayed cord clamping (DCC) [4]. Although mortality was lower in this study with DCC (6.4 vs. 9%) in unadjusted analysis (p=0.03), it was not significant (p=0.39) after post hoc adjustment for multiple secondary outcomes. [5].

There is insufficient evidence for optimal cord management in preterm infants with low heart rate (HR) needing immediate resuscitation. Two approaches to cord management – ventilation with an intact cord or umbilical cord milking (UCM) are being evaluated for infants requiring immediate resuscitation. A translational study in preterm lambs showed that when ventilation was provided before cord clamping, there was a significant improvement in cerebral and systemic oxygenation [6]. A feasibility study showed that DCC for 5 minutes improved blood pressure and cerebral oxygenation in term neonates at risk of resuscitation [7].

Studies evaluating UCM in preterm infants have demonstrated both benefits and risks. In an RCT of infants <32 weeks, UCM (20 cm stripped three times) led to higher heart rate, oxygen saturation, and lower oxygen requirement in the first 10 min [8]. Preterm neonates who had undergone UCM had better cognitive and language scores at 22–26 months when compared to DCC [9]. In infants requiring resuscitation, milking the cord and allowing it to refill before stripping each time could aid in placental transfusion without delay in initiating resuscitative measures. The UCM in a preterm lamb model had shown fluctuations in carotid pressure and flow, especially when placental refill was not practiced [10]. Katheria et al., in an RCT of preterm infants, have demonstrated high severe IVH in extremely preterm infants with UCM when compared to DCC [9].

The cord management strategies (DCC or UCM) in asphyxiated preterm neonatal models with perinatal metabolic acidosis requiring immediate resuscitation have not been evaluated. Our objectives were to study the effect of early cord clamping followed by ventilation (ECCV), ventilation with DCC for 5 minutes (DCCV), umbilical cord milking with placental refill (UCM), UCM with the placental refill, and concomitant ventilation (UCMV) in the immediate resuscitation phase in an asphyxiated preterm model on red cell volume (RCV), gas exchange, cerebral and pulmonary hemodynamics.

## Methods

Time-dated pregnant ewes at 127–128d gestation (term ~ 145–150 days) were studied as per ARRIVE guidelines after approval by the Institutional Animal Care and Use Committee of the University at Buffalo. The pregnant ewes did not receive antenatal steroids. As described previously, the preterm lamb fetus was instrumented while on placental circulation and with

the ewe under general anesthesia [11]. A jugular venous line is placed for access and blood draw. The right carotid artery is catheterized for pressure monitoring and for arterial blood gas draws. Blood flow transducers are placed to monitor the left common carotid blood flow, left pulmonary artery blood flow, and ductal flow. The lambs were intubated with a cuffed endotracheal tube (ETT), and lung liquid was passively drained by gravity. ETT was occluded before delivery to prevent air entry during gasping. Asphyxia was induced by cord occlusion until the HR dropped below 90 beats per minute (bpm). We defined asphyxia in our group as a HR <100 bpm. To ensure that the preterm lambs HR remained less than 100 bpm, we targeted an HR of 90 bpm. After asphyxia, the lambs were randomized to one of the four protocols: i) ECCV, ii) DCCV, iii) UCM, and iv) UCMV.

**i.** ECCV – Following asphyxia by cord occlusion, the cord was clamped and ventilation was initiated with change in inspired oxygen targeting preductal saturations as per neonatal resuscitation program (NRP) recommendations.

**ii.** DCCV - Following asphyxia, compression of the umbilical cord was released and ventilation was initiated with an intact cord for 5 minutes before clamping the cord.

**iii.** UCM – Following asphyxia, the umbilical cord compression was released and milking was performed by stripping the umbilical cord four times and allowing the cord to fill with the placental flow by pinching the cord on the fetal side to prevent blood from returning from the fetus. Each stripping of the cord and allowing the cord to refill with placental blood took approximately 10 seconds. No ventilation was provided for approximately 40 seconds. The cord was clamped, and ventilation was initiated.

**iv.** UCMV – Following asphyxia, compression of the umbilical cord was released, ventilation was initiated, followed by milking for 40 seconds (as described above but with ventilation), after which the cord was clamped.

Red cell volume (RCV) estimation was done based on a previous study by Strauss et al. [12]. Circulating RCV was measured using biotinylated red blood cells (RBCs) in the neonatal lamb as expressed a percentage of feto-placental volume to assess the magnitude of placental transfusion [12]. Low and high-density biotinylated RBCs were used to estimate feto-placental baseline volume and the newborn volume after the intervention. The effect of the initial intervention was measured at 2 hours (at the end of the study period) to allow for postnatal equilibration. During this period, the intravenous fluids were infused at 120ml/kg/ day, and similar volumes of blood were drawn for blood gas sampling in all groups.

Left carotid, left pulmonary, and ductal flows and blood pressures were continuously monitored throughout the period of observation. In addition, during cord milking, the fluctuations in carotid, pulmonary blood flows with each milk from baseline were measured and represented as a percentage change (fluctuation with cord milking = increase in flow in ml/min at the peak during milking ÷ baseline flow before milking).

Right carotid arterial blood gases were collected at asphyxia, 1, 2, and 5 min of ventilation. The oxygen was titrated based on saturations ($SpO_2$) targets as recommended by NRP.

Supplemental $O_2$ was initiated at 21% and titrated up by ten percent every 30 seconds to achieve the desired targets. The positive pressure ventilation was initiated with peak inspiratory pressures of 30 cmH$_2$O with a peek end-expiratory pressure of 5 cmH$_2$O targeting tidal volumes 8 ml/kg. The pressures were adjusted in order to achieve target tidal volumes every 15s.

### Sample size estimation:

The sample size for this study was based on a difference in the percentage of red cell volume (RCV) between the four groups. Based on our preliminary study, to see a difference in RCV as a percentage of fetomaternal volume between four groups, with an average difference of 15% and a standard deviation of 10% in RCV, with coefficients of 1 −1 −1 −1 (ECCV as control group), 6 lambs in each group by ANOVA were needed for this study, with a power of 0.92 and a type I probability of 0.05 (total n=24). This sample size has adequate power to detect a 20 ml/kg/min difference in pulmonary blood flow, a 5 ml/kg/min difference in carotid blood flow, and a 15 mmHg difference in partial pressures of carbon dioxide (PaCO$_2$) between the groups.

### Statistical analysis:

The Kolmogorov-Smirnov test was done to determine the parametric/non-parametric distribution of the data, and the analysis was done accordingly. For all four groups - We used two-way repeated measures ANOVA for continuous parametric variables. Post-hoc tests were done using the Bonferroni correction. The differences were for the overall profile and not the specific time points. Categorical variables were analyzed using multiple group chi-square test.

UCM & UCMV - Since the comparisons between the fluctuations were only done during milking, we used the unpaired t-test. The significance was set at a probability of less than 5%.

## Results

A total of 23 preterm lambs were evaluated in this study. They were randomized as follows: Six in ECCV, UCM, and UCMV groups and 5 in the DCCV group (due to arrest in one lamb). The characteristics of the lambs randomized are shown in table 1. Following asphyxiation by cord occlusion, preterm lambs in all groups had similar combined respiratory and metabolic acidosis (Table 2).

After initiating PPV, the HRs by 1 min were not significantly different (figure 1A).

### Red cell volume and hemoglobin:

The post-intervention neonatal red cell volumes between each group as a percentage of fetomaternal volume are shown in figure 1B. The UCMV had the highest red cell volume compared to the other groups.

### Oxygenation and ventilation:

After asphyxiation and initial resuscitation with 21% $O_2$ and titration, 75% $SpO_2$ was achieved by 5 min in 3/5 (60%) of DCCV, and 1/6 (17%) of UCMV and none in ECCV and UCM groups. The cumulative oxygen requirement during 5 minutes of resuscitation is shown as box plots (figure 1C). In the first 5 minutes, 75% of lambs in the DCCV group needed <50% oxygen, which was significantly different from the other groups. The arterial oxygenation was not statistically different between the groups (figure 2A). Carbon dioxide levels were lowest with DCCV, while UCM without ventilation had significantly higher $PaCO_2$ levels (figure 2B). The pH significantly improved in the DCCV group and was significantly higher at 5 minutes than the rest (figure 2C).

### Pulmonary and carotid blood flow:

Pulmonary blood flow increased with ventilation in all lambs (figure 3A). The pulmonary blood flows were lowest in the UCM group. DCCV had significantly higher pulmonary flows when compared to other groups. The carotid blood flow was significantly higher in the UCM group compared to other groups (figure 3B). Ductal flows in the fetal lambs were right-to-left (pulmonary-to-systemic circulation). At 5 min, the ductal shunting reversed left-to-right (systemic-to-pulmonary) in the DCCV and UCMV groups but not in ECCV and UCM groups (figure 3C). The flows across the ductus were not significantly different. The mean blood pressures were similar in all groups (figure 3D).

### Flows during umbilical cord milking:

Fluctuations in carotid and pulmonary blood flows during milking were assessed with (UCMV) and without ventilation (UCM) (figure 4) and expressed as a percentage of baseline flows during the four cord-stripping maneuvers. During milking of the cord with and without ventilation, the fluctuations in carotid flow (UCMV 6.9±2.6 % vs. UCM 24.3±6.1 % respectively, p<0.0001) and pulmonary flow (UCMV 4.8±4.5% vs. UCM 22.7±13.6% respectively, p=0.023) from baseline (figure 5).

## Discussion

Placental transfusion facilitates transition at birth by three distinct mechanisms – volume effect by adding circulating blood volume to the neonate, oxygenation by providing umbilical venous return (with $SO_2$ ~80–85%) and circulatory stabilization by contributing to left ventricular preload [13]. Initiating positive pressure ventilation in a depressed preterm infant is the most important step to facilitate transition [14]. In term infants, with the initial cries, the lungs take over the function of gas exchange from the placenta. As a result, the PVR decreases and pulmonary blood flow increases, and the shunt across the ductus is from systemic to pulmonary circulation [15, 16]. However, in a depressed premature neonate, acidosis, hypoxia, poor respiratory effort, immature lung architecture, and function may delay the transition. A metanalysis by Oei et al. has shown that preterm neonates who do not achieve saturations of >80% by 5 min and bradycardic are at risk of IVH and death [17]. In another retrospective analysis, preterm neonates remaining bradycardic for 5 min (with HR < 60 bpm) with $SpO_2$ <80% were at 18 times higher risk of mortality [18]. Thus,

avoiding delays in ventilation with simultaneous placental transfusion in preterm neonates may potentially lead to better outcomes.

Two approaches have been suggested to avoid ventilation delays due to placental transfusion. One approach is to provide PPV during DCC, and the second approach is to accelerate placental transfusion by cord milking. Previous studies in preterm lambs have shown that ventilation during DCC (physiological-based cord clamping) provided circulatory stability but did not lead to a net transfer of blood volume from the placenta to the neonate [10]. Cord milking would be a logical step to provide additional blood volume immediately after birth and continue with resuscitation but is associated with fluctuations in cerebral blood flow and IVH in preterm [9]. In this study, we evaluate cord milking with placental refill with simultaneous ventilation and demonstrated significantly higher transfusion of red cell volume and minimized fluctuations in cerebral blood flow with milking.

Increasing the transfer of placental blood volume to the neonate is a hematologic benefit of placental transfusion. Blank et al. have elegantly shown that in a non-asphyxiated preterm model, milking the cord eight times with placental refill significantly increased the blood volume [10]. However, DCC with ventilation did not result in a net transfer of blood to the non-asphyxiated neonatal lamb [10]. Our study confirmed these findings in an asphyxiated preterm lamb model (figure 1). In addition, clinical trials among preterm infants predominantly delivered by cesarean section demonstrate higher hemoglobin with UCM but not with DCC, consistent with this finding [19, 20].

The second benefit of placental transfusion is the delivery of oxygenated umbilical venous blood with the placenta continuing to serve as a site of gas exchange [6, 21–26]. Lambs in the DCCV group had the highest pH, lowest $PaCO_2$, and less inspired oxygen load. This approach of ventilation before clamping the cord is known as physiological-based cord clamping [6]. It has been associated with higher cerebral oxygen saturations than UCM in non-asphyxiated preterm lambs [10]. In non-asphyxiated [10] and asphyxiated preterm lambs, physiological-based cord clamping (DCCV) appears to be the most effective technique to improve oxygenation and ventilation due to "dual-site" (placenta and lungs) gas exchange and improved pulmonary blood flow. Milking with placental refill without ventilation (UCM) deprives the lamb of this benefit and is associated with high $PaCO_2$, low pH, and low pulmonary blood flow. During milking (UCMV), ventilation did not significantly improve pulmonary blood flow and did not appear to have the same benefits as physiological-based cord clamping. However, the benefits of providing additional oxygen-carrying capacity with red blood cell volume transfused with UCMV will potentially benefit tissue oxygen delivery and could be more clinically relevant in extremely preterm infants than just increasing pulmonary blood flow by DCCV [27].

The third benefit of placental transfusion is circulatory stabilization. In preterm neonates, excessive fluctuations and rapid increases in cerebral blood flow can potentially be associated with IVH [28]. Studies in non-asphyxiated preterm lambs have suggested that milking of the cord could lead to rapid increase and fluctuations in carotid blood flow [10]. Stenning et al. demonstrated that infusion of blood to a non-asphyxiated preterm lamb

(10ml/kg of blood over 90 s) led to a transient increase in carotid blood flow and arterial pressure, and these values decreased after ventilation onset [29]. These findings suggest that increased circulatory volume due to blood infusion without simultaneous ventilation can lead to abrupt increases in cerebral blood pressure and flow. Similar increases in carotid blood flow were observed following UCM [10].

Our study found decreased fluctuations in both carotid and pulmonary blood flow when the cord was milked with placental refill and ventilation compared to UCM alone. The UCM with ventilation led to similar carotid flows to DCCV and ECCV, with a change in the direction of ductal flows to the left to right at 5 min. We speculate that ventilation of the lung and decreased pulmonary vascular resistance minimized fluctuations in carotid blood flow. The fluctuations in both carotid and pulmonary blood flow from the baseline when the cord was milked with placental refill but without ventilation was similar to a previous study in a non-asphyxiated preterm ovine model (figure 4) [10].

Delaying the ventilation for 40 seconds until UCM was performed led to significantly higher carbon dioxide levels. In an asphyxiated preterm infant, higher $CO_2$ levels and hemodynamic fluctuations incurred during milking of the umbilical cord could affect cerebral blood and be detrimental in premature neonates. In a post hoc analysis of a randomized control trial, UCM (milking 3 times with 2 seconds refill from placenta) in preterm infants was associated with a higher rate of severe IVH compared to delayed umbilical cord clamping [30] [31]. With the available evidence from clinical and translational studies, UCM without ventilation does not demonstrate any benefit in an asphyxiated depressed preterm neonate without respiratory effort (except for a non-significant increase in blood volume), and could lead to further complications such as severe IVH. In contrast, physiological-based cord clamping (DCCV) did not increase blood volume but had better hemodynamics and gas exchange.

There are several limitations of our study. This model of asphyxiated preterm lamb is delivered by cesarean section under general anesthesia without uterine contractions. Inherent species differences exist since the ovine umbilical cord is short and has 2 arteries and 2 veins. The lung's developmental stage in lambs is closer to human neonates than that of brain. Regarding instrumentation, a limitation is cannulation of the contralateral carotid artery that could have interfered with the common carotid artery blood flow measurement. To compare the milking with milking and ventilation, we did allow the cord to refill, and the entire procedure took around 40 seconds, which could be shorter in human neonates given the longer length of the umbilical cord. The hemodynamic advantages of continuous ventilation during DCC and UCM over the respective group, compared with early cord clamping and ventilation (ECCV), unfortunately, did not reach significance. The optimal clinical alternative to UCM would be DCC without ventilation or 40–60 seconds of DCC followed by ventilation. One limitation with the model of cord compression for asphyxia is that it takes a few seconds for the umbilical veins to open up (with improving heart rate) before placental transfusion is established. Following the release of the umbilical cord compression, a short delay in the clamping of the cord in this model could have worsened bradycardia and led to higher $PaCO_2$ levels with no placental flow in the absence of ventilation. The initiation of ventilation improved the heart rate that led to the establishment

of blood flows across the umbilical cord. Thus, the $PaCO_2$ was significantly lower with ventilation. In the UCM group, milking was performed, allowing refill manually. While our study demonstrated the hemodynamic advantages of continuous ventilation during DCCV and UCMV over the respective group, the comparison with early cord clamping and ventilation (ECCV) unfortunately did not reach statistical significance. Nevertheless, our study provides mechanistic evidence of various approaches to cord management in asphyxiated bradycardic preterm neonates.

## Conclusion

Various modalities of cord management have distinct physiological effects on transfusion volume, gas exchange, and transitional hemodynamics. In an asphyxiated preterm lamb model, physiological-based cord clamping by providing positive pressure ventilation with an intact umbilical cord (DCCV) led to better gas exchange and hemodynamics but did not increase red cell volume. Cord milking with ventilation (UCMV) led to higher blood cell volume and reduced fluctuation in carotid flow. Cord milking without ventilation did not significantly increase red cell volume but was associated with increased carotid flow fluctuations. Results of our study and the ongoing clinical trials of cord management in preterm infants will provide more evidence for clinical practice.

## Financial support:

### Conflict of interest:

SL is a neonatal resuscitation program steering committee member. *All other authors report no conflicts of interest.* The research reported here is not endorsed by the funding institutions or the neonatal resuscitation program.

## References:

1. Perlman JM et al. Part 7: Neonatal Resuscitation: 2015 International Consensus on Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science With Treatment Recommendations. Circulation. 2015;132(16 Suppl 1):S204–241. [PubMed: 26472855]

2. Rabe H, Diaz-Rossello JL, Duley L, Dowswell T. Effect of timing of umbilical cord clamping and other strategies to influence placental transfusion at preterm birth on maternal and infant outcomes. Cochrane Database Syst Rev. 2012(8):CD003248.

3. Rabe H, Reynolds G, Diaz-Rossello J. Early versus delayed umbilical cord clamping in preterm infants. Cochrane Database Syst Rev. 2004(4):CD003248.

4. Tarnow-Mordi W et al. Delayed versus Immediate Cord Clamping in Preterm Infants. N Engl J Med. 2017;377(25):2445–2455. [PubMed: 29081267]

5. Katheria AC. Delayed cord clamping may not be beneficial in the premature infant. J Pediatr. 2018;196:324–327.

6. Polglase GR et al. Ventilation onset prior to umbilical cord clamping (physiological-based cord clamping) improves systemic and cerebral oxygenation in preterm lambs. PLoS One. 2015;10(2):e0117504.

7. Katheria AC et al. Delayed Cord Clamping in Newborns Born at Term at Risk for Resuscitation: A Feasibility Randomized Clinical Trial. J Pediatr. 2017;187:313–317 e311. [PubMed: 28526223]

8. Katheria A, Blank D, Rich W, Finer N. Umbilical cord milking improves transition in premature infants at birth. PLoS One. 2014;9(4):e94085.

9. Katheria A et al. A Randomized Clinical Trial of Umbilical Cord Milking vs Delayed Cord Clamping in Preterm Infants: Neurodevelopmental Outcomes at 22–26 Months of Corrected Age. J Pediatr. 2018;194:76–80. [PubMed: 29246467]

10. Blank DA et al. Haemodynamic effects of umbilical cord milking in premature sheep during the neonatal transition. Arch Dis Child Fetal Neonatal Ed. 2018;103(6):F539–F546. [PubMed: 29208663]

11. Chandrasekharan P et al. Effect of various inspired oxygen concentrations on pulmonary and systemic hemodynamics and oxygenation during resuscitation in a transitioning preterm model. Pediatr Res. 2018;84(5):743–750. [PubMed: 29967523]

12. Strauss RG et al. Circulating RBC volume, measured with biotinylated RBCs, is superior to the Hct to document the hematologic effects of delayed versus immediate umbilical cord clamping in preterm neonates. Transfusion. 2003;43(8):1168–1172. [PubMed: 12869126]

13. Bhatt S et al. Delaying cord clamping until ventilation onset improves cardiovascular function at birth in preterm lambs. The Journal of physiology. 2013;591(Pt 8):2113–2126. [PubMed: 23401615]

14. Textbook of Neonatal Resuscitation (NRP), 7th Ed2016.

15. Lakshminrusimha S, Jobe AH. Baby's First Cries and Establishment of Gas Exchange in the Lung. American journal of respiratory and critical care medicine. 2021;204(1):11–13. [PubMed: 33684327]

16. Tingay DG et al. Imaging the Respiratory Transition at Birth: Unravelling the Complexities of the First Breaths of Life. American journal of respiratory and critical care medicine. 2021.

17. Oei JL et al. Outcomes of oxygen saturation targeting during delivery room stabilisation of preterm infants. Arch Dis Child Fetal Neonatal Ed. 2017.

18. Kapadia V et al. Outcomes of delivery room resuscitation of bradycardic preterm infants: A retrospective cohort study of randomised trials of high vs low initial oxygen concentration and an individual patient data analysis. Resuscitation. 2021;167:209–217. [PubMed: 34425156]

19. March MI, Hacker MR, Parson AW, Modest AM, de Veciana M. The effects of umbilical cord milking in extremely preterm infants: a randomized controlled trial. Journal of perinatology : official journal of the California Perinatal Association. 2013;33(10):763–767. [PubMed: 23867960]

20. Katheria AC, Truong G, Cousins L, Oshiro B, Finer NN. Umbilical Cord Milking Versus Delayed Cord Clamping in Preterm Infants. Pediatrics. 2015;136(1):61–69. [PubMed: 26122803]

21. Andersson O et al. Intact cord resuscitation versus early cord clamping in the treatment of depressed newborn infants during the first 10 minutes of birth (Nepcord III) - a randomized clinical trial. Matern Health Neonatol Perinatol. 2019;5:15. [PubMed: 31485335]

22. Chandrasekharan P et al. Resuscitation with an Intact Cord Enhances Pulmonary Vasodilation and Ventilation with Reduction in Systemic Oxygen Exposure and Oxygen Load in an Asphyxiated Preterm Ovine Model. Children (Basel). 2021;8(4).

23. Lakshminrusimha S, Vali P, Chandrasekharan P, Rich W, Katheria A. Differential Alveolar and Systemic Oxygenation during Preterm Resuscitation with 100% Oxygen during Delayed Cord Clamping. Am J Perinatol. 2021.

24. Padilla-Sanchez C et al. Delayed vs Immediate Cord Clamping Changes Oxygen Saturation and Heart Rate Patterns in the First Minutes after Birth. J Pediatr. 2020;227:149–156 e141. [PubMed: 32710909]

25. Rudolph AM, Heymann MA. Cardiac output in the fetal lamb: the effects of spontaneous and induced changes of heart rate on right and left ventricular output. Am J Obstet Gynecol. 1976;124(2):183–192. [PubMed: 129010]

26. Yigit B, Tutsak E, Yildirim C, Hutchon D, Pekkan K. Transitional fetal hemodynamics and gas exchange in premature postpartum adaptation: immediate vs. delayed cord clamping. Matern Health Neonatol Perinatol. 2019;5:5. [PubMed: 31011431]

27. Katheria AC, Brown MK, Rich W, Arnell K. Providing a Placental Transfusion in Newborns Who Need Resuscitation. Front Pediatr. 2017;5:1. [PubMed: 28180126]

28. Chawla S, Chock VY, Lakshminrusimha S. Intraventricular hemorrhage and white matter injury: is persistent cerebral desaturation a missing link? Pediatr Res. 2021;89(4):727–729. [PubMed: 33247218]

29. Stenning FJ et al. Transfusion or Timing: The Role of Blood Volume in Delayed Cord Clamping During the Cardiovascular Transition at Birth. Front Pediatr. 2019;7:405. [PubMed: 31649907]

30. Katheria A et al. Association of Umbilical Cord Milking vs Delayed Umbilical Cord Clamping With Death or Severe Intraventricular Hemorrhage Among Preterm Infants. JAMA. 2019;322(19):1877–1886. [PubMed: 31742630]

31. Rabe H, Andersson O. Maternal and Infant Outcomes After Different Methods of Umbilical Cord Management. JAMA. 2019;322(19):1864–1865. [PubMed: 31742617]

**Impact Statement:**

- The best practice of placental transfusion in a depressed preterm neonate remains unknown

- Ventilation with an intact cord improves gas exchange and hemodynamics in an asphyxiated preterm model

- Cord milking without ventilation led to lower red cell volume but higher carotid blood flow fluctuations compared to milking with ventilation

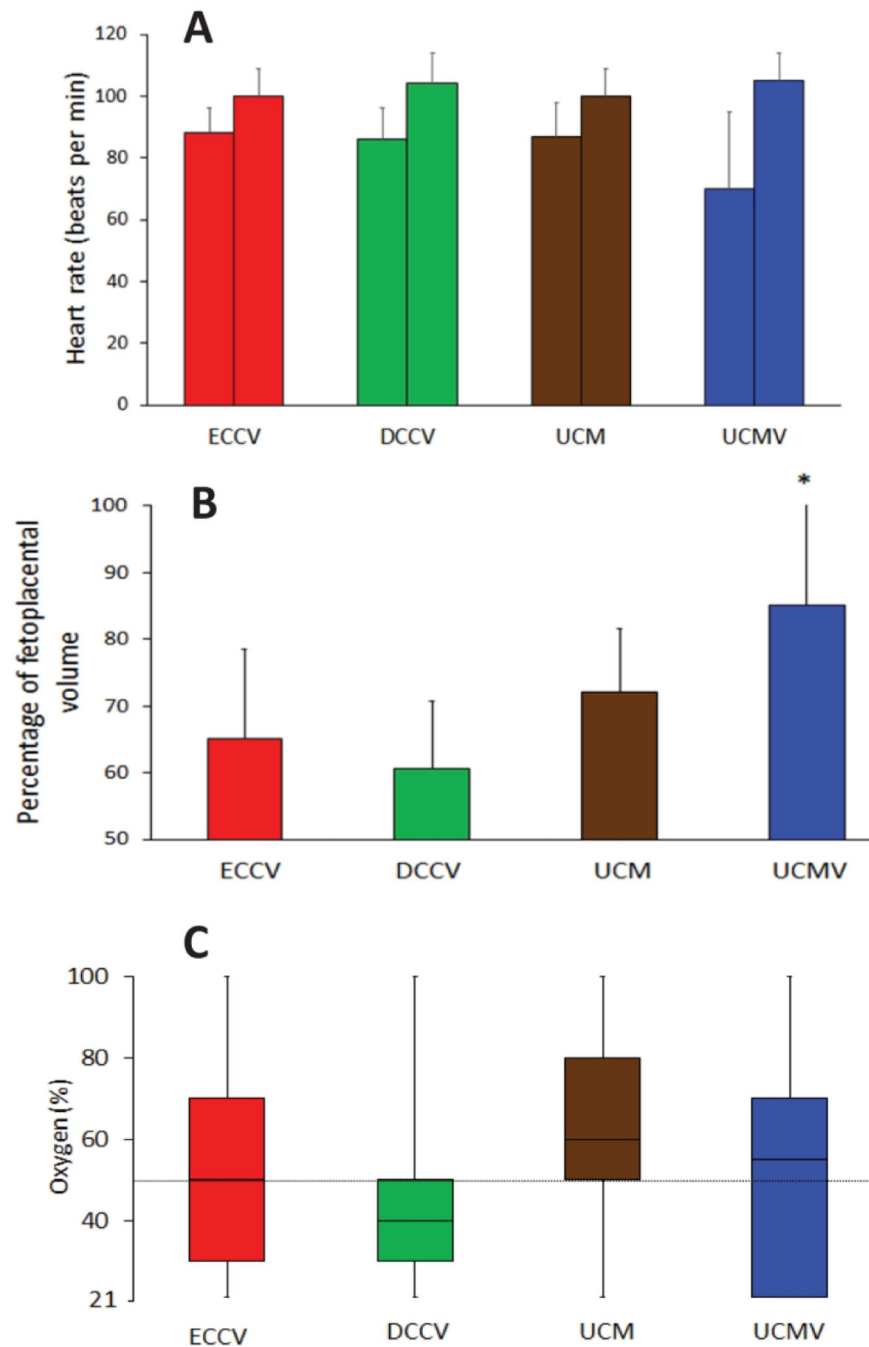- Our data can be translated to bedside and could impact preterm resuscitation

**Figure 1.**
A. Shows the heart rate (HR) at asphyxia and 1 min in each group. The HRs were not different between the groups at asphyxia and at 1 min. B. Neonatal blood volume as a percentage of fetoplacental volume; * represents statistical significance p<0.01. C. Cumulative inspired oxygen concentration during 1 to 5 min in the four groups of lambs. The horizontal dotted line represents 50% inspired oxygen.
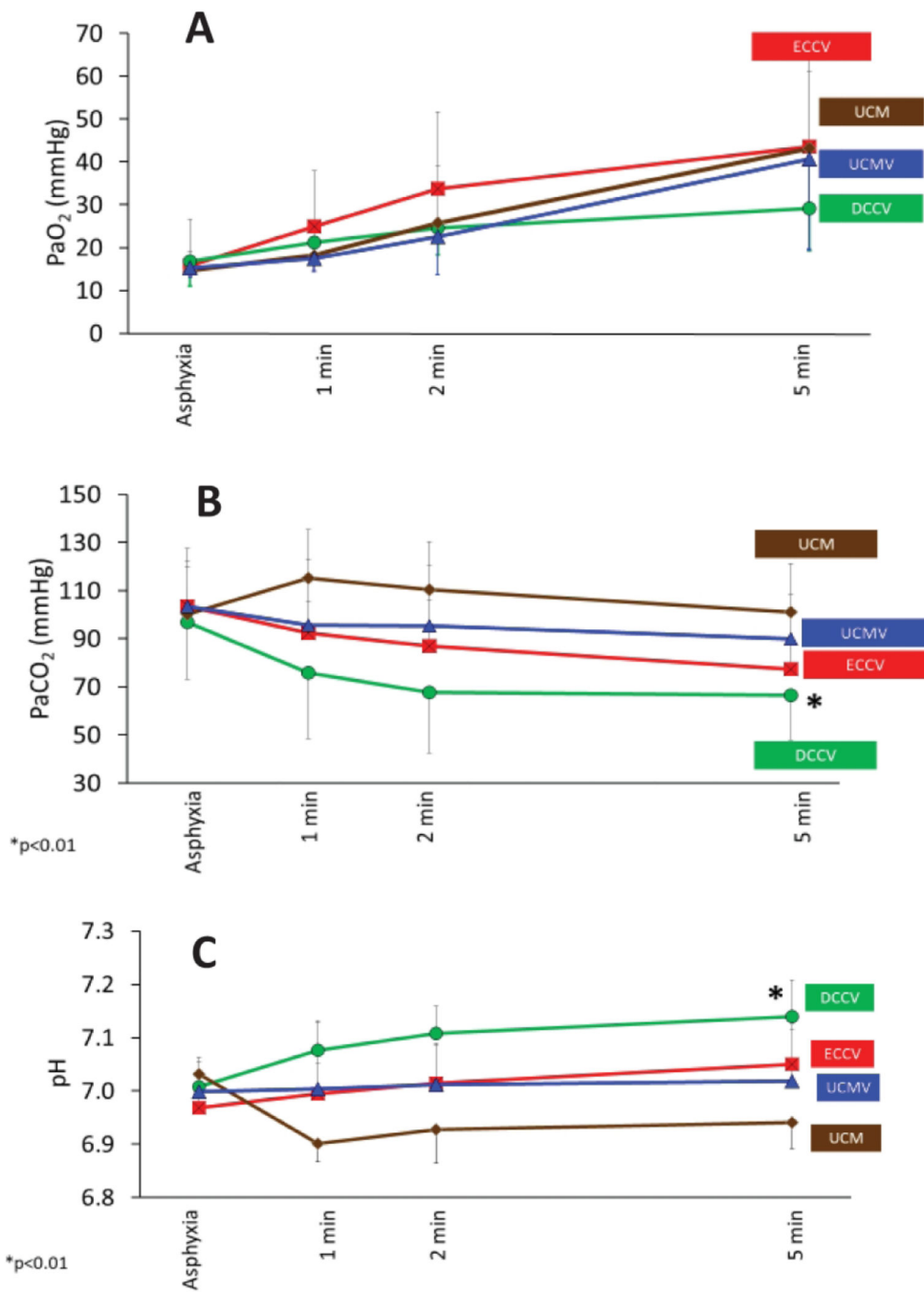
**Figure 2:**

Arterial blood gas results from asphyxia to 5 min in the four groups of lambs. Square marker – ECCV, circle – DCCV, triangle – UCMV, diamond – UCM. * p<0.01 represents statistical significance by ANOVA repeated measures. A. Arterial oxygenation; B. Carbon dioxide (PaCO$_2$); C. Arterial pH.
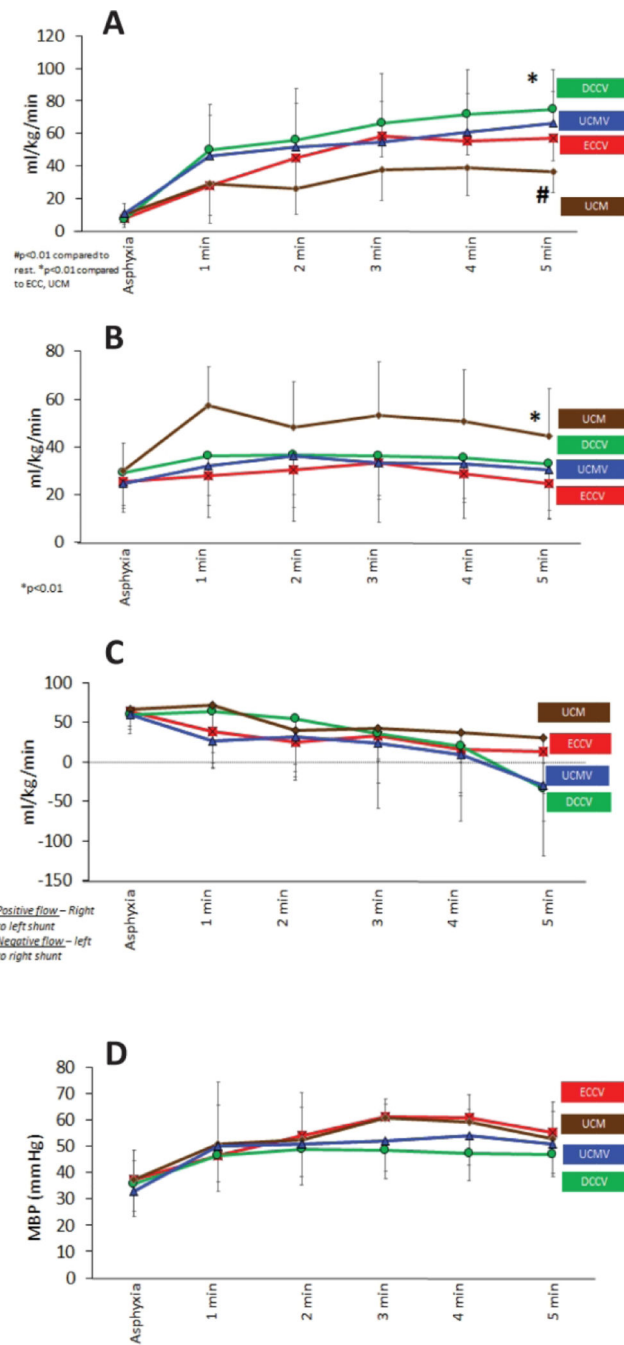
**Figure 3:**
The line graph represents the four groups square – ECCV, circle – DCCV, triangle – UCMV, diamond – UCM. * p<0.01, # p <0.01 – statistical significance. A. Left pulmonary blood flow; B. Left carotid blood flow; C. ductal blood flow (positive values – right-to-left shunt and negative values – left-to-right shunt or systemic-to-pulmonary shunting); D. mean blood pressure (MBP).
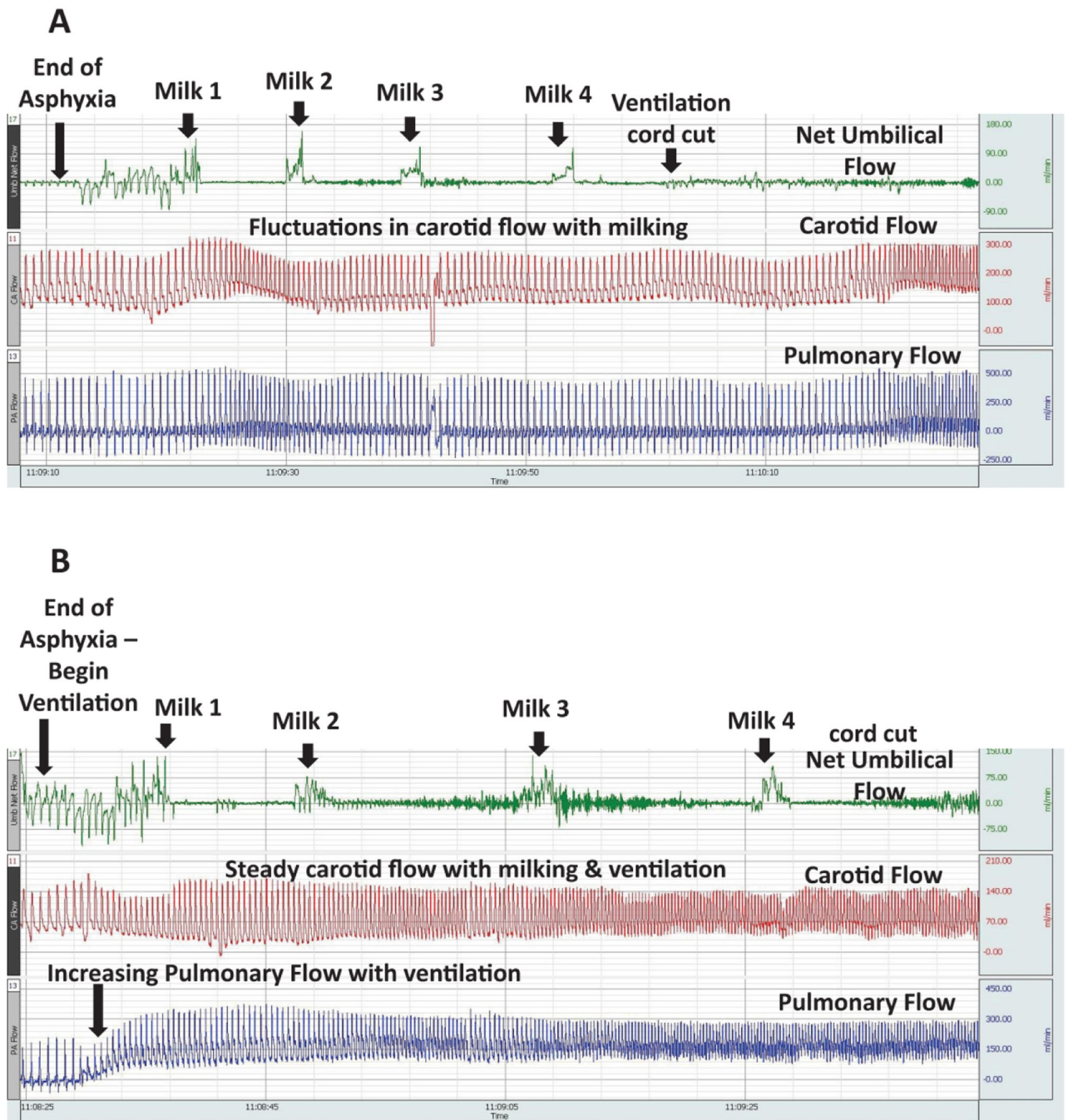
**Figure 4:**
Representative hemodynamic chart (from BIOPAC snapshot) with umbilical, carotid, and pulmonary blood flows. A. UCM without ventilation demonstrates fluctuations in carotid flow with each instance of cord milking. B. UCMV shows the effect of milking with simultaneous ventilation, showing minimal fluctuations in carotid flow and increased pulmonary blood flow with ventilation.
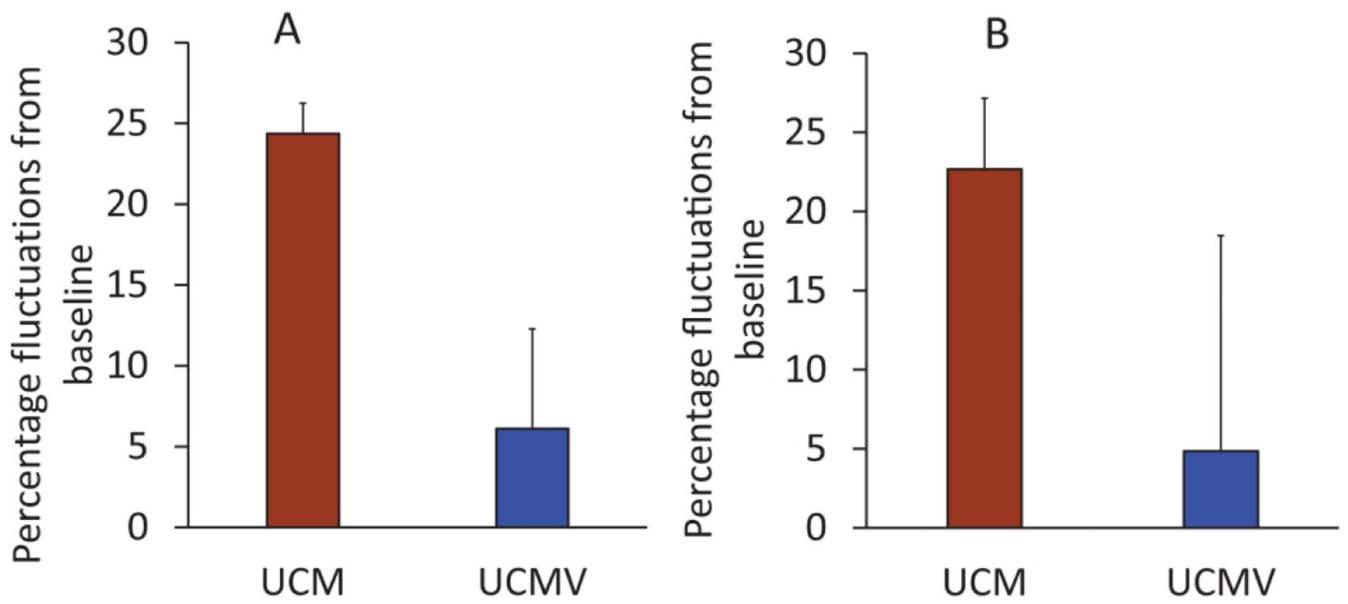
**Figure 5:**
The bar graph represents the percentage fluctuation during the four milks from the baseline blood flow. The carotid and pulmonary flow fluctuations were significantly higher when milking was performed without ventilation. *p-value <0.05 – represents statistical significance.
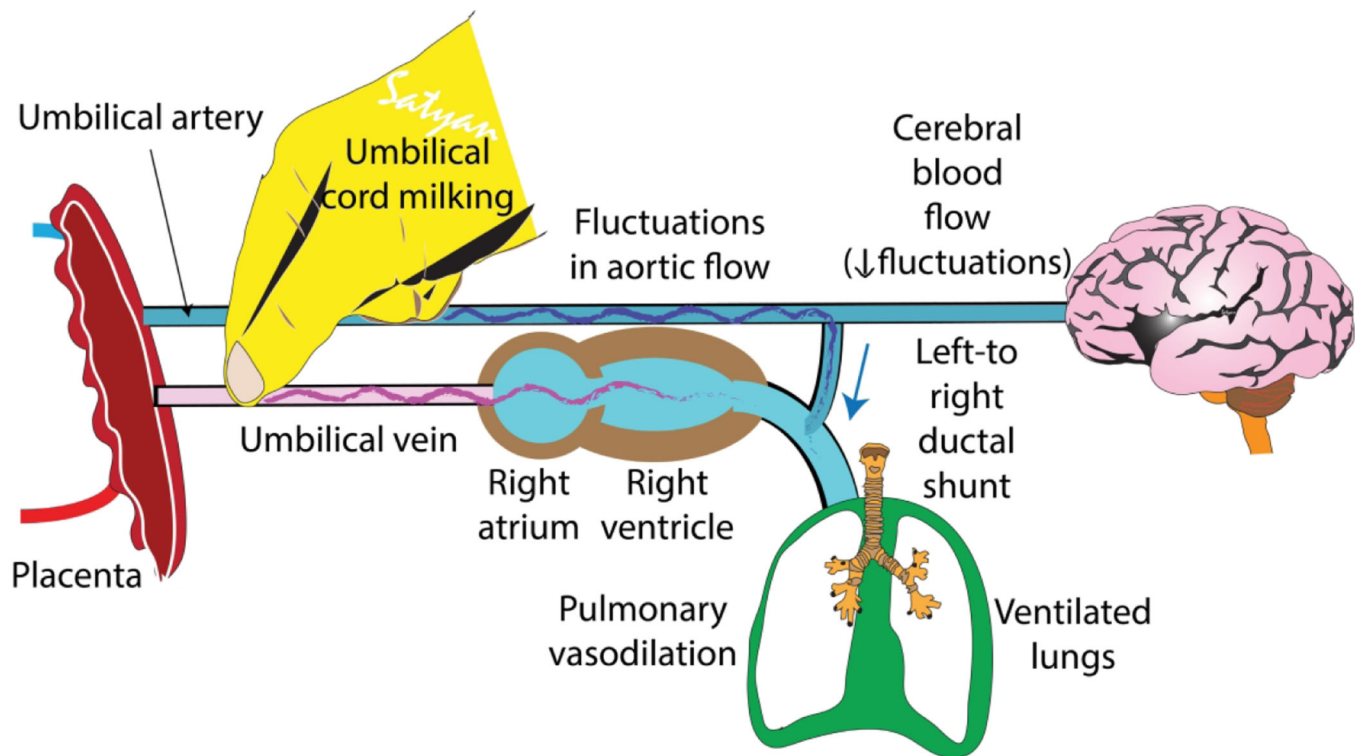
**Figure 6:**
Graphic summary showing a speculative mechanism for hemodynamic changes when umbilical cord milking is performed with ventilation. Umbilical cord milking produces fluctuations in flow in the descending aorta. With the initiation of ventilation, there is increased pulmonary blood flow and a left-to-right ductal shunt. The left-to-right ductal shunt and increased pulmonary blood flow buffer these changes in flow, minimizing fluctuations in carotid blood flow compared to cord milking without ventilation (copyright Satyan Lakshminrusimha).

**Table 1:**

Baseline characteristics of the preterm lambs

| Parameters | Early cord clamping & ventilation ECCV (n=6) | Delayed cord clamping with ventilation DCCV (n=5) | Umbilical cord milking UCM (n=6) | Umbilical cord milking with ventilation UCMV (n=6) |
|---|---|---|---|---|
| Gestational age (days) (mean ± SD) | 127 ± 0.4 | 127 ± 0.5 | 127 ± 0.4 | 127 ± 0.4 |
| Birth weight (kg) (mean ± SD) | 3.6 ± 0.5 | 3.1 ± 0.6 | 3.3 ± 0.5 | 3.0 ± 0.6 |
| Sex - Female (%) | 3 (50%) | 3 (60%) | 3 (50%) | 4 (67%) |
| Multiples (n) | 2 | 1 | 2 | 2 |
| Hemoglobin (g/dl) | 12 ± 0.9 | 12 ± 1.2 | 12 ± 1.1 | 12 ± 1.0 |
| pH | 7.23 ± 0.08 | 7.24 ± 0.02 | 7.23 ± 0.10 | 7.24 ± 0.06 |
| $PaO_2$ (mmHg) | 26.5 ± 5.4 | 24.2 ± 5.5 | 23.7 ± 6.4 | 26.0 ± 6.7 |
| $PaCO_2$ (mmHg) | 63.7 ± 6.8 | 64.4 ± 10.1 | 66.5 ± 10.7 | 66.5 ± 11.4 |
| Pulmonary blood flow (ml/kg/min) | 24 ± 10 | 22 ± 13 | 26 ± 8 | 24 ± 10 |
| Common carotid flow (ml/kg/min) | 28 ± 13 | 33 ± 15 | 30 ± 14 | 30 ± 18 |

**Table 2:**

Parameters at asphyxia

| Parameters | ECCV (n=6) | DCCV (n=5) | UCM (n=6) | UCMV (n=6) |
|---|---|---|---|---|
| Heart rate (per min) | 88 ± 8 | 86 ± 10 | 87 ± 11 | 70 ± 25 |
| Mean BP (mm Hg) | 36 ± 8 | 34 ± 10 | 30 ± 10 | 37 ± 6 |
| pH | 6.97 ± 0.06 | 7.01 ± 0.05 | 7.03 ± 0.04 | 7.00 ± 0.06 |
| PaCO$_2$ (mmHg) | 95 ± 15 | 97 ± 18 | 99 ± 13 | 96 ± 18 |

Data presented as mean and SD