

Umbilical Cord Management at Birth Douglas Andrew Blank, MD The Ritchie Centre, Hudson Institute of Medical Research

A thesis submitted for the degree of Doctor of Philosophy at Monash University in 2018 Faculty of Medicine, Nursing and Health Sciences Department of Obstetrics and Gynaecology

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# 1 Acknowledgements

The work presented in this thesis is the continuation of many studies at the Ritchie Centre and the Royal Women's Hospital designed to improve the health of newborns. I have been given a unique opportunity to join an elite team of doctors, nurses, and scientists who have led nearly every important advancement in our field over the last few decades. When I broached the idea of moving from California to Melbourne, on October 31<sup>st</sup> 2013, to begin an academic career, my mentor, Neil Finer, said that if I truly wanted to pursue academics, there was no better place in the world he could recommend and that the people I would be working with are the nicest I could hope to encounter. I really set out to chase the accomplishments of Georg S, but my experience has surpassed Dr Finer's recommendation.

I owe deep gratitude to many. Thank you to my parents, Howard and Leta, who instilled in me an ethos of challenging and questioning every theory and idea I learned until I felt I had comfortably understood the crucial concepts. My parents encouraged me to have freedom of thought and empowered me to seek out my own education. They ensured that I was placed in nurturing educational environments, where I was taught and coached by adults who respected the curiosity of a student. Thank you to my sisters, Meridith and Kara, who set a high expectation of achievement for me and gave me the confidence that I could exceed their expectations.

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This work would be irrelevant and impossible without love and support of my amazing wife, Paisley. You constantly challenge me and help me to become a better version of myself. I love you more every day. To my sons, Gardner, Levi, and Jett, thank you for being so full of enthusiasm and joy. Gardner, I admire your combination of discipline and curiosity. Levi, I hope that I can one day become as compassionate and as imaginative as you are. Jett, your determination, resilience, and humour always bring a smile to my face.

Be well,



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# 3 Abstract: Improving Neonatal Resuscitation for 800,000 Lost Infants

Eight hundred thousand infants die annually because they do not breathe adequately after birth. Ten percent of infants born worldwide require assistance breathing in the first minutes after birth.<sup>8-14</sup> Recently, there has been a resurgence of interest in delayed cord clamping (DCC) after delivery.

The ideal conditions to clamp the umbilical cord in infants who need help initiating breathing after birth is unknown. For months, the placenta supports fetal circulation and provides oxygen and nutrients ideal for growth and development. Studies show that preterm infants receiving DCC have less transfusions, lower rates of intraventricular haemorrhage, and lower rates of necrotizing enterocolitis, while term infants have higher iron stores and likely lower mortality. <sup>15,16</sup> In these studies, all infants receiving DCC were breathing spontaneously.

If an infant does not breathe at birth, current guidelines recommend immediate cord clamping (ICC) so the infant can be moved to a resuscitation platform for respiratory support.<sup>8,12,14,17</sup> The hemodynamic instability and poor condition at birth underlies intraventricular haemorrhage, hypoxic ischemic encephalopathy, and mortality.<sup>17-24</sup> It is widely recognized that the key to regaining physiologic stability during neonatal resuscitation is the establishment of lung aeration and pulmonary blood flow.<sup>8,12,14,25-31</sup> I hypothesise that if the apnoeic, non-vigorous newborn remained connected to the placental circulation via the umbilical cord during neonatal resuscitation, the oxygen levels, cerebral and systemic perfusion, and other markers of haemodynamic status will remain stable despite the infant not breathing.

Therefore, the central purpose of this thesis is to determine if establishment of lung aeration, pulmonary gas exchange, and the increase in pulmonary venous return prior to umbilical cord clamping increases haemodynamic stability in newborns that require assistance to initiate breathing after birth. We have named this technique physiologically-based cord clamping (PBCC) and baby-directed umbilical cord clamping (Baby-DUCC).<sup>7,32</sup>

The physiological changes at birth during ICC, DCC, and novel techniques like Baby-DUCC and umbilical cord milking (UCM) were previously not well understood. The "right time" to clamp the umbilical cord and separate a newborn from the placental circulation and the mother has previously been an arbitrarily prespecified time after birth. I have set out to determine the most logical and simple physiologic target that indicates that the newborn is ready to be independent from the placental circulation.

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# 4 Thesis Outline

This thesis is a combination of experiments in newborn lambs and observational studies in newborn infants. These studies were conducted to better understand the physiologic changes that occur during birth to determine an appropriate physiologic target to indicate when the newborn is ready for umbilical cord clamping. Included is a review chapter of the haemodynamic changes that occur at birth and a review of the findings from umbilical cord management trials (Chapter 6). Next, I present several observational studies in newborns and in preterm sheep that examine physiologic changes that occur immediately after birth as the infant initiates breathing. In addition, these studies search for candidate physiological markers that could indicate the infant is ready for umbilical cord clamping. Specifically, the utility of lung ultrasound (LUS) and exhaled carbon dioxide (ECO<sub>2</sub>) are investigated. Later, ventilation prior to umbilical cord clamping (PBCC) is compared to immediate cord clamping and umbilical cord milking in preterm lambs. Finally, I present a feasibility study in which delayed cord clamping persists until the infant is either breathing spontaneously or is receiving effective ventilation (baby-directed umbilical cord clamping or Baby-DUCC). Baby-DUCC is similar to physiologically based cord clamping described in the previous lamb study and ensures continued placental circulation until the infant's lungs are functioning properly. In Baby-DUCC, ECO<sub>2</sub> is used to determine readiness for umbilical cord clamping in apnoeic infants receiving respiratory support.

# Physiologic targets to determine newborn readiness for umbilical cord clamping

Lung ultrasound (LUS) has shown promise as a diagnostic tool for the evaluation of newborns with respiratory distress. Following birth, the organ responsible for gas exchange transitions from the placenta to the lungs. To establish pulmonary gas exchange, the airways must be cleared of liquid to allow the entry of air. LUS may be able to characterize this process. It can be performed at the bedside in real time, may be easily repeated during clinical changes and treatments, and does not expose the infant to ionizing radiation. Ultrasound beams penetrating an unaerated, liquid-filled lung create true ultrasound images as the density of liquid changes between the tissue layers. In contrast, ultrasound beams passing through an aerated lung produce artefacts. Traditionally, the interference of sound waves caused by air in the lungs has discouraged the use of LUS as a diagnostic tool. However, an understanding of these characteristic artefacts has led to the recognition that they are consistent and have diagnostic importance. In addition to the findings presented in **Chapters 7, 8, and 9**, these studies provided an opportunity to gain experience using LUS in the delivery room. I evaluated the ability of LUS to detect different stages of neonatal adaption after birth to determine when the newborn is ready for umbilical cord clamping.

In the delivery room, leading resuscitation programs recommend clinicians consider pneumothorax if extensive resuscitation is required for a baby with bradycardia but does not recommend clinical signs or tests to aid in the diagnosis of pneumothorax in the delivery room. In **Chapter 7**, I investigate the accuracy of LUS to detect pneumothorax in preterm, newborn lambs compared to post-mortem examination and report the test characteristics of LUS.<sup>2</sup>

In **Chapters 8 and 9**, LUS is used to describe initial lung aeration and lung liquid clearance, starting with the initiation of breathing in term and near-term infants.<sup>3,4</sup> Over 100 infants are enrolled in these observational studies in which serial lung ultrasound assessments were continued until neonatal adaption to birth was complete. In these three chapters, I also reviewed the literature regarding the use of LUS to describe neonatal adaption, diagnosis of pulmonary pathology, and detection of pneumothorax.

In **Chapter 10**, I present an observational study, measuring exhaled carbon dioxide, exhaled tidal volume, and respiratory rate from the first breath after birth until cardiopulmonary transition is complete in 100 healthy, term newborns.

The physiologic effects of umbilical cord milking (UCM) and delayed cord clamping (DCC)

Delayed umbilical cord clamping (DCC) improves outcomes for preterm infants. It results in fewer blood transfusions, lower rates of intraventricular haemorrhage and necrotising enterocolitis, improved haemodynamics, and improved motor function at 18–22 months of age.<sup>16</sup> However, if the newborn doesn't breathe after birth, resuscitation guidelines recommend immediate cord clamping (ICC) and moving the infant to a resuscitation platform. It is widely assumed that the main benefit of DCC arises from a placental to infant blood transfusion. This has led to the suggestion that umbilical cord milking (UCM) may be an alternative that does not delay the infant's transfer to a warming bed for respiratory support.<sup>33-35</sup> In physiologic based cord clamping (PBCC) the lung is aerated prior to umbilical cord clamping, stimulating a large increase in pulmonary blood flow and pulmonary gas exchange, so that pulmonary venous return can immediately replace umbilical venous return as the primary source of left ventricular preload following cord clamping.<sup>32,36,37</sup> No information was previously available on the physiological effects of UCM and whether it is physiologically similar to PBCC/DCC. **Chapter 11** presents the results of this animal experiment.

# Feasibility for Baby-Directed Umbilical Cord Clamping (Baby-DUCC)

If the newborn still has access to the placental circulation until the lungs become functional, I hypothesise that the newborn will maintain physiologic stability in the form of a steady, normal heart rate, higher oxygen saturation, and higher cerebral oxygen delivery as is apparent in newborn lambs.<sup>6,32,36,37</sup> The feasibility and safety of Baby-DUCC is reported in **Chapter 12** and heart rate values of the enrolled infants that receive Baby-DUCC are reported to determine haemodynamic stability.

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# 5 Declarations

This thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.



Print Name: Douglas Andrew Blank

Date: 11<sup>th</sup> of October 2018

Thesis including published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes 6 original papers published in peer reviewed journals and 1 review chapter accepted for publication in a neonatal text book. The core theme of the thesis is Umbilical Cord Management at Birth. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the Monash University, Faculty of Medicine, Nursing, and Health Sciences, Obstetrics and Gynaecology under the supervision of Professor Stuart Hooper (main supervisor) and A/Prof Graeme Polglase.

# The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of seven studies contributing to my PhD thesis, my contribution to the work involved the following:

Thesis Chapter	Publication Title	Status	Nature and % of student contribution	Co-author name(s) Nature and % of Co- author's contribution*	Co-author(s), Monash student Y/N*
6	Chapter 5: Hemodynamic Significance and Clinical Relevance of Delayed Cord Clamping and Cord Milking	Accepted	60%, concept, data collection, first draft, and revision	Katheria AC: 40%, concept, review of material, and revision	No
7	Lung Ultrasound Accurately Detects Pneumothorax in a Preterm Newborn Lamb Model	Published	60%, concept, data collection, first draft, and revision	Hooper S: 5% Binder-Heschl C: 5% Kluckow M: 5% Gill A: 5% LaRosa D: 2% Rodgers K: 2% Zahra V: 2% Inocencio I: 5% Moxham A: 2% Davis P: 2% Polglase: 5%	Yes: Inocenio
8	Lung ultrasound during the initiation of breathing in healthy term and late preterm infants immediately after birth, a prospective, observational study	Published	65%, concept, data collection, first draft, and revision	Rogerson S: 10% Kamlin O: 10% Fox L: 2% Lorenz L: 2% Kane S: 2% Polglase G: 2% Hooper S: 2% Davis P: 5%	No
9	Lung ultrasound immediately after birth to describe normal neonatal transition: an observational study	Published	65%, concept, data collection, first draft, and revision	Kamlin O: 10% Rogerson S: 10% Fox L: 2% Lorenz L: 2% Kane S: 2% Polglase G: 2% Hooper S: 2% Davis P: 5%	No
10	Respiratory changes in term infants immediately after birth	Published	63%, concept, data collection, first draft	Gaertner V: 15% Kamlin O: 5% Nyland K: 2% Eckard N: 2% Dawson J: 2% Kane S: 2% Polglase G: 2% Hooper S: 2% Davis P: 5%	No
11	Haemodynamic effects of umbilical cord milking in	Published	65%, concept, data collection, first draft, and revision	Polglase G: 10% Kluckow M: 2% Gill A: 2%	Yes: Inocencio and Stenning

	premature sheep during the			Crosslev K: 2%	
	neonatal transition			, LaRosa D: 2%	
				Rodgers K: 2%	
				Zahra V: 2%	
				Inocencio I: 2%	
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12				Badurdeen S: 10%	
				Kamlin O: 5%	
				Jacobs S: 2%	
	Baby-Directed Umbilical Cord Clamping: A Feasibility Study	Published	63%, concept, data collection, first draft	Thio M: 2%	
				Dawson J: 2%	No
				Kane S: 2%	
				Polglase G: 2%	
				Hooper S: 2%	
				Davis P: 10%	

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Date: 11<sup>th</sup> of October 2018

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor signature:

Date: 11<sup>th</sup> of October 2018

6 Hemodynamic Significance and Clinical Relevance of Delayed Cord Clamping and Cord Milking<sup>1</sup>

This chapter is presented as the accepted manuscript to be published as a chapter in the text: Seri: Hemodynamics and Cardiology: NEONATOLOGY QUESTIONS AND CONTROVERSIES, 3E. Elservier, Saunders, Philadelphia, USA. Accepted Jan 2018.

# CHAPTER 5

# Hemodynamic Significance and Clinical Relevance of Delayed Cord Clamping and Umbilical Cord Milking

Anup C. Katheria and Douglas Blank

- Both delayed cord clamping and umbilical cord milking provide a placental transfusion at birth.
- An important benefit aside from volume may be the stabilization of the transitional circulation.
- There is a good physiologic rationale for delaying umbilical cord clamping until after the infant begins to breathe.
- Umbilical cord milking may be superior at providing a placental transfusion at cesarean section and may provide a transfusion more quickly in nonbreathing infants but this needs more study.

Delayed cord clamping (DCC) and umbilical cord milking (UCM) are two techniques that provide placental transfusion to the newborn infant. Increasing fetal hemoglobin and blood volume by placental transfusion is an extremely effective method of enhancing arterial oxygen content, increasing cardiac output, and improving oxygen delivery to the tissues. Placental transfusion is the transfer of residual placental blood to the infant during the first few minutes after delivery. DCC is the practice of waiting to clamp the umbilical cord after birth for at least 30 seconds or longer. Studies have shown that DCC benefits in preterm infants including improved hemodynamics, less blood transfusions, lower rates of intraventricular hemorrhage, and necrotizing enterocolitis, as well as improved motor function at 18 to 22 months of age.<sup>1-3</sup> Term infants receiving DCC have higher hemoglobin levels at 24 hours after birth and improved iron stores at 3 to 6 months without an increase in reported maternal morbidities.<sup>4</sup> In resource-limited settings, there is observational evidence to suggest that mortality is reduced if umbilical cord clamping (UCC) occurs after the initiation of spontaneous respiration.<sup>5</sup> International recommendations advocate for a delay in UCC for 30 seconds to more than 60 seconds after birth if the infant is vigorous.6-10

It is primarily believed that, after delivery, the major recipient of placental blood is the pulmonary bed. Normally, as the infant initiates spontaneous breathing and establishes lung aeration, the pulmonary blood vessels dilate and the infant will draw blood from the placenta into the dilated pulmonary blood vessels.<sup>1,2,11,12</sup> If infants do not breathe at birth, guidelines recommend immediately clamping the umbilical cord and moving the infant to a resuscitation platform in order to provide positive pressure ventilation (PPV).<sup>13–15</sup> In the largest randomized clinical trial investigating delivery room respiratory support in infants less than 29 weeks' gestation at birth, over 60% received PPV. This outcome suggested that many infants would receive immediate cord clamping prior to inflation of the lungs.<sup>16</sup>

UCM is a procedure in which the clinician milks or pushes the blood in the umbilical cord from the placenta to the infant. There are two established techniques

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of UCM that have been described in the literature. One method is "intact umbilical cord milking." In this technique, the clinician milks 20 cm of the umbilical cord over 1 to 2 seconds, and releases the umbilical cord after each milk to allow the cord to refill with blood. This process is repeated 2 to 4 times prior to UCC. $^{17-22}$  Another UCM technique, called "cut-umbilical cord milking," is clamping the umbilical cord close to the placenta and milking the residual volume of blood in the umbilical cord after cord clamping.3,23-25 Due to insufficient evidence, international recommendations currently discourage the use of UCM outside of clinical studies.6-10

The primary advantage of UCM over DCC is the rapid blood transfer from the placenta to the infant immediately after birth without interfering with the evaluation and the resuscitation of the newborn. In several trials, authors have concluded that UCM appears to confer the same benefits as DCC.<sup>1,18-20,26,27</sup> In addition, UCM may be a more effective method to transfer blood during cesarean deliveries because the uterus is not vigorously contracting.20

In this chapter, we will review the relevant literature regarding the effects of DCC and UCM in the delivery room on hemodynamics measurements in the first hours after birth, and on blood volume measurements. Finally, we will discuss outstanding questions and future directions of DCC and UCM.

# **Transitional Physiology and Animal Studies of Delayed Cord Clamping and Umbilical Cord Milking**

Human studies have a limited ability to accurately measure physiologic changes immediately after delivery. Animal models can use invasive monitoring prior to delivery to study the effects of umbilical cord management strategies, specifically to understand the effects on cerebral and pulmonary blood flow.

In the fetal phase, the placenta performs the function of gas exchange, providing the fetus with oxygen and eliminating carbon dioxide. The lungs are filled with liquid secreted by the lungs and pulmonary blood flow is low. The umbilical circulation, via the umbilical vein, ductus venosus, and foramen ovale, provides the majority of blood flow to the left ventricle. The placental circulation is a lowresistance pathway that receives up to 50% of the fetal cardiac output. UCC dramatically affects the neonatal circulation by increasing peripheral resistance (afterload), as the low resistance placenta pathway is removed, and by the loss of umbilical venous supply to the left ventricle (preload). As the infant initiates breathing and the lungs aerate, pulmonary blood flow increases, replacing the umbilical venous flow to supply the left ventricle and providing adequate preload. In theory, increasing pulmonary blood flow and ensuring a pathway for a sustained left ventricular preload before UCC would better prepare the infant for the hemodynamic changes of UCC (Fig. 5.1).28,29

Animal models have shown benefits of initiating ventilation to increase pulmonary blood flow prior to UCC in preterm, anesthetized newborn lambs.<sup>30,31</sup> Newborn lambs at 126 days' gestational age (equivalent to ~26 weeks in humans) with UCC prior to ventilation had dangerous swings in cerebral blood flow, arterial blood pressure, heart rate, and cerebral oxygenation. Lambs that received ventilation prior to UCC had a much smoother transition to ex utero life, including attenuated changes in cerebral perfusion and blood pressure and increased levels of oxygenation. A major limitation is that the fetal lambs in these studies were under anesthesia and paralyzed without the ability to breathe spontaneously. In addition, lambs had their lung fluid drained and in some cases received a 20-second sustained inflation breath prior to being placed on a ventilator. Whereas this model provides important information regarding hemodynamics during placental transfusion, the model does not provide answers in regards to whether ventilation is beneficial or even required during DCC in premature newborns

Human studies have suggested that gravity affects the amount of placental transfusion at vaginal birth.32 Holding the neonate high above the placenta (head 40 to 60 cm above) decreases placental transfusion similar to immediate cord clamping (ICC).

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Fig. 5.1 Diagram of the fetal heart showing the percentages of combined ventricular output ejected by each ventricle and traversing the major vascular pathways. *Ao*, Aorta; *DA*, ductus arteriosus; *LA*, left atrium; *LV*, left ventricle; *MPT*, main pulmonary trunk; *PA*, pulmonary artery; *RA*, right atrium; *RV*, right ventricle. (From Rudolph AM: Distribution and regulation of blood flow in the fetal and neonatal lamb. *Circ Res* 57(6):811–821, 1985.)

A recent study found no difference in infant weights after DCC for 2 minutes with infants placed on the maternal abdomen versus at the introitus.33 However, total weight gain was half of what was previously found,<sup>34,35</sup> indicating that 2 minutes may not be enough time for a full placental transfusion for the term infant. Mercer et al. found that term infants placed on the maternal abdomen immediately after birth who were assigned to DCC for 5 minutes received a significantly larger placental transfusion than those with a 2-minute delay.36 However, the same may not be true for cesarean section (C/S). The effect of gravity on umbilical blood flow during DCC was investigated by measuring umbilical venous and arterial blood flow using ultrasonic flow probes and biotin labeled blood to measure placental transfusion volumes in preterm fetal lambs delivered by C/S.37 Anesthetized fetal lambs were placed 10 cm above and 10 cm below the ewe during DCC and received subsequent mechanical ventilation prior to UCC. The onset of mechanical ventilation resulted in a decrease in both umbilical venous and arterial flow. The decrease in umbilical arterial and venous blood flow was proportional; therefore the net flow of blood to the fetal lamb did not change. There was no observed increase in blood volume during the period of DCC, therefore no placental transfusion was detected. In addition, the position of the fetal lamb in relation to the ewe did not affect the net flow of umbilical blood to the fetal lamb. Further animal studies are needed to explore physiologic changes during spontaneous breathing after birth and vaginal delivery.

Improved physiologic stability may play an important role in the benefits of DCC observed following C/S. In the animal model described earlier, ventilation prior to UCC increased pulmonary blood flow and resulted in less fluctuations in blood pressure, cerebral blood flow, and cerebral oxygenation; however, significant placental transfusion resulting in increased blood volume was not observed (Fig. 5.2). A possible explanation for these findings is the lack of spontaneous breathing in the anesthetized animals. Spontaneous breathing is commonly observed after delivery even in the most premature human infants. It may also stimulate placental transfusion by opening pulmonary capillary beds.<sup>38</sup> In a recent trial of premature infants randomized to assisted ventilation or tactile stimulation there was no difference in resuscitation interventions, transitional hemodynamics, or neonatal outcomes.<sup>38</sup> However, over 90% of premature infants had spontaneous ventilation whether they were provided with tactile stimulation alone or

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**Fig. 5.2** Umbilical blood flow measured during ventilation prior to umbilical cord clamping in anesthetized preterm lambs. Umbilical venous and umbilical arterial blood flow were significantly reduced after initiation of ventilation (P < .001). However, net umbilical blood flow did not change during this time (P = .99), resulting in no placental transfusion. *UA*, Umbilical artery; *UV*, umbilical vein. (From Blank DA, Polglase GR, Kluckow M, et al.: Haemodynamic effects of umbilical cord milking in premature sheep during neonatal transition. *Arch Dis Child Fetal Neonatal Ed* Dec 5, 2017. doi 10.1136/archdischild-2017-314005.)

assisted ventilation (Fig. 5.3). It may be that a mechanism of benefit of DCC is allowing time for spontaneous breathing prior to the clamping of the cord and thus, by maintaining left ventricular preload, ensuring an unperturbed hemodynamic transition. Further clinical trials are needed to better determine whether assisted ventilation (with continuous positive airway pressure [CPAP] and positive pressure ventilation [PPV]) provides benefit during a placental transfusion.

# Cardiovascular Effects of Delayed Cord Clamping and Umbilical Cord Milking in the Delivery Room

Despite the difficulties in obtaining accurate physiologic data immediately after birth of human infants, there are a few studies which have investigated the cardiovascular adaptation in newborns during DCC. In a cohort of healthy term, vaginally-delivered infants, arterial and venous umbilical blood flow was measured using Doppler ultrasound starting 30 seconds after birth until the umbilical cord was clamped.<sup>39</sup> Several different patterns of umbilical cord blood flow were observed, emphasizing that the physiology of umbilical cord blood flow is complex. The initiation of breathing appeared to promote venous flow to the newborn; however, crying often caused a reversal of flow. Arterial blood flow was observed to continue after umbilical cord pulsations ceased and umbilical arteria and venous blood flow often stopped at different times. The potential volume of the placental transfusion is increased if there is differential constriction of the umbilical arteries prior to the umbilical vein.<sup>40</sup> In another cohort of healthy term infants, continuously monitored cardiac output was measured via electrical impedance during DCC starting 90 seconds after birth. Every minute of postnatal life that the cord



Fig. 5.3 Number of infants breathing at different time points with stimulation during delayed cord clamping. (Modified from Katheria AC, Lakshminrusimha S, Rabe H, McAdams R, Mercer JS. Placental transfusion: a review. *J Perinatol* 178:75–80, 2016, drawing by S. Lakshminrusimha.)

Table 5.1 HEART	RATE COMPARIS	Sons in stu	DIES COMPARII	NG IMMEDIATE C	ORD CLAMPING
AND	DELAYED CORD	CLAMPING.	THE EXPECTED	HEART RATE RA	NGE FOR INFANTS
WHO	D BREATHE SPON	TANEOUSLY	AND RECEIVED	IMMEDIATE COP	RD CLAMPING IS
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Study	N	Time of Cord Clamping (s)	GA or BW	HR@1 min, BPM	SpO₂@ 1 min,%
Dawson, 201074,75	468	<20	38 weeks (25–42)	96 (65–127)	66 (55–75)
Linde, 2016 <sup>76a</sup>	55	44 (29-59)	$3100 \pm 428 \text{ g}$	$149 \pm 33$	NA
Katheria, 201638	150	$64 \pm 9$	$28 \pm 2$ weeks	$115 \pm 32$	NA
Katheria, 201541	20	$318 \pm 32$	$39 \pm 1$ weeks	$176 \pm 15$	NA
Smit, 201477	109	300 (180-420)	40 weeks	61 (42–146)	78 (67-87)
			(37 - 42)		

<sup>a</sup>Gestational age was not reported in this study; heart rate reported at 40 seconds. *BW*, Birth weight; *BPM*, beats per minute; *GA*, gestational age; *HR*, heart rate; *SpO*<sub>2</sub>, saturation of oxygen.

was kept unclamped, the stroke volume increased and cardiac output increased 13% compared to baseline. The increase in stroke volume and cardiac output was observed even after umbilical cord pulsation ceased.<sup>41</sup> Again, these findings underscore the importance of allowing for a smooth change from placental to pulmonary blood flow supplying the left ventricular preload during the immediate transitional period.

In animal studies, DCC has been shown to improve oxygen saturations  $(SpO_2)$  and attenuate the swings in blood pressure and cerebral blood flow seen in ICC. After birth, if the infant is still connected to a functioning placenta that continues to provide gas exchange and left ventricular preload, one might expect infants with DCC to have higher heart rates and higher SpO<sub>2</sub>. A few small studies have reported the heart rate and SpO<sub>2</sub> immediately after birth, in preterm and term infants who receive DCC. Compared with normative data, the studies have conflicting results of the effects of DCC (Table 5.1). Winter et al. reported all infants had a heart rate greater than 100 BPM at 60 seconds in a small pilot trial in which respiratory support could be provided during DCC in infants less than 32 weeks.<sup>42</sup> Finally, there is very little data on the effects of UCM on SpO<sub>2</sub> immediately after birth.

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#### Table 5.2 COMPARISON OF ECHOCARDIOGRAPHIC FINDINGS IN STUDIES COMPARING IMMEDIATE CORD CLAMPING AND DELAYED CORD CLAMPING OR INTACT UMBILICAL CORD MILKING

Study	N	GA Weeks	Intervention	SVCICC mL/kg/min	SVC Intervention mL/kg/min	Hours After Birth
Meyer, 2012 <sup>78</sup>	30	26 (25-28)	DCC: 30-40s	52 (42-100)	91 (81–101) <sup>a</sup>	16
Sommers, 2012 <sup>49</sup>	51	$28\pm1$	DCC: 41 ± 9s	$89\pm24$	$112\pm30^{a}$	6
Katheria, 2014 <sup>19</sup>	60	$28\pm3$	Intact UCM	$69\pm22$	$94\pm27^{a}$	6
Popat, 201650	266	$28 \pm 2$	DCC: 60s (0-70)	$92 \pm 35$	$95 \pm 41$	3-6

 $^{a}P < .05$  compared to ICC.

DCC, Delayed cord clamping; GA, gestational age at birth; ICC, immediate cord clamping; SVC, superior vena cava flow; UCM, umbilical cord milking.

A small, randomized, controlled trial showed that heart rate and SpO<sub>2</sub> were higher immediately after birth, requiring lower amounts of inspired oxygen (FiO<sub>2</sub>) and mean airway pressure, in preterm infants receiving intact UCM versus infants receiving ICC.<sup>18</sup> This suggests that cord milking may enhance early pulmonary blood flow and decrease pulmonary pressures. The decreased need for oxygen and mean airway pressure coincides with the finding that these infants also had a lower incidence of oxygen requirement at 36 weeks' postmenstrual age. There are no published results of the physiologic effects of cut-UCM in the delivery room.

# Hemodynamic Measurements in the First Hours After Birth Following Delayed Cord Clamping and Umbilical Cord Milking

Studies have demonstrated that low systemic blood flow during the first 24 hours after birth increases the risk of peri/intraventricular hemorrhage (P/IVH), neurode-velopmental impairment, and death in extremely preterm infants.<sup>43–45</sup> Assessment of systemic blood flow, such as superior vena cava (SVC) flow, potentially provide a more accurate assessment of cardiovascular adequacy than blood pressure alone.<sup>44,46</sup> In trials, cardiovascular support with vasopressor-inotropes or inotropes and fluid boluses may increase low systemic blood flow but these interventions have not prevented adverse consequences such as the development of P/IVH nor have they improved neurodevelopmental outcomes (see Chapters 1, 3, 6 and 7).<sup>47,48</sup> As suggested earlier, DCC and UCM may aid in improving immediate hemodynamic transition and thus circulatory stability and also increase circulating blood volume after birth. Consequently, there is interest in studying umbilical cord management strategies and their role in prevention of low systemic blood flow states and subsequently decreasing the risk of P/IVH.

Recent studies measuring systemic blood flow by echocardiography that compare DCC to ICC have conflicting results (Table 5.2). In a small study by Sommers et al., DCC improved SVC flow compared to ICC.<sup>49</sup> However, in a much larger multicenter randomized controlled trial, DCC failed to show any improvement in SVC flow and infants randomized to DCC demonstrated lower right ventricular output.<sup>50</sup> Unfortunately, 22% of the infants allocated to the DCC arm had cord clamping prior to the targeted clamping at 1 minute mainly due to the obstetricians being uncomfortable with waiting when infants were less vigorous. However, it is in these very infants where DCC might be most advantageous. Several trials have shown that DCC in preterm infants leads to higher blood pressure in the first hours after birth and less treatment for low blood pressure, compared with infants receiving ICC.<sup>51–54</sup>

Cord milking may also be associated with improvements in hemodynamics particularly after C/S. UCM has been shown to improve systemic blood flow, cerebral oxygenation, and myocardial function compared to ICC.<sup>19,26</sup> Previous studies have suggested that placental transfusion may be impaired after C/S compared to vaginal Hemodynamic Significance and Clinical Relevance of Delayed Cord Clamping

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birth.<sup>4,55,56</sup> In a comparison of intact UCM versus DCC for at least 45 seconds in 154 infants less than 32 weeks gestational age at birth delivered by cesarean section, SVC flow, right ventricular output, and blood pressure were all significantly higher in the infants receiving intact UCM than DCC in the first 12 hours after birth.<sup>20</sup> However, neither group had an excess of abnormal systemic flow and there were no differences in clinical outcomes. The hemodynamic changes of cut-UCM have not been studied. Finally, one study compared DCC for 60 seconds versus DCC for 60 seconds with respiratory support (mask ventilation/CPAP) if indicated in infants less than 32 weeks' gestation.<sup>38</sup> There were no differences in systemic blood flow measurements or cerebral oxygenation between the groups during the first 12 hours after birth.

# Neonatal Blood Volume After Delayed Cord Clamping and Umbilical Cord Milking

In a randomized trial, Aladangady and colleagues used a biotin-labeled autologous red blood cell dilution method to compare red blood cell volume between very preterm infants receiving 30 to 90 seconds of DCC versus ICC.56 At 4 hours after birth, the DCC infants had a significantly higher red blood cell volume than ICC (74 mL/ kg vs. 63 mL/kg, P < .001). The difference was more pronounced in infants delivered vaginally. The authors found no difference in hematocrit levels at 1 hour after delivery despite measuring an increase in red blood cell volume. As the increased volume of blood providing the placental transfusion would be a proportionate transfer of red blood cells and plasma, no increase in hematocrit would be immediately detectable. A study by Strauss and colleagues also showed that despite an increase in red blood cell volume, initial hematocrit was not higher in preterm infants receiving DCC.55 However, the infants receiving DCC had higher hematocrit levels at 7 days after birth. The higher hematocrit persisted during the first month after birth, and the authors speculated that this finding is potentially due to the increased blood volume coupled with improved hemodynamics leading to enhanced excretion of free water and less dilution from intravenous fluids in the DCC group.55 However, a metaanalysis has found that preterm infants with DCC have higher hematocrit levels at 4 and 12 hours after birth, and a decreased need for red blood cell transfusions for either hypotension or anemia.

The total placento-fetal blood volume is reported to be 115 mL/kg at term of which 70 mL/kg is in the fetus and 45 mL/kg in the placenta.<sup>57</sup> Accordingly, the timing of cord clamping has the potential to influence neonatal blood volume in the term infant. If the umbilical cord is clamped immediately after delivery, the hematocrit in the newborn averages 48% to 51%.<sup>57</sup> However, waiting to clamp the cord 3 to 4 minutes after delivery increases the blood volume by 30%.<sup>32</sup> The hematocrit increases over the subsequent 30 minutes to 2 hours to average 59% to 62% at 6 hours of age and then decreases to 52% to 59% at 24 hours of age.<sup>58</sup>

A potential advantage of UCM over DCC for providing increased placental transfusion is that UCM is not dependent on uterine tone and thus UCM may be a more effective technique after cesarean delivery. The placental blood flow at 24 to 29 weeks' gestation is about 8 mL/s in the umbilical vein which increases to 10 mL/s at term.<sup>59</sup> Milking the preterm umbilical cord provides a blood volume of about 18 mL/kg over 2 seconds.<sup>60</sup> In a 1-kg infant this would provide a similar physiologic infusion of 9 mL/s. A recent study comparing UCM to DCC in premature infants born by C/S demonstrated an improved initial hematocrit, systemic blood flow, blood pressure, and urine output.<sup>61</sup> Milking the cord has also been shown to increase blood volume in term infants born via C/S. In one trial, performing UCM 5 times resulted in a mean hematocrit of 57% compared with 51% after ICC at 36 to 48 hours of age.<sup>62</sup>

# Long-Term Outcomes and Mortality After Delayed Cord Clamping and Umbilical Cord Milking

There are a few studies reporting neurodevelopmental outcomes of DCC and UCM in preterm infants. Mercer and colleagues have published two studies finding that

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DCC up to 45 seconds versus ICC improves motor function at 18 months as assessed by the Bayler Scales of Infant and Toddler Development in preterm infants less than 32 weeks' gestation.<sup>2,63</sup> Interestingly, improvement in motor function after DCC was more profound in male infants. In the only study reporting long-term outcomes comparing intact-UCM versus a 30-second delay in cord clamping, there was no difference in infants born at less than 33 weeks' gestation on Bayley III scores at 2 and 3.5 years.<sup>22</sup>

Andersson and colleagues conducted a randomized trial of over 380 healthy, term infants born in Sweden comparing DCC for  $\geq$ 180 seconds to ICC ( $\leq$ 10 seconds).<sup>64</sup> Although neonatal hemoglobin and immunoglobulin G (IgG) levels and 4-month iron stores were higher in the DCC group, no differences in the iron status and neurodevelopment, assessed by the Ages and Stages Questionnaire (ASQ), were seen at 12 months. Male infants who received DCC had higher ASQ scores than male infants who received ICC, but female infants who received DCC had lower ASQ scores than female infants who received ICC. These intriguing gender differences have not been further evaluated. Finally, there are no published studies reporting long-term outcomes comparing UCM to ICC or after cut-UCM.

Two large, prospective, observational studies in resource-limited settings conducted by Ersdal and colleagues have investigated the relationship between DCC and death. In a cohort of over 12,000 spontaneously breathing term and late preterm infants, delaying UCC until after the initiation of spontaneous respirations reduced the risk of death and the combined risk of death and hospital admission at 24 hours after birth.<sup>5</sup> This happened despite the fact that the number of infants who died in this cohort was small (0.2% of the infants enrolled). A second observational study described the timing of UCC in over 1200 infants who received PPV for apnea after birth.65 Initiating PPV prior to UCC did not significantly reduce the combined risk of death and admission at 24 hours after birth (18% if cord clamping occurred before initiating PPV vs. 14% if cord clamping occurred after initiating PPV, P = .328). The delay in the onset of PPV was significantly associated with the combined risk of death and admission at 24 hours. Of note, the timing of cord clamping was  $39 \pm 35$  seconds in infants who received PPV. No significant difference in mortality has been observed between very preterm infants receiving DCC, UCM, or ICC.4,54 However, as the incidence of death in very preterm infants was less than 5%, a larger cohort of infants is needed to appropriately address this auestion.

# **Conclusions and Future Directions**

ICC was introduced with no prior evidence as an attempt to reduce the risk of postpartum hemorrhage. Both DCC and cord milking had been practiced and described centuries before the introduction of ICC. Aristotle described the cord milking procedure<sup>66</sup> in 350 BC: "It often happens that the child appears to have been born dead when it is merely weak, and when before the umbilical cord has been ligatured, the blood has run out into the cord and its surroundings. But experienced midwives have been shown to squeeze blood into the child's body from the cord, and immediately the child that a moment before was bloodless came back to life again." Improved shortterm outcomes have already been demonstrated in several studies with UCM but long-term safety studies are lacking.<sup>67</sup> For DCC, there have never been any adverse maternal outcomes seen between the early and late cord clamping groups including severe postpartum hemorrhage, postpartum hemorrhage of 500 mL or more, mean blood loss, or maternal hemoglobin levels.<sup>4</sup> There has never been a trial published that demonstrates any benefit of ICC, and the majority of trials when compared to DCC and UCM suggest that it may actually be harmful.

Although a number of human studies suggest that the predominant benefit of DCC and UCM is the increase in blood volume, animal studies and one epidemiological study suggest that achieving lung aeration and pulmonary blood flow prior to cord clamping may be important.<sup>13–15,68–73</sup> Polglase and colleagues suggested

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the term "physiology-based cord clamping," in which a physiologic target indicating readiness for UCC is achieved prior to clamping the umbilical cord.<sup>31</sup> Delaying UCC until after the lungs are aerated, gas exchange is established, and pulmonary blood flow is increased could maintain physiologic stability by improving immediate hemodynamic transition and optimizing the potential for placental transfusion after birth. While promising, physiology-based cord clamping makes an appropriate target for the majority of clinicians challenging. A delay for perhaps 60 seconds in infants that demonstrate adequate breathing may be an appropriate starting point. Further studies are needed to determine whether cord milking is superior to DCC in neonates born via C/S, and whether it can be considered an alternative approach when DCC cannot be performed.

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Lung Ultrasound Accurately Detects Pneumothorax in a Preterm Newborn Lamb Model<sup>2</sup> 7

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# ORIGINAL ARTICLE

# Lung ultrasound accurately detects pneumothorax in a preterm newborn lamb model

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Aim: Pneumothorax is a common emergency affecting extremely preterm. In adult studies, lung ultrasound has performed better than chest x-ray in the diagnosis of pneumothorax. The purpose of this study was to determine the efficacy of lung ultrasound (LUS) examination to detect pneumothorax using a preterm animal model.

Methods: This was a prospective, observational study using newborn Border–Leicester lambs at gestational age = 126 days (equivalent to gestational age = 26 weeks in humans) receiving mechanical ventilation from birth to 2 h of life. At the conclusion of the experiment, LUS was performed, the lambs were then euthanised and a post-mortem exam was immediately performed. We used previously published ultra- sound techniques to identify pneumothorax. Test characteristics of LUS to detect pneumothorax were calculated, using the post-mortem exam as the 'gold standard' test.

Results: Nine lambs (18 lungs) were examined. Four lambs had a unilateral pneumothorax, all of which were identified by LUS with no false positives.

Conclusions: This was the first study to use post-mortem findings to test the efficacy of LUS to detect pneumothorax in a newborn animal model. Lung ultrasound accurately detected pneumothorax, verified by post-mortem exam, in premature, newborn lambs.

Key words: bubble test; lung aeration; lung injury; post-mortem exam; respiratory distress syndrome.

What is already known on this topic

- 1 Pneumothorax is a frequently encountered newborn emergency, chest x-ray is the currently preferred mode of diagnosis.
- 2 Adult studies have demonstrated lung ultrasound performs better than chest x-ray to diagnose pneumothorax in emergency room settings.
- 3 The efficacy of lung ultrasound to detect pneumothorax in newborn animals had not been validated.

What this paper adds

- 1 Lung ultrasound can accurately detect pneumothorax, verified by post-mortem examination, in premature ventilated newborn lambs.
- 2 We have described a novel gold standard technique for evaluating air leak syndromes on post-mortem examination in an animal model.
- 3 We did not have any false positive diagnoses of pneumothorax using lung ultrasound.

incidence of pneumothorax at entry into the trial was 19%.4

The risk of pneumothorax is highest during the first 48 h of life. In large trials of extremely low gestational age newborns, the incidence of pneumothorax was 7-9% with 79% of the air leaks occurring in the first 48 h of life.<sup>1-3</sup> In a study of term babies with severe respiratory distress shortly after birth, the

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Pneumothorax places babies at increased risk for death and significant morbidities, and prompt recognition of pneumothorax is desirable.<sup>5</sup> In the delivery room, the Neonatal Resuscitation Program

recommends consideration of pneumothorax if extensive resuscitation is required for a baby with bradycardia, but does not recommend clinical signs or tests to aide in the diagnosis of pneumothorax in the delivery room.<sup>6,7</sup> Lung ultrasound (LUS) potentially offers a superior alternative to x-ray for the diagnosis of pneumothorax because it can be performed and interpreted by the treating clinician at the bedside, can be easily repeated and does not expose the baby to radiation.

Ultrasound beams penetrating a fluid filled lung create ultrasound images as the density of fluid changes between the

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pleural line and the lung parenchyma. The attenuation of sound waves caused by air in the lungs has discouraged the use of LUS. However, ultrasound beams passing through an aerated lung will produce characteristic artifacts that are now recognised as having diagnostic importance.<sup>8–10</sup>

Standard LUS signs have been established in adult studies using CT scans for validation and all signs arise from the pleural line.  $^{\overline{8},9,11-14}$  In a well-aerated lung, the pleural line is bright because of the difference in acoustic impedance between the visceral pleura and the aerated lung parenchyma.9 This reflector is at 90° to the angle of insonation and may give rise to a reverberation artifact, A-lines, whereby the pleural line is regularly repeated through the depth of the ultrasound window. In a pneumothorax the discrepant acoustic boundary occurs between the parietal pleura and the pleural air, producing strong reverberation artifact represented as A-lines. Healthy lung is seen as the 'seashore sign' on M-mode or characteristic movement of the pleural line with respiration in 2-D clips called lung sliding. If there is a pneumothorax, only A-lines are seen and lung sliding is obliterated. The 'seashore sign' is replaced with a 'stratosphere sign' (Fig. 1). The primary aim of this study was to determine the accuracy of LUS in detecting a pneumothorax in a ventilated preterm, newborn lamb model.

# Methods

This was a prospective observational study of premature, newborn lambs with a gestational age of 126 days (equivalent to 26-week gestation in humans) conducted at the Hudson Institute at Monash University. The experimental protocol was performed in accordance with guidelines established by the National Health and Medical Research Council of Australia and was approved by the Monash Medical Centre animal ethics committee.

# Surgical preparation

At 126  $\pm$  2 days gestation Border–Leicester ewes were anaesthetised with an intravenous bolus of 5% sodium thiopentone (Pentothal; 1 g in 20 mL) and, following intubation, maintained with inhalation of 1.5–3% halothane in air. The fetal head and neck were exposed via hysterotomy for surgical insertion of carotid artery flow catheters, jugular vein catheters and oral intubation with a 4.0 cuffed endotracheal tube (Coviden, Dublin, Ireland). Immediately after instrumentation, lambs were delivered surgically, quickly weighed then moved to a warming bed, dried and ventilation initiated. The lambs were enrolled in a primary study of ventilator associated lung injury.

All lambs received a 30-s sustained inflation of 35 cmH<sub>2</sub>O (Neopuff; Fisher & Paykel, Auckland, New Zealand) followed by an injurious ventilation strategy consisting of 15 min of ventilation with pressures of  $40/0\ cmH_2O$  at a rate of 60 breaths per minute, followed by 105 min of volume guarantee ventilation of 7 mL/kg/breath with a positive end expiratory pressure of 5 cmH<sub>2</sub>O (Babylog 8000, Dräger, Lübeck, Germany). Ventilator rate and peak inspiratory pressure were titrated to achieve a PaCO2 of 35-60. Lambs were ventilated with warmed, humidified gas starting at a  $FiO_2$  of 0.21, which was adjusted to targeted pre-ductal transcutaneous oxyhemoglobin saturation of 85-95% from 5 min of age (Radical 7, Massimo, California, USA). The lambs did not receive prenatal steroids prior to delivery, and surfactant was not administered. Alfaxalone in 4% glucose was administered by continuous infusion for anaesthesia, anal- gesia and suppression of spontaneous breathing.

## LUS examination

LUS exams were conducted prior to 60 min, at 90 min and at 2 h of life. We used previously published ultrasound techniques to



Fig. 1 Negative pneumothorax on LUS and autopsy (a, b) versus positive pneumothorax on LUS and autopsy (c, d). (a) LUS M-mode and 2-D images showing the 'seashore sign' of 'waves' on top hitting the 'beach' underneath which represents normal lung sliding. The image also shows consolidated B-lines creating a white- out appearance of a lung with RDS (type 3). (b) Corresponding negative post-mortem gross examination of the lungs, the right and left diaphragm are clearly seen in contact with the lungs. (c) The 'seashore' is obliterated by horizontal lines creating the 'stratosphere sign' on M-mode, difficult to see the pleural line and a lack of lung movement. The corresponding 2-D image shows A-lines with the absence of B-lines. A-lines with a lack of B-lines and no lung sliding indicates a pneumothorax. (d) Corresponding positive post-mortem gross examination, looking superiorly from the umbilicus of the lamb towards the inferior portion of the diaphragm/ lungs, a tension pneumothorax seen on the right side of the chest with no evidence of tension pneumothorax of the left chest.

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identify pneumothorax (stratosphere sign on M-mode, lack of lung sliding and the presence of A-lines with a lack of B-lines on 2-D images).<sup>8,9,11-14</sup> The Philips Compact Extreme Ultrasound Machine (CX-50, Koninklijke Philips, Netherlands) was used to collect images, using L12-3, S12-4 and C5-1 probes, with the L12-3 preferred when available. In order to minimise interference with the primary experiment, we did not compare images from different probes on the same animal. The initial settings for the examinations were a depth of 4 cm for all probes, a gain of 100 for the L12-3, 75 for the C5-1 and 60 for the S12-4. Exams were performed with the lambs in the supine position. The ultrasonographers (DB, CB) examined the anterior lung fields with the probe positioned in a longitudinal orientation with the notch towards the lamb's head. The ultrasound exam consisted of capturing 2-D clips and M-mode images of the right upper and lower and left upper and lower anterior lung fields. The ultrasonographer performing the exam recorded the presence and location of the pneumothorax prior to autopsy.

# Post-mortem examination

Immediately after obtaining the images at 2 h the lambs were euthanised (sodium pentobarbitone i.v. 100 mg/kg) and a postmortem examination performed. The abdomen was carefully opened and the diaphragm inspected for tension pneumothorax, demonstrated by air causing downward displacement of the diaphragm.<sup>15,16</sup> The chest was then opened, the lungs and trachea dissected and inspected for blebs (air pockets trapped beneath the visceral pleura), pulmonary interstitial emphysema

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(PIE) and other evidence of air leak. A cut 4.0 endotracheal tube (ETT) was inserted into the intact trachea, the cuff gently inflated and secured with suture to prevent air leak. A syringe was placed into the proximal end of ETT such that the entire system was air tight, the lungs were submerged in a clear container of water and air was slowly injected in a stepwise fashion until a maximum volume of 10 mL/kg was obtained. A pneumothorax was positively identified if we observed downward displacement of the diaphragm after opening the abdomen (tension pneumothorax, Fig. 1) or if air bubbles were observed escaping during the bubble test (on-going leak, Fig. 2). In addition, we considered the autopsy positive for air leak syndrome if we noted pneumothorax, blebs or PIE on gross inspection of the lung performed both before and after the bubble test (Fig. 3).

# Statistical analysis

Vital signs, respiratory parameters and blood gas values in lambs before and after pneumothorax and in lambs without pneumothorax were compared using a one-way ANOVA. Means with SD are reported for normally distributed continuous variables and medians with 25–75% interquartile ranges are reported when the distribution was not normal. The sensitivity, specificity, positive predictive value and negative predictive value of LUS to detect pneumothorax were tested using the post-mortem exam results as the 'gold stand' test. Each lung was considered individually. To test inter-rater reliability, two expert ultrasonographers, who were not present during data collection, (MK, AG) independently evaluated images obtained from each



Fig. 2 Example of a positive (a, b, c) and negative (d, e) bubble test for ongoing air leak. The lungs and trachea were carefully dissected out of the chest. We replaced and secured a cuffed 4.0 endotracheal tube (ETT) into the intact trachea to create an air tight system. We then submerged the lungs in water and slowly injected into the ETT in a stepwise fashion until a maximum volume of 10 mL/kg was reached. (a, b, c) Positive bubble test depicting bubbles escaping from the right middle lobe with 3 mL/kg of air injected (a, b), the lungs rise slightly as up to 10 mL/kg is reached. (d, e) negative bubble test. The unaerated lung sinks to the bottom of the container, with injection of 10 mL/kg of air, the lung rises to the top of the container. No bubbles are seen escaping indicating a negative test for ongoing air leak.

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Fig. 3 Post-mortem gross examination examples from the two subjects with false negative LUS exams for air leak syndrome (a, b: Subject 5 and c, d: Subject 8). (a) Posterior view of whole lungs with negative findings prior to injection of air. (b) Close up of the posterior LUL showing PIE after injection of 5 ml/kg, the bubble test was negative for ongoing air leak. (c) Anterior view of the lungs. Prior to injection of air, a bleb in the upper margin of the posterior aspect of the LLL was seen. After injection of 10 mL/kg of air, the LLL bleb is still difficult to see looking at the anterior view of the lungs. (d) Posterior view of the lungs with a large bleb early noted. LLL, left lower lobe; LUL, left upper lobe; RLL, right lower lobe; RML, right middle lobe; RUL, right upper lobe.

hemi-thorax of all subjects and graded the images 'yes' or 'no' for the presence of pneumothorax blinded to the post-mortem result. Statistical significance was accepted as P < 0.05. STATA software was used for statistical analysis (StataCorp, Texas, USA).

# Results

Nine lambs were included in the study. The median weight was 2.5 kg (IQR 2.4-2.7), and four of the lambs were males. There were no demographic differences of the lambs between those who developed pneumothorax and those that did not. LUS correctly identified all four cases of pneumothorax, with no false positives. The sensitivity, specificity, negative predictive value and positive predictive value for pneumothorax were 100%

(Table 1). All cases of pneumothorax involved the upper and lower lung fields and in all cases both 2-D clips (showing A-lines with a lack of lung sliding) and M-mode (showing a 'stratosphere' sign) were positive. The contralateral lung was negative on LUS and autopsy in all cases. The primary experiment allowed for one attempt at drainage of the pneumothorax via needle aspiration. Therefore, needle aspiration was attempted in three of the four identified pneumothoraxes with transient improvement in HR, SpO<sub>2</sub> and MAP. The fourth lamb had the pneumothorax identified at the time of scheduled euthanasia, so needle decompression was not attempted.

On post-mortem examination, three cases of pneumothorax were confirmed as tension pneumothorax with downward displacement of the diaphragm seen when opening the abdomen. In the final case of tension pneumothorax, needle

Table 1 Test characteristics of lung ultrasound to detect tension pneumothorax using the post-mortem examination as the 'gold standard'

	Autopsy (tension PTX)		
LUStest result	Positive	Negativ	
Positive	4 = TP (4 tension PTX)	e 0=FP	PPV (TP/(TP + FP))=100%
Negative	0 = FN	14 = TN	NPV (TN/FN + TN) = 100%
	Sens (TP/(TP + FN)) = 100%	Spec (TN/(FP + TN)) =100%	

LLS was interpreted prior to the autopsy was considered positive if there were A-lines with no lung sliding or B-lines on 2-D cine or if there was the 'stratosphere' sign on M-mode. Post-mortem exam was considered positive for tension pneumothorax if there was air displacing the diaphragm inferiorly on opening of the abdomen (tension pneumothorax), or if there was ongoing air leak seen while injecting the dissected lungs with up to 10 mL/kg of air with the lungs submerged underwater (bubble test). FN, false negative; FP, false positive; PTX, pneumothorax; TN, true negative; TP, true positive.

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aspiration resulted in 80 mL of air was drained with immediate improvement in bradycardia, hypoxia and hypotension until the experiment concluded 8 min later. Autopsy showed a positive bubble test, confirming ongoing air leak.

In addition to the four cases of correctly identified pneumothorax, there were two cases of non-pneumothorax air leak syndrome that were not detected on LUS. One lamb had PIE involving the posterior aspect of the left upper lobe (Fig. 3, A and B). A second had a bleb involving the superior posterior aspect of the left lower lobe (Fig. 3, C and D). In both cases the bubble test for ongoing air leak was negative. There were no false positive LUS exams. No subject had bilateral air leak syn- dromes on autopsy. Two expert ultrasonographers (MK, AG) independently identified all four pneumothoraxes without any false positives after evaluation of 34 images each.

# Comparison of physiologic parameters

Comparisons of physiologic parameters between lambs prior to pneumothorax, lambs after pneumothorax was recognised on LUS and lambs that never developed pneumothorax are presented in Table 2. There were no significant differences in any variable between the lambs that never developed pneumothorax to the lambs prior to pneumothorax. A pneumothorax resulted in a significantly lower heart rate, saturation of peripheral oxygen and mean arterial blood pressure and a higher alveolar-arterial concentration of oxygen gradient compared to values prior to the pneumothorax.

# Discussion

The validity of ultrasound to detect pneumothorax in preterm neonates is unknown. We have shown that LUS can accurately detect a ventilator associated pneumothorax in an animal model

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that represents extremely low gestational age newborns. This is the first study that has used an immediate post-mortem examination to verify an air leak syndrome in newborns. We performed the post-mortem immediately following the conclusion of the experiment, completing the examination of the lungs within 30 min of euthanasia. By viewing the diaphragm from an inferior approach we could reliably detect a tension pneumothorax. We believe our bubble test technique functions as an adequate confirmatory test to exclude a pneumothorax which is not under tension.

Two lungs demonstrated non-pneumothorax air leak syn-

dromes (PIE and bleb). These were not misclassified as pneumothorax by LUS exams. In both cases the bubble test for an ongoing air leak was negative. Future studies are needed to differentiate between air leak syndromes that warrant treatment and those that warrant observation.

In newborns, LUS has been reported to predict which babies will need admission to the NICU, surfactant, reduce the number of x-rays taken, and can characterise the appearance of transient tachypnea of the newborn, meconium aspiration syndrome and the appearance of the lungs after surfactant administration.<sup>10,17–22</sup> LUS has been used by emergency and intensive care services to diagnose, characterise and treat pneu-mothorax in adult populations.<sup>12–14,23–25</sup> Using CT scans as the gold standard to diagnose pneumothorax, recent meta-analyses have confirmed that LUS was superior to x-ray.<sup>23,24</sup> The accuracy of LUS to diagnose and characterise pneumothorax compared favourably to CT scan in an adult porcine model.<sup>11,26</sup>

# Limitations

There were several limitations of this study. The incidence of air leak syndromes in our study was higher than would be expected in the NICU because our subjects received an injurious

Table 2 Comparison of vital signs, respiratory parameters and blood gas analysis between lambs prior to pneumothorax, lambs after pneumothorax was recognised and lambs that never developed pneumothorax

	Pre PTY n = 4	Post PTX n =4	No PTX n=5	
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Mean airway pressure (cmH <sub>2</sub> O)	- 18 ± 2.7	17.8 ± 2.9	17.6 ± 3	0.98
Tidal volume (mL/kg)	6.8 ± 1.3	6.8 ± 1.3	$5.4 \pm 1.8$	0.33
FiO <sub>2</sub>	$0.55 \pm 0.13$	$0.9 \pm 0.2$	$0.72 \pm 0.23$	0.08
Heart rate (bpm)	170 ± 42	84 ± 64*	151 ± 22	0.047
SpO <sub>2</sub> (%)	93 ± 5	63 ± 20**	93 ± 2	0.01
NIRS (%)	67 ± 5	57 ± 12	73±7	0.06
Mean arterial pressure (mmHg)	49 ± 7	25 ± 9**	49±6	0.00
pН	7.21 ±0.1	7.15 ±0.16	7.11 ±0.17	0.64
PaO <sub>2</sub> (mmHg)	38 ± 11	36 ± 19	40 ± 7	0.92
PaCO <sub>2</sub> (mmHg)	50 ± 16	62 ± 11	$88 \pm 40$	0.17
A–a gradient (mmHg)	293 ± 88	600 ± 21**	351 ± 137	0.01
Oxygen index	27 ± 8	52 ± 22	31 ± 14	0.11
Cerebral blood flow (mL/min)	$45 \pm 30$	27 ± 19	51 ± 23	0.36
EtCO <sub>2</sub> (mmHg)	$37 \pm 30$	19±4	26 ± 7	0.4

\*Significant difference between Pre-PTX and Post-PTX. \*\*Significant difference between both Pre-PTX and Post-PTX and Pre-PTX and No-PTX. Analysis conducted using a one-way ANOVA and Tukey post-hoc test. A-a gradient, alveolar – arterial concentration of oxygen gradient; EtCO<sub>2</sub>, end tidal carbon dioxide; FiO<sub>2</sub>, fraction of inspired oxygen; NIRS, near infrared spectroscopy; PaCO<sub>2</sub>, partial pressure of carbon dioxide dissolved in arterial blood; PaO<sub>2</sub>, partial pressure of oxygen dissolved in arterial blood; SpO<sub>2</sub>, saturation of inspired oxygen.

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ventilation strategy. We used an immediate post-mortem exam as a gold standard for diagnosing pneumothorax. We believe that an immediate post-mortem exam is valid and easy to inter- pret. However, we are the first to report diagnosis of pneumo- thorax employing this technique. Our subjects had severe respiratory distress syndrome (RDS). It may be easier to distin- guish between a lung with severe RDS and pneumothorax than a lung with mild RDS and a pneumothorax. We did not compare transillumination or chest x-ray to LUS. Trans-illumination has been shown to be an accurate method to diagnose pneumotho- rax in neonates using chest x-ray as the gold standard.<sup>27-29</sup> Ul- trasound may be superior to trans-illumination in newborns with more subcutaneous fat and muscle or edema, but we did not make that comparison. Addressing the limitations of this study will serve as the basis for improvement of future studies to test the accuracy of LUS in diagnosis of air leak syndromes.

# Conclusion

LUS can accurately detect pneumothorax, verified by postmortem examination, in premature ventilated newborn lambs. We have described a novel gold standard technique for evaluating air leak syndromes on post-mortem examination in an animal model. Future studies in both humans and in animal models are warranted to further explore the potential utility of LUS in the detection and treatment of pneumothorax.

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Journal of Paediatrics and Child Health (2016) © 2016 Paediatrics and Child Health Division (The Royal Australasian College of Physicians) 8 Lung ultrasound during the initiation of breathing in healthy term and late preterm infants immediately after birth, a prospective, observational study<sup>3</sup>

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Clinical paper

Lung ultrasound during the initiation of breathing in healthy term and late preterm infants immediately after birth, a prospective, observational study<sup>u</sup>



**EUROPEAN** 

RESUSCITATION COUNCIL

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Introduction: Lung ultrasound (LUS) has shown promise for evaluation of newborns with respiratory distress. However, no study has described the appearance of LUS during the initiation of breathing. We used LUS to describe the appearance of the lungs in healthy infants immediately after birth, starting with the infant's first breath, through the first 20 min after birth.

Methods: This was a single-center observational study enrolling neonates born at  $\ge$  35 weeks. We obtained LUS video recordings with the initiation of breathing. Recordings that captured one of the 1st four breaths after birth were included. We also obtained recordings at 1-10 and 11-20 min after birth. Recordings were graded using a modified version of a previously published system, with additional grades to describe the appearance of the lungs prior to establishment of the pleural line.

*Results:* We studied 63 infants, mean gestational age =  $39^{1/7} \pm 2$  days, mean weight = 3473 g  $\pm 422$ , 33 infants were delivered vaginally and 30 via cesarean section. We captured the first breath after birth in 28 infants and within the first four breaths from the remaining 35 infants. The pleural line was established by a median of 4 breaths (3-6). At the 1-10 min examination, all infants had an established pleural line and 89% demonstrated substantial liquid clearance. At the 11–20 min examination, all infants had substantial liquid clearance.

Conclusion: Establishment of the pleural line, indicating lung aeration and substantial liquid clearance is achieved with the first few breaths after birth in term and near term infants.

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# Introduction

Lung ultrasound (LUS) has shown promise as a diagnostic tool for evaluation of newborns with respiratory distress.<sup>1-15</sup>Following birth, the organ responsible for gas exchange transitions from the placenta to the lungs.<sup>16</sup> To establish pulmonary gas exchange, the airways must be cleared of liquid to allow the entry of air. LUS

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may be able to characterize this process. LUS can be performed at the bedside in real time, may be easily repeated during clinical changes and treatments, and does not expose the infant to ionizing radiation.<mark>17.18</mark>

Ultrasound beams penetrating an unaerated, liquid-filled lung create true ultrasound images as the density of liquid changes between the tissue layers. In contrast, ultrasound beams pass- ing through an aerated lung produce artifacts.<sup>1.8.19.20</sup> Traditionally, the interference of sound waves caused by air in the lungs has discouraged the use of LUS as a diagnostic tool. However, an understanding of these characteristic artifacts has led to the recognition that these artifacts are consistent and have diagnostic importance. 1.6.8.9.12.13.21 Our hypothesis is that we can use lung ultrasound to describe initial lung aeration and liquid clearance as healthy term and near term infants initiate breathing after birth.

<sup>&</sup>lt;sup>U</sup> A Spanish translated version of the summary of this article appears as Appendix in the final online version at <u>http://dx.doi.org/10.1016/j.resuscitation.2017.02.017</u>.

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### Methods

This was a prospective, observational study of newborn term and late preterm infants. We used ultrasound to characterize the appearance of the lungs as infants initiate breathing after birth. The study was conducted at the Royal Women's Hospital, in Melbourne, Australia, a regional referral hospital averaging more than 7000 deliveries per year.

#### Inclusion criteria and consent

Infants born at >35 weeks without an antenatal diagnosis of significant pulmonary pathology (i.e. diaphragmatic hernia) were eligible for participation. Written, informed, antenatal consent was obtained prospectively from expecting mothers. We also obtained oral permission from the delivering obstetrician or midwife to be present at deliveries and conduct the study. The patients included in this study represent a convenience sample, with a target enrollment of 50 infants, half delivered vaginally and half delivered via elective cesarean section (CS) without labor. In addition, we studied a third group of infants, not included in our goal sample size of 50, who were born via unplanned CS after

labor (cervical dilation  $\geq 5 \text{ cm}$  and  $\geq 2 \text{ h}$  of uterine contractions). The infants in this study represent a subset of a larger cohort to describe the changes using LUS through the first 24 h after birth (ANZ Clinical Trials Registry Number 12615000380594). Our goal was to obtain 10-20 s lung ultrasound video recordings as the infant initiated breathing after birth. Patients were also included if we were able to obtain lung ultrasound video recordings beginning prior to the 4th breath after birth. The study was approved by the Royal Women's Hospital human research ethics commit- tee.

## Data collection

We obtained serial LUS video recordings, using a GE Venue 50 ultrasound machine (GE Healthcare, Chicago, USA) and a "hockeystick," L8-18i linear transducer with a depth of 2.5 cm and a gain of 60.

As soon as the delivering infant's chest was exposed, the researcher placed the transducer on the chest and obtained a  $\,$  10–  $\,$  $20\ s$  video recording using B-mode and M-mode capturing the initiation of breathing, referred to as the "birth" examination. For the birth exam, we obtained video recordings of either the right or left chest, depending on which side was available. In the event of a cesarean delivery, the researcher scrubbed in using sterile technique to enter the surgical field. The ultrasound transducer was placed in a sterile plastic sheath and sterile ultrasound gel was used. Infants were either being held by the delivering midwife or obstetrician or were placed on their mother's chest during the birth examination. In addition to the birth examination, we also obtained 3 s video recordings from both the right and left side of the chest using B-mode and M-mode at 1-10 min after birth and 11-20 min after birth. In subsequent examinations, infants were either held by their mother or placed on a warming bed.

During each examination, the LUS transducer was placed in the infant's axillae with the notch pointed superiorly towards the infant's head. The transducer was 3.5 cm long, typically capturing images from 2 to 3 intercostal spaces. We placed the LUS transducer in the infant's axillae because we could obtain consistent images regardless if the infant was prone or supine, while minimizing handling. The transducer was then adjusted until a "bat sign" was achieved and the lungs appeared as aerated and dry as possible.<sup>8,19,22</sup> We also collected clinical and demographic information until discharge from the hospital.

#### Lung ultrasound grading

Ultrasound examination of the lung depends on attenuation of sound waves and interpretation of characteristic artifacts. In healthy, aerated lungs, the ultrasound beam passes through the pleural line and encounters air. The air scatters the ultrasound beam and gives rise to horizontal, hyperechoic reverberation artifacts called "A-lines," in which the pleural line is regularly repeated through the depth of the ultrasound window [Figs. 1 & 2].<sup>1,6,8,19,20</sup> A healthy lung has characteristic movement of the pleural line with respiration, seen as "lung sliding" on B-mode video recordings and the "seashore sign" on M-mode.<sup>22–26</sup> Retention of lung liquid after birth is associated with the presence of hyperechoic vertical projections, called "B-lines," that arise from the pleural line and extend through the ultrasound image [Figs. 1 & 2].<sup>3,5,8,15,19,20,27</sup>

We modified a previously validated lung ultrasound grading system described by Raimondi et al. (Fig. 2).<sup>1-3</sup> Type 3 is seen in healthy lungs with full aeration and liquid clearance. Type 3 is characterized by a lack of B-lines and the presence of horizontal, hyperechoic A-lines that repeat regularly through the ultrasound window.<sup>8</sup> We evaluated each video recording for the presence of "lung sliding" on B-mode video recordings and the "seashore sign" on M-mode to rule out pneumothorax.<sup>23-26,28</sup> Type 2 is characterized by the presence of discrete vertical B-lines, arising from a clearly defined pleural line. Type 1 images are created by the coalescence of vertical B-lines that blunt the pleural line and produce a uniform, hyperechoic image called the "white-out" lung. Type 1 is associated with respiratory distress syndrome.<sup>1,2,6,8,13</sup>

We added two additional grades to describe the appearance of the lung prior to the establishment of the pleural line. Type 0 is seen prior to the initiation of breathing and establishment of the pleural line. This is a true US image (no air artifacts) as the US beam passes exclusively through liquid. The ribs and acoustic shadow of the ribs are still visible; the pleural line is either too hypoechoic to be seen or appears as an extremely thin line. This image is also described as "hepatization" of the lungs as they appear to have the same US feature as the liver. [Video examples 1 & 2 in the online version at DOI: 10.1016/j.resuscitation.2017.02.017] Type 0.5 depicts the pleural line with a patchy appearance with poor definition, consisting of speckled hyperechoic areas mixed with hypoechoic lung tissue. This image was visible, transiently, after the initiation of breathing, before the establishment of the pleural line. We also considered the LUS grade to be 0.5 if one area of the ultrasound window showed an established pleural line and another area of the window showed lung without an established pleural line [Fig. 1.3 and 1.4 and Video example 2 in the online version at DOI: 10. 1016/j.resuscitation.2017.02.017].

#### Analysis and statistics

The birth examinations were graded on the above scale at the beginning and end of the video recording. We recorded the number of breaths the infant had taken prior to placing the transducer on the infant's chest to determine if we captured the initiation of breathing. Recordings that started within the infant's first 4 breaths were included for analysis. At the 1–10 min and 11–20 min examination, the LUS grade from each side was combined for analysis, i.e. infants could have a grade of type 3/3, type 2/3, type 2/2, type 2/1, type 1/1, etc.

Means are reported for normally distributed continuous variables and medians with 25–75% interquartile ranges are reported when the distribution was skewed. The Friedman test was used to analyze changes in LUS grades at different time points for the whole cohort. We compared LUS grades at the start and end of the birth exam and each side of the chest at the 1–10 min and 11–20 min exams. The Kruskal–Wallis H Test using Dunn's proce-

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Fig. 1. Characteristic features seen in lung ultrasound. (1.1) Example of type 3.<sup>2</sup> An established, sharp pleural line (SPL) seen in intercostal spaces between ribs (R). The ultrasound beam does not penetrate beyond the ribs, creating acoustic shadows seen as black, hypoechoic, spaces below the ribs. After the ultrasound beam has encountered the pleural line (SPL), the ultrasound beam is scattered and reverberation artifacts are seen as horizontal A-lines (A), that are parallel to the pleural line and repeated through the window. (1.2) Example of type 2. An established, sharp pleural line (SPL) seen in intercostal spaces between ribs (R). B-lines (B), horizontal, hyperechoic projections that arise from the pleural line and project through the images. B-lines, obscuring any potential A-lines, are associated with retention of lung fulci. <u>1-8</u> (1.3) Example of 0.5, the lung in intercostal space "D" has the beginning of a hyperechoic pleural line, but otherwise does not have a defined pleural line. (1.4) Example of 0.5, intercostal spaces "C" and "D" show examples of a speckled, ragged pleural line.

dure with Bonferroni adjustment was used to compare LUS grades between infants delivered vaginally, CS with labor, and elective CS (CS without labor). Mean and standard deviations (SD) are reported for normally distributed continuous variables and medians and 25–75% interquartile range (IQR) for skewed variables. Four blinded observers independently evaluated 20 first breath videos, assigning a grade at the beginning and end of each video, for a total of 40 grades. The evaluation was repeated with a 3-month gap between the first and second evaluation. An intraclass correlation coefficient with a two-way random model and a one-way model was used to calculate inter-rater and intra-rater reliability, respectively. Statistical significance was defined as p < 0.05. Descriptive statistics were performed using IBM SPSS Statistics 21.0 (SPSS, Inc, Chicago, USA).

# Results

Between April 2015 and February 2016, we attempted to capture the initiation of breathing in 76 infants. We enrolled and studied 63 infants (83% of attempted infants). We missed the initiation of breathing in 13 infants: 9 because they had taken  $\geq$ 5 breaths before the chest was available to the researcher, there was a recording error in 2 infants, 1 infant did not breath initially and required positive pressure ventilation, and 1 infant because the researcher was not in the room when the infant was born. We successfully captured the 1st breath at birth in 28 infants. We included an additional 35 infants who had LUS recordings starting at breath 2–4 after birth

(breath 2, N = 21, breath 3, N = 11, breath 4, N = 3). <u>Table 1</u> displays demographic information.

We obtained and analyzed 315 LUS video recordings. We found no evidence of pneumothorax in any infants enrolled in the study. In the delivery room, 2 infants received positive pressure ventilation for apnea after initially crying and 1 infant received CPAP for poor respiratory effort. Only 1 infant was admitted to the nursery with tachypnea, which started at 17 h after birth and was thought to be due to suspected sepsis. The infant was placed on antibiotics and the tachypnea resolved without respiratory support.

#### Lung ultrasound grading

At the birth examination, we recorded a median of 8 s (IQR 6-10) and 6 breaths (IQR 5-8) after the initiation of breathing. In 56% of the birth examinations, the transducer was placed on the infant's right chest. The pleural line was first visible (type  $\geq 0.5$ ) and first established (type  $\geq 1$ ) at a median of 2 breaths (IQR 1-3) and 4 breaths (IQR 3-5) after birth, respectively. By 5 breaths after birth, all infants had a visible pleural line and by 7 breaths after birth, 90% of infants had an established pleural line [Fig. 4]. The Friedman test showed that LUS grades increased over time (p < 0.005). Birth exam start grades were significantly lower than birth exam end grades (p = 0.01) and both the right and left side of the chest at the 1-10 and 11-20 min exams (p < 0.005 for birth exam start versus 1-10 or 11-20 min). LUS at the birth exam end were also significantly

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# Lung ultrasound grades



Type 0: Hepatization

A true US image with no artifacts, taken prior to initiating breathing, no air in the lung, no defined pleural line



Type 0.5: Speckled pleural line

Seen after breathing is initiated, prior to establishment of a defined pleural line



Coalescence of Blines from fluid retention creating a white-out image, pleural line is defined, but often blunted



Type 2: B-lines Distinct vertical Blines, indicating residual fluid, mixed with horizontal A-lines, the pleural line is sharp



Type 3: A-lines

Horizontal A-lines, sharp pleural line, normal lung sliding, no B-lines

# More aeration and fluid clearance

Fig. 2. Grading system for lung ultrasound video recordings modified from Raimondi et al.<sup>1-3</sup> Type 0: "hepatization," the appearance of the lungs after delivery, prior to the infant initiating breathing. This is a true US image, i.e. no artifacts. The US beam does not encounter air, as it passes through liquid and soft tissue. The ribs and acoustic shadow of the ribs is still visible. The pleural line is either extremely thin or hypoechoic. Type 0.5: the pleural line has a speckled, patch hyperechoic appearance with poor definition and is not horizontal in orientation. This image was transiently visible after the initiation of breathing, but before the establishment of the pleural line. Type 1: "the white-out lung", B-lines, or vertical projections arising from the pleural line, have coalesced, creating a uniform white-out image consistent with significant liquid retention. The pleural line may be blunted. The white-out appearance is associated with respiratory distress syndrome.<sup>2,3,6</sup> Type 2: discrete vertical B-lines arising from the pleural line indicating lung aeration and incomplete liquid absorption. Type 3: horizontal A-lines with a lack of B-lines and a sharp pleural line, indicating lung amount on with respirations, called lung sliding of the pleural line should be seen in videos recordings of type 1-3 lungs, and is needed to distinguish type 3 lungs from pneumothorax.

#### Table 1

Demographic and clinical information based on mode of delivery. Although there was a trend for infants delivered vaginally to have higher SpO<sub>2</sub> at the 1-10 min exam (median time 2 min after birth), there were no statistical differences in HR or SpO<sub>2</sub> at the 1-10 min exam or 11-20 min exams. Infants that were delivered via caesarean section had a shorter length of time between delivery and umbilical cord clamping.

	Total	Vaginal delivery	Elective CS	CS with labor	P-value
N GA weeks <sup>days</sup>	$63 \\ 39^{1/7} \pm 2 days$	33 39 <sup>3/7</sup> ±11days	22 38 <sup>6/7</sup> ±7days	8 39 <sup>2/7</sup> ±10days	0.08
Weight (g)	$3473 \pm 422$	3501±356	$3422 \pm 449$	$3493 \pm 616$	0.75
Gender, male (%) Antenatal steroids Time of cord clamping (s) APGAR 1 m APGAR 5 m	54% 20 (20-90) 8 (8-8) 9 (9-9)	55% 6% 90 (40-180) 8 (7-8) 9 (9-9)	55% 23% 20 (13-20) 8 (8-8) 9 (9-9)	63% 0% 20 (20-23) 8 (8-9) 9 (9-9)	0.92 0.08 <0.001* 0.24 0.77
Time of 1-10 min exam (m) HR 1-10min (BPM) SpO <sub>2</sub> 1-10 min (%)	2 (1-3) 170 (154-180) 80%±18	2 (1-4) 178 (167-191) 85% ±18	2 (1-3) 165 (151-170) 78% ±13	2 (1-2.3) 173 (160-179) 82% ±8	0.77 0.08 0.07
Time of 11-20 min exam (m) HR 11-20min (BPM) SpO <sub>2</sub> 11-20 min (%)	14 (12-16) 160 (148-171) 97% ± 3	12 (11-14) 160 (147-174) 97% ± 3	16 (14-18) 152 (144-152) 96% ±3	16.5 (14-17) 172 (168-179) 98% ± 2	<0.001* 0.07 0.10

lower than the 1–10 and 11–20 min exams (p < 0.005 for all comparisons). No differences were seen in grades between the 1–10 and 11–20 min exams. Table 2 shows the proportion of LUS grades at each time point. It took an average of  $111 \pm 46$  and  $110 \pm 40$  s to complete the examinations (capturing 2 video recordings of 3 s in length) at 1–10 min and 11–20 min, respectively.

# Comparisons based on mode of delivery

We compared infants delivered via elective CS and infants delivered after labor (combining the infants born vaginally with the infants born via CS after labor). There was no difference

in LUS grades at the beginning and the end of the birth video between infants delivered after labor and infants delivered via elective cesarean section (p = 0.85 and p = 0.075, respectively). Infants delivered after labor had higher LUS grades at 1-10 mins and at 11-20 min (p = 0.03, p = 0.017, respectively) [Fig. 4]. Table 1 shows the demographic and clinical differences between different modes of delivery. Intraclass correlation coefficient confirmed there was a strong positive correlation for inter-rater reliability (K = 0.89, 95% CI = 0.83-0.94) and intra-rater reliability (rater 1: K = 0.89, 95% CI = 0.80-0.94, rater 2: K = 0.77, 95% CI = 0.61-0.87, rater 3: K = 0.74, 95% CI = 0.56-0.85, rater 4: K = 0.72, 95% CI = 0.54-0.84).

# Percent of infants with visible and established pleural line after each breath at birth

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Fig. 3. Percent of infants with visible pleural line (>type 0) and established pleural line (>type 0.5) after each breath at birth. After 1 breath, 41% (26/63) of the infants had a visible pleural line and 16% (10/63) of the infants had an established pleural line. After the 7th breath, 100% (63/63) of the infants had a visible pleural line and 90% (57/63) of the infants had an established pleural line.

Table 2

Lung ultrasound grades at each time point. Type 0 was only seen at the initiation of breathing. At the 1-10 min examination, all infants had a defined pleural line. At the 11-20 min examination, all infants demonstrated substantial liquid clearance (LUS grade  $\geq$  type 2).

	Birth exam start (≤1st 4 breaths after birth)	Birth examend	
Туре 0	33 (52%)	0 (0%)	
Type 0.5 Type 1	11 (17%)	5 (8%)	
Type 1 Type 2	13 (21%)	38 (60%)	
Type 3	1 (2%)	2 (3%)	
	1-10 min	11-20 min	
Type 0	0 (0%)	0 (0%)	
Type 0.5	0 (0%)	0 (0%)	
Type 1/1 or 1/2	7 (11%)	0 (0%)	
Type 2/2 or 2/3	43 (68%)	42 (67%)	
Type 3/3	13 (21%)	21 (33%)	

## Discussion

This is the first study that has used ultrasound to describe the appearance of the lungs prior to the initiation of breathing, while the lung was gasless, and during the first breaths. In the 1960s, Karlberg and Lind used serial chest X-rays of infants after birth as they aerated their lungs.<sup>29,30</sup> More recent studies using phase contrast imaging in spontaneously breathing newborn rabbits have to demonstrate that lung aeration occurs within the first few breaths after birth.31-33 These studies suggest that large transpulmonary pressures generated during inspiration drive the movement of liquid from the airways into the surrounding lung tissue. Infants generate large inspiratory pressures during the initiation of breathing. We believe that the first detection of the pleural line represents the first entry of air into the distal lung regions. By the 5th breath, all infants in this study had a visible pleural line and by the 7th breath, 89% of infants had an established pleural line. No type 0 lung was seen after the 4th breath and no type 0.5 was seen at

the 1–10 min examination, confirming that lung aeration in infants occurs quickly. Inspiratory efforts appear to be one of the most important mechanisms of driving airway liquid clearance after birth in normal healthy infants.  $\frac{34-37}{2}$ 

There were several limitations to this study. Our observations were made on a small number of infants and we were unable to capture the first breath in over half the infants enrolled in the study. It was challenging to assign a grade to the birth examination videos

because the appearance of the lungs in these dynamic recordings changed dramatically over the 8 s (IQR 6–10) after the initiation of breathing [Fig. 3]. Nevertheless, the high inter-rater and intra-rater reliability scores are reassuring and studies suggest that LUS is easy to learn.<sup>24,38,39</sup>

Twenty-seven of 28 infants with birth recordings starting prior to the initiation of breathing began with a LUS grade of type 0.

One infant started with a grade 0.5 at breath 1 and likely took a breath immediately after delivery that was not observed. Also, we only captured a finite window consisting of 2-3 intercostal spaces on one side of the chest. It is possible that our limited ultrasound window may not be representative of changes occurring throughout the lungs. However, at term, both animal and human imaging studies show lung aeration to occur quickly and uniformly after the initiation of breathing.<sup>29,31,34</sup>

The "white out" lung (Type 1/1) has been predictive of respiratory failure requiring NICU admission and intubation and surfactant administration.<sup>2,3,6</sup> Type 1/1 lung likely represents liquid retention, whereas type 0 and type 0.5 represent a gasless and partially aerated lung, respectively. Our study provides a catalog of images describing the transition in healthy infants, which can serve as a basis for comparisons to determine if LUS can predict respira- tory failure or respiratory distress syndrome in the delivery room. Additionally, LUS in the delivery room may be able to guide respiratory support. For example, the lack of an established pleural line despite positive pressure ventilation, may indicate that air is not penetrating into the distal airways and adjustments may be required. Further studies are needed to test the utility of LUS to guide treatment decisions in the minutes after birth.






Fig. 4. Lung ultrasound grades, comparing infants exposed to labor versus infants delivered via elective cesarean section. Top panel, percent of infants with LUS grades 0-3 at the beginning of the birth clip and the end of the birth clip. There was no difference in LUS grades at the beginning and the end of the birth video between infants delivered after labor and infants delivered via elective cesarean section (p = 0.85 and p = 0.075, respectively). Infants delivered after labor had higher LUS grades at 1-10 min and at 11-20 min (p = 0.03, p = 0.017, respectively).

# Conclusions

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This study demonstrates that lung ultrasound can be used to observe the initiation of breathing in healthy term infants. Establishment of the pleural line, indicating lung inflation, and substantial clearance of liquid is seen during the first few breaths after birth. Studies investigating the use of lung ultrasound in the delivery room to evaluate the infant who requires respiratory support are warranted.

# **Conflict of interest statement**

The authors confirm there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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9 Lung ultrasound immediately after birth to describe normal neonatal transition: an observational study<sup>4</sup>

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# Original article

# Lung ultrasound immediately after birth to describe normal neonatal transition: an observational study

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**Objective** Lung ultrasound (LUS) has shown promise as a diagnostic tool for the evaluation of the newborn with respiratory distress. No study has described LUS during 'normal' transition. Our goal was to characterise the appearance of serial LUS in healthy newborns from the first minutes after birth until airway liquid clearance is achieved.

study design Prospective observational study. setting Single-centre tertiary perinatal centre in Australia.

Patients Of 115 infants born at ≥35 weeks gestational age, mean (SD) gestational age of 38  $\frac{6}{12}$  deks±11 days, mean birth weight of 3380±555 g, 51 were delivered vaginally, 14 via caesarean section (CS) after labour and 50 infants via elective CS.

**Interventions** Weobtained serial LUS videos via the right and left axillae at 1–10 min, 11–20 min and 1, 2, 4 and 24 hours after birth.

Main outcome measures LUS videos were graded for aeration and liquid clearance according to a previously validated system.

**results** Weanalysed 1168 LUS video recordings. As assessed by LUS, lung aeration and airwayliquid clearance occurred quickly. All infants had an established pleuralline at the first examination (median=2 (1–4) min). Only 14% of infants had substantial liquid retention at 10 min after birth. 49%, 78% and 100% of infants had completed airwayliquid clearance at 2, 4 and 24 hours, respectively.

**conclusions** In healthy transitioning newborn infants, lung aeration and partial liquid clearance are achieved on the first minutes after birth with complete liquid clearance typically achieved within the first 4 hours of birth.

trial registration number ANZCT 12615000380594.

# bAckgrOund

Lung ultrasound (LUS) has shown promise as a diagnostic tool for evaluation of the newborn with respiratory distress. Specifically, LUS has been able to distinguish between respiratory distress syndrome, pneumothorax and transient tachypnoea of the newborn.<sup>1–15</sup> During birth, the infant must transition from dependency on the placenta for gas exchange to lungs that successfully perform this function.<sup>16</sup> This process involves inflation and liquid clearance of a gasless lung resulting in fully aerated lung with an established functional residual capacity. LUS may be able to characterise this transition. LUS can be performed by the bedside clinician

# What is known on this subject?

- Ultrasound beams passing through an aerated lung produces characteristic artefacts that can be interpreted for diagnostic purposes.
- Lung ultrasound has been able to distinguish between respiratory distress syndrome, pneumothorax and transient tachypnoea of the newborn in infants with respiratory distress.
- Todate, no studies describe the healthy transitioning newborn lung using ultrasound from the first minutes after birth through complete lung liquid clearance.

# What this study adds?

- In a population of healthy newborns, all infants achieved lung aeration and partial airway liquid clearance within the first 20 min after birth.
- Serial lung ultrasound can be used to monitor changes in lung aeration and airway liquid clearance after birth.
- Complete airway liquid clearance is typically achieved within the first 4 hours after birth.

in real time with minimal delay, may be easily repeated during clinical changes and treatments and does not expose the infant to radiation.  $^{17\,18}$ 

Ultrasound beams passing through an aerated lung produce characteristic artefacts.  $\frac{1 \cdot 8 \cdot 19 \cdot 20}{100}$  Traditionally, the interference of sound waves caused by air in the lungs has discouraged the use of LUS as a diagnostic tool. However, these characteristic artefacts are now recognised as having diagnostic importance.  $\frac{1 \cdot 6 \cdot 89 \cdot 12 \cdot 13}{100}$ 

No studies describe the appearance of the normally transitioning newborn lung using ultrasound on a healthy population of infants from the first minute after birth until full lung aeration and liquid clearance are achieved. We hypothesise that we can use LUS to describe lung aeration and liquid clearance starting in the delivery room through the first hours after birth in healthy term and near term infants.

# MethOds

This was a single centre, prospective, observational study of term and late preterm conducted

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at the Royal Women's Hospital, in Melbourne, Australia, a regional referral hospital with a high-risk perinatal service averaging >7000 deliveries per year.

Participants were eligible for the study if they were  $\geq 35$  weeks gestation at delivery and did not have an antenatal diag- nosis of significant pulmonary pathology (ie, diaphragmatic hernia). Written, informed, antenatal consent was obtained from expecting mothers prior to delivery. We also obtained oral permission from the delivering obstetrician or midwife to be present at deliveries and conduct the study. Patients were recruited as a convenience sample, with a target enrollment of 100 infants, 50 born via elective caesarean section (CS) (without labour) and 50 born via vaginal delivery. In addition, we studied a third group of infants, not included in our goal sample size of 100, who were born via unplanned CS after a period of labour (minimum cervical dilation of at least 5 cm and more than 2 hours of uterine contractions). The study was approved by the Royal Women's Hospital ethics committee and registered with the Australian and New Zealand Clinical Trials Registry: 12615000380594.

# data collection

We obtained serial LUS video recordings, using a GE Venue 50 ultrasound machine (GE Healthcare, Chicago, Illinois, USA) and a 'hockey-stick,' L8-18i linear transducer with a depth of 2.5 cm and a gain of 60. Images were interpreted and graded during or immediately after each LUS examination by the researcher. We obtained 3 s video recordings of the right and left side of the chest using B-mode and M-mode. Images were collected at 1–10 min, 11–20 min, 1 hour, 2 hours and 4 hours after birth. Vital signs (heart rate, respiratory rate and SpO<sub>2</sub>) were also assessed at each time point. At the 4-hour examina- tion, if the infant had achieved a LUS grade consistent with full liquid clearance and complete aeration and the postductal SpO<sub>2</sub> was >97%, no further ultrasounds were performed. If not, a

repeat examination was performed at 24 hours after birth. We collected clinical and demographic information until hospital discharge.

# Lus examination

Infants were examined while being held by their mother or on a warming bed. Healthy infants born vaginally were placed on the mother's chest immediately after birth. During each examination, the LUS probe was placed in the infant's axillae with the notch pointed superiorly. The probe was then adjusted until a 'bat sign' was achieved and the lungs appeared as aerated and dry as possible.<sup>819,21</sup> The probe was 3.5 cm long and a typical image included the lung parenchyma from two to three rib spaces. We choose to place the LUS probe in the infant's axillae because we

could obtain consistent images regardless if the infant was prone or supine, while minimising handling. We used a previously validated LUS grading system described by Raimondi and colleagues,<sup>1–3</sup> assigning a grade of type 1, 2 or 3 to each

video (figure 1). In addition, we evaluated for the presence of lung sliding on B-mode video loops and the 'seashore sign' on M-mode to rule out pneumothorax. $\frac{21-26}{2}$ 

# Analysis and statistics

The LUS grade from each side of the chest at a single time point was combined for analysis. Infants could have a grade of type 3/3 (type 3 on both sides of the chest), type 2/3 (type 2 on one side and type 3 on the other side of the chest), type 2/2 (type 2 on both sides of the chest), type 1/2 (type 1 on one side and type 2 on the other side of the chest), type 1/1 (type 1 on both sides of the chest) and so on. The Friedman test was used to compare LUS grades between time points for the whole cohort. The Kruskal-Wallis H Test using Dunn's procedure with Bonferroni adjustment was used to compare results based on mode

# Lung Ultrasound Grading



Type 1: Fluid retention, pleural line blunt, "white-out" appearance from coalescence of B-lines (CBL)



Type 2: Establishment of a clear, sharp pleural line (SPL) and partial fluid clearance, vertical B-lines (B) obscuring horizontal A-lines (A) are seen



Type 3: Full aeration and fluid absorption, sharp pleural line (SPL), only horizontal A-lines seen

# More aeration and fluid clearance

**Figure 1** Grading system for lung ultrasound from Raimondi *et al.*<sup>1-3</sup>Left: type 1 or 'white-out' lung, significant liquid retention associated with respiratory distress syndrome (type 1) is seen between the acoustic shadow cast by the ribs (R). White-out is produced by the coalescence of B-lines (CBL) and the pleural line is blunt. Centre: type 2, retention of lung liquid after birth is characterised by the presence of hyperechoic vertical projections called 'B-lines' (B) that arise from the sharp pleural line (SPL), extend through the ultrasound image and obscure the 'A-lines' (A).<sup>3.5.15.2033</sup> This represents an intermediate step in the progression of liquid-filled to air-filled lungs of the transitioning newborn. Right: type 3 or aerated neonatal lung with horizontal A-lines, which are hyperechoic reverberation artefact produced by the ultrasound beam encountering air.<sup>16.8.19.20</sup>Other ultrasound signs indicating a healthy lung include characteristic movement of the pleural line with respiration, seen as 'lung sliding' on B-mode video recordings and the 'seashore sign' on M-mode. A-lines, with no B-lines, a lack of lung sliding (abnormal movement) and the 'stratosphere' sign on M-mode, is indicative of pneumothorax.<sup>2123-26</sup>

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table 1 Demographic information					
	total	Vaginal delivery	elective cs	cs after labour	p Value
n	115	51	50	14	
GA weeks days	38 <sup>6/7</sup> ±11 days	39 <sup>2/7</sup> ±12 days	39 <sup>1/7</sup> ±15 days	38 <sup>3/7</sup> ±8 days	<0.003*
Weight, g	3380 g±555g	3419 g±469g	3346 g±676g	3315 g±596g	0.32
Gender, male, %	54%	55%	48%	71%	0.49
Antenatal steroids	23%	10%	38%	14%	0.001*
Time of cord clamping, s	20 (20–60)	90 (20–160)	20 (10–20)	20 (10–20)	<0.001*
Apgar at 1 minute	8 (8–8)	8 (7.5–8)	8 (8–8)	8 (8–9)	0.54
Apgarat 5 minutes	9 (8–9)	9 (9–9)	9 (9–9)	9 (9–9)	0.30
Time of 1–10 min examination, min	2 (1–4)	2.5 (1–4)	2 (1–4)	2 (1.3–3.8)	0.62
HR 1–10 min, bpm	165±23	173±25	157±21	164±18	0.02*
SpO <sub>2</sub> 1–10 min,%	81±13	86±13	78±13	81±9	0.16
RR 1–10 min	57±16	64±14	52±17	55±12	0.42
Time of 11–20 min examination, min	13 (11–16)	12 (11–14)	15 (12–18)	13.5 (13–17)	0.001*
HR 11–20 min, bpm	158±19	160±22	154±16	161±21	0.29
SpO <sub>2</sub> 11–20 min, %	97±3	97±3	96±4	97±2	0.16
RR 11–20 min	57±13	61±13	55±13	47±10	0.42
Time of 1-hour examination, min	61 (50–73)	65 (56–79)	60 (50–70)	63 (42–73)	0.495
HR 1 hour, bpm	144±16	148±15	142±16	136±16	0.052
SpO <sub>2</sub> 1 hour, %	98±2	98±2	99±1	99±1	0.16
RR 1 hour	51±15	56±14	48±14	50±19	0.42

Demographics and clinical results between modes of delivery.

\*p<0.05. By 2 hours after birth, all infants had SpO₂≥93% and five had a RR 70–80. By 4 hours after delivery, one infant had a RR >70 (=72).

bpm, beats per minute; CS, caesarean section; GA, gestational age; HR, heart rate; RR, respiratory rate.

of delivery. Mean and SD are reported for normally distrib- uted continuous variables and medians and 25%–75% IQR for skewed variables. Four blinded observers independently evaluated 30 video recordings and repeated the evaluation with a 3-month gap between the first and second evaluation. An intraclass correlation coefficient with a two-way random model and a one-way model was used to calculate inter-rater and intrarater reliability, respectively. Statistical significance was defined as p<0.05. Descriptive statistics were performed using IBM SPSS Statistics V.21.0.

# resuLts

Between March 2015 and February 2016, we studied 115 patients and analysed 1168 videos. Mean gestational age was  $38^{6/7}$  weeks±11 days, and mean birth weight was  $3380\pm555$  g (table 1). Fifty-one infants were delivered vaginally, 50 via elective CS and 14 via CS after labour (table 1). Infants delivered vaginally had a significantly higher gestational age than infants born via elective CS and more time prior to umbilical cord clamping than infants delivered via CS. Infants born via elective CS had more exposure to antenatal steroids than infants born after labour. All other parameters were non-significant.

Vital signs and APGAR scores are shown in table 1. Infants who delivered vaginally had higher HRs initially, which may reflect the stress of labour; no subsequent difference was seen. We found no evidence of pneumothorax in any infants enrolled in the study. Only one infant was admitted to the nursery with tachypnoea, which started at 17 hours after birth and was thought to be due to suspected sepsis. The infant was placed on antibiotics and the tachypnoea resolved without respiratory support.

# Lus grades

The timing of the LUS examinations is shown in table 1. The median (IQR) time of the 2-hour, 4-hour and 24-hour ultrasound

examinations was 131 (120–150) min, 259 (233–300) min and 28 (24–48) hours after birth, respectively, with no differences between groups.

At the 1–10 min examination, only 3% had type 1/1 and 13% had type 1/2. By 11–20 min examination, no infants had type 1/1 or type 1/2. By 4 hours and 24 hours after birth, 69% and 85% of infants had a type 3/3 grade, respectively. In the first 2 hours after birth, LUS grades at the different examination time points did not differ significantly. However, the LUS grades at 4 hours and 24 hours were significantly higher than at 1–10 min, 11–20 min, 1 hour and 2 hours (comparing 2 hours with 4 hours, p=0.009, p<0.001 for all other significant comparisons) (figure 2). Although a type 1/3 was possible, we did not observe a type 1/3 at any LUS examination.

We compared LUS grades for infants delivered vaginally, via elective CS and via CS after labour. Infants delivered via elective CS had significantly lower LUS grades than infants born vaginally and infants born via CS after labour at 1–10 min (elective CS vs vaginal delivery, p=0.001 and elective CS vs CS after labour, p=0.01) and 11–20 min (elective CS vs vaginal delivery, p<0.001 and elective CS vs CS after labour, p=0.03). At 1 hour, infant born via elective CS had non-significantly lower LUS scores (elective CS vs vaginal delivery, p=0.09 and elective CS vs CS with labour, p=0.06; figure 3). There were no differences in LUS grades between vaginally delivered infants and infants delivered via CS after labour (p=1 at 1–10 and 11–20 min).

We also compared infants delivered via elective CS and infants delivered after labour (combining vaginal birth with CS after a period of labour); infants delivered after labour had higher LUS grades at 1–10 min, 11–20 min and at 1 hour after birth (p<0.001, p<0.001, p=0.009, respectively). After the 1-hour examination, no statistical difference was seen between these two groups (p=0.09 at 2 hours, p=0.6 at 4 hours and p=0.46 at 24 hours).

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Figure 2 Lung ultrasound (LUS) grading over 24 hours for all subjects. Three-second B-mode and M-mode video loops were acquired at the midaxillary line of the right and left chest. By 10 min after birth, all infants had type 2/2 or higher. The LUS grade at 4 and 24 hours was significantly higher than at 10 min, 20 min, 1 hour and 2 hours (comparing 2 hours with 4 hours, p=0.009, p<0.001 for all other significant comparisons).

# 'backsliding'

Several infants had achieved a type 3 grade on one side of the chest, but then had a type 2 grade on that side in subsequent examinations. We called this 'backsliding', which refers to the LUS grade worsening from one time point to the next. Overall, backsliding occurred at least once in 47% of the infants, with 10% backsliding twice. Backsliding occurred less frequently at each subsequent time point (table 2). Backsliding was seen in 51%, 38% and 57% of infants delivered vaginally, via elective CS and via CS after labour, respectively, and did not differ based on mode of delivery (p=0.14). No infant regressing to type 2/1 grade. This occurred between the 20 min and 1-hour examination. The infant then was graded as type 2/2 on the 2-hour examination. Between the 4-hour and 24-hour examinations, no backsliding was observed.

# Inter-rater and intrarater reliability

Intraclass correlation coefficient confirmed that there was a strong positive correlation for inter-rater reliability (k=0.96, 95% CI 0.93 to 0.98) and intrarater reliability (rater 1: k=0.96, 95% CI 0.91 to 0.98, rater 2: k=0.9, 95% CI 0.8 to 0.95, rater 3: k=0.85, 95% CI 0.72 to 0.93, rater 4: k=0.79, 95% CI 0.61 to 0.9).

# discussion

This is the earliest study, starting within 10 min after birth, to use ultrasound to describe the temporal change in airway liquid clearance in healthy term and near term infants until neonatal adaption to birth is complete. LUS has been used previously to predict which infants will need admission to the neonatal intensive care unit (NICU), which preterm infants will receive surfactant, and to describe transient tachypnoea of the newborn,



Figure 3 Lung ultrasound (LUS) grading over 24 hours based on mode of delivery. Infants born vaginally and infants born via caesarean section (CS) after a trial of labour had significantly higher LUS grades than infants born via elective CS (ie, CS without labour) at 1–10 min and 11–20 min. No differences were seen between vaginally delivered infants and infants born via CS after a trial of labour.

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table 2 'Backsliding,' the percentage of infants at each time point that had a 'lower' lung ultrasound (LUS) grade at the subsequent examination						
Lus grade	1–10 to 11–20 min (%)	11–20 min to 1 hour(%)	1 to 2 hours (%)	2 to 4 hours (%)	4 to 24 hours (%)	
Type 3/3 to 3/2	26	29	9	6	0	
Type 3/2 to 2/2	43	42	20	10	0	
Type 3/3 to 2/2	11	16	4	3	0	
Type 2/2 to 2/1	0	2	0	0	0	
Type 2/2 to 1/1	0	0	0	0	0	
Total	18	27	9	6	0	

meconium aspiration syndrome, pneumothorax and the appearance of the lungs after surfactant administration. $\frac{2-7 \ 11 \ 24}{24}$  The present study might be used as a reference for identifying airway liquid retention and monitoring the progress of lung aeration after birth.

Animal studies indicate that the primary mechanism of airway liquid clearance is likely due to the negative pressure generated during inspiration, which drives the liquid out of the airways.<sup>27=</sup> <sup>30</sup> Infants born after a period of labour likely had some liquid clearance before birth, which may explain why they had higher LUS grades in the first hour after birth compared with infants born via elective CS. Although our numbers are small, infants in this study who were exposed to labour had similar LUS grades at all time points, regardless if they were delivered vaginally or via CS. Of note, 38% of the infants delivered by elective CS in this study had received antenatal steroids to reduce the risk of respiratory distress after delivery. The mechanism may be due to enhancing lung liquid clearance, potentially explaining why we only saw a difference in LUS grades between different modes of delivery initially, but no difference by 2 hours after birth.<sup>11.31.32</sup>

We observed partial or complete liquid clearance occur within the first minutes after birth in this cohort of healthy infants. After establishment of lung inflation, residual lung liquid may take several hours to clear. In addition to intrapleural pressure differences, secondary mechanisms of lung liquid absorption likely contribute to this process. Most of the infants in this study were crying vigorously during the 1-10 and 11-20 min examinations, then transitioned to quiet breathing by the 1-hour and 2-hour examinations. 'Backsliding' from type 3 and type 2 was seen in 47% of the infants, likely due to liquid re-entry into the airways. Fluctuation in LUS grades may be dependent on the infant transitioning from vigorous crying immediately after birth to quiet, regular breathing observed several minutes after birth. Quiet breathing may not generate the same intrathoracic pressure to drive the lung liquid into the interstitial space. In the current study, only one infant showed backsliding from type 2/2 transiently to a type 1/2. No infants had a type 1/1 after 10 min after birth. Only three infants had a type 1/1 grade at the first examination. All were born via elective CS and all had the first LUS examination at 1 min after birth.

A limitation of this study was the lack of infants with tachypnoea or respiratory distress. We can only speculate on the utility of LUS in the delivery room to detect and manage common neonatal pathologies. Raimondi and colleagues<sup>2</sup> - 3 reported that a type 1/1 grade at 120 min after birth correlates with respiratory distress syndrome and the need for support. In addition, 4 out of 46 infants with a type 2 grade in that study were subsequently admitted to the NICU for continuous positive airway pressure and oxygen. No infant in the current study was admitted for respiratory distress and very few had tachypnoea after birth, despite the prevalence of infants with a type 2/2 grade (38% at 2 hours). The difference in clinical outcomes between studies may be due to a more mature patient population, differences in interpretation, chance variation or error in the ability of LUS to detect respiratory distress. Other studies support that there may be no clinical difference between infants with type 2/2 and better LUS grades in the first hours after birth.<sup>561315</sup>We only observed type 1/1 or 1/2 transiently in 14% of infants; it is possible that these images are subtly different than images due to respiratory distress syndrome. However, the strong agreement in LUS grades (inter-rater and intrarater reliability) was reassuring.

# cOncLusIOn

In a population of healthy newborns, all infants achieved lung aeration and partial airway liquid clearance within the first 20 min after birth. Serial LUS can be used to monitor changes in lung aeration and airway liquid clearance after birth. Complete airway liquid clearance is typically achieved within the first 4 hours after birth. Further studies using LUS in the delivery room for diagnosis and management pathological conditions of the newborn are warranted.

contributors All authors have made significant contributions to the conception and design of the study, acquisition of data, data analysis and interpretation, drafting and revising the manuscript and final approval. All research was conducted at the Royal Womenâ€<sup>™</sup>s Hospital, Melbourne, Victoria, Australia. DAB wrote the first draft of the manuscript. No author received payment to produce the manuscript. All authors have reviewed and approve of the submitted version of the manuscript and they take full responsibility for the content.

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#### Respiratory changes in term infants immediately after birth<sup>5</sup> 10

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Clinical paper

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# ABSTRACT

Keywords: Exhaled carbon dioxide Tidal volume Respiratory rate Newborn Resuscitation Delivery room

A R T I CLEIN FO

Introduction: Over 5% of infants worldwide receive breathing support immediately after birth. Our goal was to define references ranges for exhaled carbon dioxide (ECO<sub>2</sub>), exhaled tidal volume (VTe), and respiratory rate (RR) immediately after birth in spontaneously breathing, healthy infants born at 36 weeks' gestational age or older. Methods: This was a single-centre, observational study at the Royal Women's Hospital in Melbourne, Australia, a busy perinatal referral centre. Immediately after the infant's head was delivered, we used a face mask to measure ECO2, VTe, and RR through the first ten minutes after birth. Respiratory measurements were repeated at one hour. Results: We analysed 14,731 breaths in 101 spontaneously breathing infants, 51 born via planned caesarean section and 50 born vaginally with a median (IQR) gestational age of 391/7 weeks (383/7-395/7). It took a median of 7 (4-10) breaths until ECO2 was detected. ECO2 quickly increased to peak value of 48 mmHg (43-53) at 143 s (76-258) after birth, and decreased to post-transitional values, 31 mmHg (28-24), by 7 min. VTe increased after birth, reaching a plateau of 5.3 ml/kg (2.5-8.4) by 130 s for the remainder of the study period. Maximum VTe was 19 ml/kg (16-22) at 257 s (82-360). RR values increased slightly over time, being higher from minute five to ten as compared to the first two minutes after birth.

Conclusions: This study provides reference ranges of exhaled carbon dioxide, exhaled tidal volumes, and re-spiratory rate for the first ten minutes after birth in term infants who transition without resuscitation

# Introduction

Over 5% of infants worldwide receive breathing support immediately after birth [1-4]. More than 800,000 infants die annually of birth asphyxia; many of these deaths may be avoided through simple resuscitation techniques [2,5-7]. Rising heart rate and oxygen saturation (SpO<sub>2</sub>) values are used as indicators of adequate ventilation [1,8-10]. Studies have shown that exhaled carbon dioxide (ECO<sub>2</sub>)

levels correlate with lung aeration and that increasing ECO2 precedes increases in heart rate and SpO2 in effectively resuscitated infants [11-<u>13</u>]

The presence of ECO2 indicates airway patency, establishment of lung aeration, pulmonary blood flow, and pulmonary gas exchange. Lung aeration leads to a reduction of pulmonary vascular resistance which in turn leads to an increase in pulmonary blood flow, now serving as the preload of the left ventricle [14-18]. Schmölzer and

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colleagues have reported a median of three breaths before any  $ECO_2$  could be detected, supporting that functional residual capacity needs to be established before gas exchange begins [19].

Monitoring exhaled carbon dioxide and exhaled tidal volume (VTe) may guide ventilation of the compromised newborn [11,12,20-24]. However, studies defining reference ranges of ECO<sub>2</sub>, VTe, and respiratory rate (RR) in the delivery room in spontaneously breathing infants are limited [19,25]. This study aims to fill that gap by describing the respiratory changes in term infants immediately after birth.

# Methods

This is a single centre, prospective, observational study of spontaneously breathing term infants in the birth suites and operating theatres at The Royal Women's Hospital in Melbourne, Australia, a regional referral hospital with a high-risk perinatal service averaging > 7500 deliveries per year. The study was approved by The Royal Women's Hospital Human Ethics Committee and registered with the Australia New Zealand Trials Registry (TRN: 12615000380594).

#### Population

Participants were eligible for the study if they were  $\geq$ 36 weeks gestation at birth with no known congenital disorder that might interfere with pulmonary gas exchange at birth (i.e. diaphragmatic hernia, Potter sequence, cyanotic heart lesions, etc.). Written, informed, antenatal consent was obtained from expectant mothers prior to birth and verbal permission was obtained from the delivering obstetrician or midwife to conduct the study. Infants were recruited as a convenience sample, with a target enrolment of 100 spontaneously breathing infants, 50 born via planned caesarean section (CS, without labour) and 50 born via vaginal birth. Infants were excluded if they were delivered via an unplanned CS or if they received respiratory support (positive pressure ventilation, CPAP, or supplemental oxygen) in the first 10 min after birth.

# Data collection

The NM3 respiratory function monitor (Phillips Healthcare, Massachusetts, USA) was used to measure  $ECO_2$  by infrared absorbance and gas flow, VTe and RR with a flow sensor. The combined  $CO_2$ /flow sensor has a dead space of 1 ml. Saturation of peripheral oxygen (SpO<sub>2</sub>) was measured via an incorporated Masimo pulse oximeter (Masimo, Irvine, California). The  $CO_2$ /flow sensor was attached to a face mask in a mainstream configuration which was placed over mouth and nose of the infant as soon as the head was delivered. Infants breathed through the mask during data collection. The distal end of the  $CO_2$ /flow sensor was open to air.

Respiratory values were measured breath by breath by the respiratory function monitor. These values were timestamped and automatically downloaded into an Excel data sheet. Downloaded data were verified by videotaping the monitor screen with a GoPro Hero 3 + (GoPro Inc., San Mateo, CA, USA) or by a specifically designed data acquisition system connected to the respiratory function monitor which recorded waveforms of flow, volume,  $ECO_2$  and plethysmography in real time. The sequence of events after birth (i.e. time of birth, time face mask applied, time of cord clamping, etc.) was recorded with an audio recorder via the GoPro.

Time of birth was defined as the moment when the infant's entire body was delivered. The facemask was placed on the infant, and data collection commenced, as soon as possible after the infant's head was delivered; in some infants this occurred prior to birth. Respiratory measurements were recorded continuously for the first 60 s after birth, then every 30 s for the next 3 min, and subsequently every minute from 4 to 10 min after birth. From minute 1 to minute 10, we aimed to capture at least 20 s of continuous recordings each time the facemask

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was placed on the infant. We obtained a final 30 s of continuous respiratory measurements one hour after birth, if the post-ductal SpO<sub>2</sub> was  $\geq$  97%, indicating neonatal transition. If the post-ductal SpO<sub>2</sub> was

 $\leq$  97%, we re-evaluated the infant at a later time when the SpO<sub>2</sub> was  $\geq$  97%. Umbilical arterial and venous cord blood gases were noted when available. We collected demographic information and clinical information until hospital discharge.

After vaginal births, data collection continued whilst the baby was on the mother's chest. Prior to CS births, using sterile technique, a plastic sterile sheathing was placed over the combined flow sensor, flow sensor tubing and wires. Sterile scissors were used to cut openings in the sterile sheath at both ends of the flow sensor, and the sheath was secured to the flow sensor using sterile rubber bands. A sterile facemask was then connected to one exposed end of the combined flow sensor. One of the researchers scrubbed, entered the sterile field, and stood next to the obstetrician (Supplemental Video). Data collection con- tinued for 10 min after birth, during which time midwives performed routine care, including drving the infant, administration of vitamin K. swaddling the infant, and presenting the infant to the parents for cud- dles. Data collection was ceased if felt to interfere with routine care, parental bonding, or feeding at the request of the midwife or parents. If there were any signs of respiratory compromise, support was given according to Australian Neonatal Resuscitation guidelines [26].

# Analysis and statistics

Median ECO2, VTe, and RR were calculated every 10 s from the beginning of data collection until 4 min after birth, then every 30 s from 4 to 10 min after birth. Mask leak was calculated by measuring the difference between inhaled and exhaled tidal volumes for each continuous block of time the mask was placed on the infant's face (20-60 s). Breaths were then eliminated until leak was less than 20%. Means and standard deviation (SD) were calculated for continuous variables and analysed using Student's T-Test. Medians and inter- quartile ranges (IQR) were calculated for non-normally distributed variables and analysed using Mann-Whitney U test. Correlations were assessed using Pearson's r statistic. A two-way mixed measures ANOVA with a Games-Howell post hoc analysis was used to compare continuous variables over time between groups. One-way repeated measures ANOVA was used for parametric data and a Friedman's test was used for non-parametric data to analyse the changes in continuous variables over time within each group. A Bonferroni-Holm correction was used to account for multiple testing. Measurements were analysed using IBM SPSS Statistics 24 (SPSS, Inc, Chicago, IL) and R statistics (version 3.4.1). Statistical significance was considered as p < 0.05.

# Results

# Study population

We enrolled 108 infants in the study. Seven infants received respiratory support immediately after birth and were excluded. The population characteristics and timing of events for the remaining 101 patients are summarised in Table 1. Comparing modes of delivery, there were no statistically significant differences in gestational age at birth, umbilical cord arterial and venous pH, first temperature, and time to apply the face mask, time to first breath, time to first cry, and time to umbilical cord clamping in seconds after birth. In addition, there were no differences in respiratory measurements comparing gender or gestational age ( > 38 weeks versus < 38 weeks at birth). No infant included in the analysis was admitted to the nursery for respiratory distress. Three infants were admitted, 2 for phototherapy and 1 for presumed sepsis. The facemask was placed prior to the initiation of breathing and the first breath after birth was successfully captured in 69 infants. We captured the second, third, and fourth breath in 19, 8, and 4 infants, respectively. Data collection began with the 20th breath for the

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# Table 1

Demographic, antenatal, and delivery room data presented in medians and IQR. Gender is depicted as number and percentage of male participants. \* = Statistical difference comparing modes of delivery, p < 0.05. Umbilical arterial and venous blood gas values were available for 58 and 67 of the infants, respectively. GA = Gestational age at birth; PartCO<sub>2</sub> = umbilical arterial CO<sub>2</sub>; PvenCO<sub>2</sub> = umbilical venous CO<sub>2</sub>;  $^{\circ}$ C = degrees Celsius.

Patient Characteristics	All subjects (N = 101)	Caesarean Births (N = 51)	Vaginal Births (N = 50)
Male sex, n (%)	53 (52%)	24 (47%)	29 (58%)
GA (weeks <sup>days</sup> )*	39 <sup>1/7</sup> (38 <sup>3/7</sup> -39 <sup>5/7</sup> )	38 <sup>6/7</sup> (38 <sup>1/7</sup> -39 <sup>2/7</sup> )	39 <sup>3/7</sup> (38 <sup>4/7</sup> -40 <sup>1/7</sup> )
Weight (grams)	3345 ± 494	3354 ± 500	3337 ± 494
Apgar at 1 min	8 (8-8)	8 (8-8)	9 (8-8)
Apgar at 5 min	9 (9-9)	9 (9-9)	9 (9-9)
pH (umbilical cord arterial blood)*	7.26 (7.21-7.29)	7.28 (7.25-7.3)	7.21 (7.18-7.26)
PartCO <sub>2</sub>	53 (48-59)	53 (49-59)	51 (46-59)
pH (umbilical cord venous blood)*	7.32 (7.28-7.36)	7.35 (7.34-7.36)	7.3 (7.26-7.32)
PvenCO2	42 (38-45)	43 (40-46)	40 (37-44)
FirstTemp (°C)*	36.7 (36.5-37.1)	36.7 (36.5-36.8)	37.1 (36.6-37.3)
Time to face mask application (seconds)*	1 (-5-2)	1 (15-2)	2 (1-2)
Time to first breath (seconds)*	1 (-4-3)	0 (-13-2)	2 (1-5)
Time to first cry (seconds)*	3 (0-19)	0 (-10-6)	11 (2-29)
Time to umbilical cord clamping (seconds)*	13 (6-131)	6 (4-11)	130 (91-233)

final infant. We analysed 14,731 of the total 15,877 (92.8%) breaths captured. A median of 92.3% (IQR 89.2–96.9) of breaths per infant had valid values for  $ECO_2$  and were included for analysis.

# Exhaled carbon dioxide after birth

We recorded a median of 7 breaths (IQR: 4–11) prior to the detection of ECO<sub>2</sub> (<u>Table 2</u>). There were no significant differences in ECO<sub>2</sub> based on mode of delivery. ECO<sub>2</sub> values changed significantly over time (p < 0.001), ECO<sub>2</sub> quickly increased to peak values after birth and then slowly decreased to post-transitional states (Fig. 1). There was a median of 5 breaths (1–14) per infant that had ECO<sub>2</sub> values within 5 mmHg of the max ECO<sub>2</sub> value after the max ECO<sub>2</sub> was measured. There was no significant difference in ECO<sub>2</sub> values from 7 min after birth through the post-transitional assessment (1 h after birth). When available, paired maximum ECO<sub>2</sub> values were higher than umbilical venous CO<sub>2</sub> values (61 comparisons, p < 0.001), but did not differ significantly with umbilical arterial CO2 values (52 comparisons, p = 0.07).

# Exhaled tidal volume and respiratory rate after birth

VTe increased significantly over time (p < 0.001), reaching a

plateau of 5.3 ml/kg (2.5–8.4) by 130 s and remaining constant for the remainder of the study period (Fig. 2). Maximum VTe for each infant was a median of 19 ml/kg (16–22) at 257 s (82–360) after birth (Table 2). At the post-transitional assessment, infants had a median (IQR) VTe of 5.8 ml/kg (5.2–6.9). The time to maximum VTe was significantly later than the time to maximum ECO<sub>2</sub> (p = 0.002).

The RR increased during the study period (p < 0.001, Fig. 3) and was significantly higher from minute five to ten as compared to the first two minutes after birth. At 1 h, infants had a median (IQR) RR of 57 breaths per minute (48–65). There were no differences in VTe or RR between modes of birth.

# Infants who received respiratory support after birth

Seven infants were excluded from the primary analysis because they received respiratory support immediately after birth. Five received positive pressure ventilation and 2 received continuous positive airway pressure. There were no demographic or antenatal differences between the infants included for primary analysis and the infants who received respiratory support, including gestational age, mode of delivery, umbilical cord blood gas values, timing of umbilical cord clamping, and birth weight. Infants who received respiratory support had higher max ECO2 values (63 mmHg (60–67) versus 48 mmHg (43–55), p < 0.001),

#### Table 2

Description of exhaled carbon dioxide (ECO<sub>2</sub>), exhaled tidal volume (VTe) and respiratory rate (RR) after birth. There were no statistical differences in ECO<sub>2</sub>, VTe, or RR comparing mode of delivery. Means and standard deviations and medians and interquartile range are shown. N breaths = number of breaths; mmHg = millimetres of mercury. ml/kg = milliliters per kilogram.

	Overall (N = 101)	Caesarean Births (N = 51)	Vaginal Births (N = 50)
Exhaled carbon dioxide (ECO2)			
N breaths until first ECO <sub>2</sub>	7 (4-11)	7 (4-10)	8 (5-12)
N breaths until ECO2 > 15 (mmHg)	9 (6-12)	8 (6-11)	10 (6-13)
Value first detectable ECO2 (mmHg)	15 (12-20)	15 (13-20)	15 (11-20)
Time to first ECO <sub>2</sub> (seconds)	9 (5-21)	8 (4-20)	10 (8-21)
Max ECO <sub>2</sub> level (mmHg)	48 (43-53)	45 (41-52)	50 (45-55)
Time to max ECO2 (seconds)	143 (76-258)	158 (82-305)	114 (75-193)
ECO2 value at 1 h (mmHg) Time	27 ± 3	28 ± 3	27 ± 3
at 1 h follow up (minutes)	78 (58-130)	72 (54–134)	87 (70-124)
Exhaled tidal volume (VTe)			
Value peak VTe (ml/kg)	19.3 ± 3.3	19.6 ± 3.4	19 ± 3.2
Time of peak VTe (seconds after birth)	257 (82-360)	257 (80-346)	280 (84-366)
VTe at 1 h (ml/kg)	5.8 (5.2-6.9)	5.6 (5.2-6.1)	6 (5.3-7.6)
Respiratory rate (RR)			
RR at 1 min (breaths per minute)	45 (35-60)	47 (37-59)	42 (29-76)
RR at 2 min (breaths per minute)	50 (35-61)	46 (31-57)	50 (38-64)
RR at 5 min (breaths per minute)	60 (51-75)	63 (53-83)	59 (51-69)
RR at 1 h (breaths per minute)	57 (48-66)	54 (47-62)	56 (43-66)

# Douglas Blank, MD: PhD Thesis Submission, December 2018 PhD title: Umbilical cord management during neonatal resuscitation

D.A. Blank et al. Resuscitation130(2018)105-110 Exhaled carbon dioxide over time 60 Exhaled carbon dioxide in mmHg 50 40 30 20 10 0 Birth 1Min 2Min 3Min 4 min 5 min 6 min 7 min 8 min 9 min 10 min 1 hour -- 90% -- 75% --- Median -- 25% ----- 10%



Exhaled tidal volume over time



Fig. 2. Exhaled tidal volume (VTe) in millilitres per kilogram (ml/kg) over time, all subjects (n = 101), median values and the 10th, 25th, 75th and 90th percentile are shown.



Fig. 3. Respiratory rate over time, all subjects (n = 101), median values and 10th, 25th, 75th and 90th percentile are shown.

lower 1-min Apgar (5 (4–7) versus 8 (8–8), p < 0.001) and 5-min Apgar (8 (7–8) versus 9 (9–9), p < 0.001), and a later time to initiate crying (78 s (16–156) versus 3 s (0–19), p = 0.009). Otherwise there were no differences in respiratory measurements, including time of max ECO2, ECO2 at 1 h, time or value of maximum VTe, or VTe at 1h.

#### Discussion

This is the first study measuring ECO<sub>2</sub>, VTe, and RR in spontaneously breathing infants from birth through neonatal adaption. Establishment of the expected ranges for ECO<sub>2</sub>, VTe, and RR may facilitate the of use respiratory monitoring during neonatal resuscitation. After birth the infant establishes functional residual capacity and pulmonary blood flow increases, shifting the elimination of CO<sub>2</sub> from the placenta to the lung [14,18]. We could detect ECO<sub>2</sub> after a median of seven breaths, indicating the number of breaths infants need to establish pulmonary gas exchange. We found that ECO<sub>2</sub> increased rapidly to a peak value at approximately two minutes after birth, which is consistent with previous studies [19]. After ECO<sub>2</sub> reaches a peak value, ECO<sub>2</sub> steadily decreases, which likely reflects reducing PCO<sub>2</sub> in arterial blood [11]. From 7 min after birth onwards ECO<sub>2</sub> values were the same as post-transitional values.

 $ECO_2$  has been used to indicate airway obstruction, mask leak, lung aeration, and clinical improvement during neonatal resuscitation [11,12,20,21,27,28]. Monitoring  $ECO_2$  has also been used to screen for over and under ventilation and to improve cerebral blood flow in intubated lambs [29–31]. While  $ECO_2$  monitoring has become increasingly popular in the NICU, it is scarcely used in the delivery room [30,32,33]. We have established expected values for  $ECO_2$  in healthy infants after birth.

 $ECO_2$  can help us understand when the neonatal lung is functioning properly because  $ECO_2$  detection indicates that there is lung aeration, pulmonary blood flow, and pulmonary gas exchange. The maximum  $ECO_2$  measured may reflect umbilical arterial PaCO\_2 levels. In the small group of infants that received respiratory support, maximum  $ECO_2$  was significantly higher than among infants who did not received PPV or CPAP, while other respiratory measurements were not different. During facemask ventilation, clinicians should expect extremely low values until lung aeration and pulmonary gas exchange is established, fol- lowed by an increase in  $ECO_2$  to a maximum level. Decreasing  $ECO_2$  after a maximum value is reached should be reassuring because venti- lation is effective enough to clear carbon dioxide.

We observed a slight increase in VTe within the first two minutes after birth, which then remained steady at a median of ~6 ml/kg; this is similar to published studies [34–36]. We observed a great variability in VTe that is a normal feature of breathing after birth, particularly within the first two minutes where tidal volumes ranged from 0.2 ml/kg to 24.2 ml/kg. The time to maximum VTe was significantly longer than the time to maximum ECO<sub>2</sub> and infants were commonly observed to take large tidal volumes after the maximum VTe had been reached. For example, 10% of infants had VTe > 10 ml/kg at 600 s after birth. The RR increased over the first five minutes to a plateau of 60 breaths per minute.

Comparing infants by mode of delivery, planned caesarean versus vaginal births, we found no differences in ECO2, VTe, or RR despite differences in patient characteristics (Table 1). It is known that infants born by caesarean sections have an increased risk of requiring respiratory support [37]. However, we excluded all infants who received respiratory support. Respiratory transition appears to be similar between modes of delivery for spontaneously breathing infants. The infants born via planned caesarean were born at an earlier gestational age primarily because of our institution's policy of performing repeat, planned Caesareans around 39 weeks gestational age. The median values and interquartile ranges of the infants' umbilical cord blood pH, first temperature, time to first breath, and time to first cry are all within expected ranges for healthy infants at birth and the observed

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differences are likely of no clinical significance. Lower umbilical cord blood pH in infants born vaginally may reflect the stress due to labor. Differences in time to umbilical cord clamping are due to the current standard practice at our institution of delayed cord clamping after routine vaginal births and immediate cord clamping after caesarean births.

# Limitations

This is an observational study and does not provide evidence that monitoring ECO2, VTe, or RR in the delivery room confers a clinical benefit. We obtained measurements by placing a mask over the infant's face, which may have increased vagal activity and decreased respiratory drive. There is also the risk that ECO2 values were higher because of ECO2 retention in the dead space of the facemask. We did not use a closed circuit, the distal end of the flow sensor was open to air and the facemask was placed intermittently, allowing ECO2 to diffuse into the room. At the 1-h timepoint, when ECO2 was stable, we did not observe a rising ECO<sub>2</sub> during collection, which could be as long as 60 s. Once max ECO2 was reached, we saw a consistent decrease in ECO2. We deleted breaths with excess leak, but we were unable to completely eliminate mask leak, which may have affected measurements. Infrequently, we may have missed the first breaths, underestimating the number of breaths until ECO2 was present. However, we initiated monitoring as soon as the head was delivered, prior to birth in nearly half of the infants. Intentionally, we did not attempt to capture all breaths taken during the 1st ten minutes after birth because we wanted to minimize interference with the family's experience of delivering a healthy infant. This decreased the potential volume of data to analyse. We managed to collect over 14,700 spontaneous breaths with accurate data, which we feel is adequate to define normal values of ECO2, VTe, and RR immediately after birth. Finally, our population consisted only of healthy infants  $\geq\!36$  weeks. These results may not apply to preterm infants.

# Conclusions

We demonstrated that exhaled carbon dioxide increases quickly in spontaneously breathing term infants to peak values at 2–3 min after birth followed by a slow but steady descent to post-transitional levels. Exhaled tidal volumes and respiratory rate increased after birth, then plateaued in the minutes after birth. Large tidal volumes were observed throughout the first ten minutes after birth. This study may ultimately contribute to improved interventions in non-breathing infants after birth by providing reference ranges of normal postnatal development of various respiratory parameters.

# Conflicts of interest

None.

#### Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<u>https://doi.org/10.1016/j.resuscitation.2018.07.008</u>.

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Haemodynamic effects of umbilical cord milking in premature sheep during the neonatal transition<sup>6</sup> 11

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# Haemodynamic effects of umbilical cord milking in premature sheep during the neonatal transition

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Received 24 August 2017 Revised 31 October 2017 Accepted 5 November 2017 ABSTRACT

**Objective** Umbilical cord milking (UCM) at birth may benefit preterminfants, but the physiological effects of UCM are unknown. We compared the physiological effects of two UCM strategies with immediate umbilical cord clamping (UCC) and physiological-based cord clamping (PBCC) in preterm lambs.

Methods At 126 days' gestational age, fetal lambs were exteriorised, intubated and instrumented to measure umbilical, pulmonary and cerebral blood flows and arterial pressures. Lambs received either (1) UCM without placental refill (UCMwoPR); (2) UCM with placental refill (UCMwPR): (3) PBCC, whereby ventilation commenced prior to UCC; or (4) immediate UCC. UCM involved eight milks along a 10 cm length of cord, followed by UCC. Results A net volume of blood was transferred into the lamb during UCMwPR (8.8 mL/kg, IQR 8–10, P=0.01) but not during UCMwoPR (0 mL/kg, IQR -2.8 to 1.7) or PBCC (1.1 mL/kg, IQR -1.3 to 4.3). UCM had no effect on pulmonary blood flow, but caused large fluctuations in mean carotid artery pressures (MBP) and blood flows (CABF). In UCMwoPR and UCMwPR lambs, MBP increased by 12%±1% and 8%±1% and CABF increased by 32%±2% and 15%±2%, respectively, with each milk. Cerebral oxygenation decreased the least in PBCC lambs (17%, IQR 13–26) compared with UCMwoPR (26%, IQR 23-25, P=0.03), UCMwPR(35%, IQR 27-44, P=0.02) and immediate UCC (34%, IQR 28-41, P=0.02) lambs. Conclusions UCMwoPR failed to provide placental transfusion, and UCM strategies caused considerable haemodynamic disturbance. UCM does not provide the same physiological benefits of PBCC. Further review of UCM is warranted before adoption into routine clinical practice.

# InTROduCTIOn

Delayed umbilical cord clamping (UCC) improves outcomes for preterm infants. The benefits include fewer blood transfusions, lower rates of intraventricular haemorrhage and necrotising enterocolitis, improved haemodynamics, and improved motor function at 18–22 months of age.<sup>1-4</sup> However, if the newborn remains apnoeic after stimulation, international resuscitation guidelines recommend immediate UCC and moving the infant to a resuscitation platform to provide positive pressure ventilation (PPV).<sup>5-7</sup> As over 60% of infants less than 29 weeks' gestation receive PPV in the delivery room, the opportunity for delayed UCC in these infants is limited.<sup>8.9</sup> It is widely assumed that the main benefit

# What is already known on this topic?

- Umbilical cord milking has been investigated as an alternative method to delayed cord clamping in preterm infants.
- Todate, no studies describe the physiological effects of umbilical cord milking at birth.

# What this study adds?

- Umbilical cord milking in preterm lambs caused considerable haemodynamic disturbances in carotid artery blood flow and systemic blood pressure without increasing pulmonary blood flow.
- Neither umbilical cord milking without placental refill nor delayed cord clamping resulted in a placental transfusion, whereas umbilical cord milking with placental refill did.
- Umbilical cord milking does not provide the same physiological benefits of delayed cord clamping.

of delayed UCC is placental to infant blood transfusion, which has led to the suggestion that umbilical cord milking (UCM) may be an alternative to delayed UCC.

In humans, investigators have milked 20 cm of the umbilical cord (UC) over 2 s, which is repeated two to four times.<sup>10–17</sup> Proponents suggest that the primary advantage of UCM is the rapid transfer of blood from the placenta to the infant, which then does not delay the infant's transfer to a warming bed for respiratory support. However, this assumes that placental transfusion is the primary benefit of delayed UCC. Nevertheless, several trials have now concluded that UCM appears to confer the same benefits of delayed UCC<sup>1</sup> 11 to 112 the 20 and may be more effective in infants delivered by caesarean section.<sup>13</sup>

It is widely recognised that lung aeration is the key to maintaining physiological homeostasis during neonatal resuscitation as it is a requirement for pulmonary gas exchange and stimulates an increase in pulmonary blood flow (PBF).<sup>5–7</sup> <sup>22–27</sup> Recent animal studies have demonstrated that ventilation prior to UCC ('physiological-based cord clamping' or PBCC) has benefits independent of a placental transfusion, including maintaining cardiac output

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and stabilising arterial blood pressures during transition.<sup>28</sup> <sup>29</sup> PBCC involves aerating the lung, stimulating a large increase in PBF, so that pulmonary venous return can immediately replace umbilical venous return as the primary source of left ventricular preload following UCC. PBCC also mitigates the large increase in arterial pressure and cerebral blood flow that results from the increase in afterload associated with UCC. <sup>28</sup> <sup>29</sup> As a result, lambs receiving PBCC have more stable mean systemic blood pressures (MBP), mean carotid artery blood flows (CABF), cerebral oxygenation (SctO<sub>2</sub>%) and heart rate (HR) compared with lambs receiving immediateUCC.

No information is currently available on the physiological effects of UCM and whether it is physiologically similar to delayed UCC. We used preterm lambs to study the physiolog- ical changes induced by two UCM strategies. These were UCM without placental refill (UCMwoPR), which is most similar to UCM performed in human infants,<sup>10–16</sup> and UCM with placental refill (UCMwPR) to ensure placental blood transfusion prior to UCC. To our knowledge, no clinical trial has specifically used a strategy of UCMwPR in humans. These UCM techniques were compared with PBCC and immediate UCC to investigate how each influenced PBF, MBP, CABF, SctO<sub>2</sub>%, HR and UC blood flow. We hypothesised that UCM is not physiologically similar to PBCC and would simulate the haemodynamic instability of immediate UCC.

# MeThOdS

All experimental procedures were performed in accordance with the National Health and Medical Research Council Code of Practice for the Care and Use of Animals for Scientific Purposes.

# Surgical preparation and instrumentation

Pregnant ewes underwent caesarean sections at 126±1 days' gestation (approximately equivalent to 26 weeks' gesta- tional age in humans) under general anaesthesia as previously described.<sup>28</sup> 29 Anaesthesia was induced using an intravenous bolus of 5% sodium thiopentone (pentothal; 1 g in 20 mL) and, following intubation, maintained with inhaled isoflurane (1.5%)-3%) in oxygen/air. Preterm fetal lambs were exposed by hysterotomy and polyvinyl catheters (20 gauge) inserted into the left fetal carotid artery and jugular vein. Flow probes (Tran- sonic Systems, Ithaca, New York, USA) were placed around the right carotid artery, left main pulmonary artery, one umbilical artery and one umbilical vein (sheep have two umbilical veins and arteries). The fetal trachea was intubated with a 4 mm cuffed endotracheal tube and lung liquid was passively drained prior to ventilation. A near-infrared spectroscopy sensor (Casmed FORE-SIGHT, CAS Medical Systems, Branford, Connecticut, USA) was placed over the left frontal cortex to continuously measure cerebral oxygen saturation (Sct0 %).

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# experimental intervention

Twenty-nine preterm lambs were randomised to one of four groups: UCMwoPR (n=6), UCMwPR (n=10), PBCC (n=7) and immediate UCC (n=6). UCM involved milking a 10 cm segment of UC eight times, taking 1–2 s per milk, starting at the placental end and milking towards the fetus. The UC was milked eight times because the UC is shorter in sheep than in humans, allowing only 10 cm of cord to be milked. In humans, 20 cm of the UC is commonly milked, and as we aimed to achieve a similar volume of blood transfer in lambs as in human studies, eight milks were required. In lambs receiving UCMwoPR, the UC was released in between milks so that the UC could poten- tially refill with blood from either the placental or fetal end;

this is the technique commonly reported in humans. In lambs receiving UCMwPR, the UC remained occluded after each milk at the fetal end so that blood refilled the UC only from the placental end. The UC was then occluded on the placental end to ensure the UC retained its volume before the milking procedure was repeated (online <u>supplementary videos 1 and 2</u>). In both UCM groups, the UC was clamped immediately after the final milk, without a final release period, and ventilation commenced 30 s later. In the immediate UCC group, ventilation was initiated 30 s after the UC was clamped. In the PBCC group, ventilation commenced 3 min prior to UCC.

#### Ventilation and monitoring

In all subjects, ventilation began with a sustained inflation (30  $cmH_0 \times 30$  s), followed by volume-guaranteed mechanical ventilation at 7 mL/kg with a positive end-expiratory pressure of 5 cmH 0, rate of 60 inflations per minute, inspiratory time of 0.5 sand fraction of inspired oxygen (FiO<sub>2</sub>) of 0.21 (Dräeger Babylog 8000 Plus ventilator, Dräeger, Lübeck, Germany). The peak inflation pressure was limited to a maximum of 40 cmH Q. Blood gases were recorded immediately prior to commencing the experiment and then at 10 min intervals (ABL30, Radiometer, Copenhagen, Denmark). Ventilator and FiO adjustments were made as needed to maintain arterial pH >7.25, partial pressure of carbon dioxide in arterial blood (PaCO ) 45-55, and peripheral capillary oxygen saturation (SpO<sub>2</sub>) according to published reference ranges for the first 10 min, then 85%-95% thereafter.<sup>30</sup> Vital signs and physiological parameters were monitored and recorded continuously using LabChart (ADInstruments, Bella Vista, New South Wales, Australia).

After UCC, an infusion of Alfaxan (5–15 mg/kg/hour, Jurox, Rutherford, Australia) in 5% dextrose was commenced to maintain sedation for the duration of the experiment. All lambs were ventilated for 60 min. Ewes were euthanised following UCC and lambs were euthanised at the conclusion of the experiment, both with an overdose of sodium pentobarbitone (100 mg/kg intravenously, Jurox).

# **Analysis and statistics**

Baseline and fetal data were analysed using the Kruskal-Wallis test to compare groups. A two-way mixed analysis of variance (ANOVA) with post-hoc analysis using Games-Howell was performed to compare continuous variables between intervention groups. One-way repeated-measures ANOVA with Bonferroni correction for multiple comparisons was used for parametric data, or a Friedman's test with Bonferroni correc- tion for multiple comparisons was used for non-parametric data to analyse the changes in continuous variables over time within each group. Normal data are presented as means and SEM, while nonnormalised data are presented as medians and IQRs.

There are two veins and arteries in the fetal lamb; therefore, only half of the potential umbilical blood flow was measured. The flow of umbilical blood to the lamb was assigned a positive value, whereas blood flow from the lamb to the placenta was given a negative value. We measured blood flow, in mL/min, during the intervention in each vessel in relation to its baseline. The total volume of blood to the fetal lamb was calculated by subtracting the umbilical artery volume from the umbilical venous volume in mL/kg. The umbilical flows and net blood volumes transferred to the lambs were calculated during UCM for the UCM groups and compared with the time between onset of ventilation and UC clamping in the PBCC group (3 min for PBCC). In the PBCC lambs, the average flow for the umbilical vein, artery and the difference (UV-UA) was

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Table 1 Fetal characteristics for each group reported in mean±SD								
	n	% Male	Weight (kg)	hb	SctO <sub>2</sub> %	ph	PaCO <sub>2</sub>	PaO <sub>2</sub>
UCMwoPR	6	50	3.1±0.4	13.5±1.3	45±8	7.28±0.06	52±2	22±6
UCMwPR	10	60	3.3±0.4	13.2±1	48±8	7.29±0.05	55±6	28±5
PBCC	7	43	3.2±0.6	12.2±0.8	49±1	7.23±0.05	56±5	23±6
Immediate UCC	6	50	3.2±0.7	12.2±0.6	50±2	7.29±0.06	51±2	25±9

There were no baseline differences between groups

PaO<sub>2</sub>, arterial oxygen tension; Hb, haemoglobin; PBCC, physiological-based cord clamping; SctO<sub>2</sub>%, cerebral oxygenation; UCC, immediate cord clamping; UCMwPR,

umbilical cord milking with placental refill; UCMwoPR, umbilical cord milking without placental refill.

measured before and after ventilation onset and compared using a Friedman's test.

# PBF, MBP and CABF were measured from heartbeat to heartbeat and compared between groups at the time of cord clamping in immediate UCC, during UCM and immediately before cord clamping in PBCC. HRs and SctO<sub>2</sub>% were measured every second and compared during the first 5 min after UCC.

SPSS V.24 was used for all statistical calculations. Statistical significance was accepted as P<0.05.

# ReSulTS

# **Baseline fetal characteristics**

Fetal characteristics and initial blood gas status were not different between groups (table 1). Basal PBF, MBP, CABF, HR and SctO<sub>2</sub>% were similar in all groups before the experiment. Physiological recordings showing the effects of each UC intervention are shown in figure 1.

# Placental transfusion and umbilical blood volume during milking

Net umbilical blood flow into the lamb (UV-UA) was higher in UCMwPR (8.8 mL/kg, IQR 8–10) than in UCMwoPR (0 mL/ kg, IQR -2.8 to 1.7, P=0.012) and PBCC (1.1 mL/kg, IQR

-1.3 to 4.3, P=0.049) (figure 2A, B) groups. No net placental to lamb blood transfusion was detected in UCMwoPR and PBCC lambs. The median volume of blood transfused to the lamb during each milk was 1.2 mL/kg (IQR 0.7–2.2) for the UCMwoPR group and 0.92 mL/kg (IQR 0.3–1.2) for the UCMwPR group. Following release of the UC between each milk, the volume of blood leaving the lamb (indicated by negative value) was -2 mL/kg (IQR -2.8 to -1.1) in the UCMwoPR group.

Umbilical venous and arterial blood flows were significantly reduced in PBCC lambs on ventilation onset (P<0.005 for both umbilical venous and arterial flow). As the reductions were



Figure 1 Single animal examples of all four experimental groups. (A) Umbilical cord milking without placenta refill resulted in no net placental transfusion and cerebral haemodynamic instability. (B) Umbilical cord milking with placental refill resulted in a significant increase in placental blood flow to the fetal lamb, but also significant cerebral haemodynamic instability. (C) Immediate cord clamping resulted in significant cerebral haemodynamic instability. (C) Immediate cord clamping resulted in significant cerebral haemodynamic instability. (C) Immediate cord clamping resulted in significant cerebral haemodynamic instability. (C) Immediate cord clamping resulted in significant cerebral haemodynamic instability. (D) Physiological-based cord clamping resulted in haemodynamic stability, but no placental transfusion. UV-UA, umbilical venous flow minus umbilical arterial flow to calculate the net umbilical blood flow toward the fetal lamb.

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**Figure 2** Volume of blood transferred to the lamb during umbilical cord milking and physiological-based cord clamping. (A) Volume of placental transfusion in umbilical cord milking without placental refill (UCMwoPR), umbilical cord milking with placental refill (UCMwPR) and physiological-based cord clamping (PBCC). \*Indicates significant difference (P<0.05). (B) Volume and direction of umbilical cord blood volume during UCMwoPR and UCMwPR. (C) Umbilical cord blood flow during the first 3 min of ventilation in PBCC in the umbilical vein and umbilical artery. Umbilical venous and umbilical arterial blood flow were significantly reduced after the initiation of ventilation. (D) Net umbilical blood flow during PBCC (umbilical venous minus umbilical arterial blood flow). Total umbilical blood flow to the fetal lamb did not change during the first 3 min of ventilation.

similar in both vessels, net placental to lamb blood flow did not change during PBCC (P=0.999) (figure 2C, D).

# Physiological parameters PBF, MBP, CABF, SctO<sub>2</sub>% and hR

UCM, either with or without placental refill, was not associated with a change in PBF (UCMwoPR: 2±1 mL/kg/min, P=0.21; UCMwPR: 1±1 mL/kg/min, P=0.82). While immediate UCC also did not increase PBF (3±2 mL/kg/min, P=0.37), PBF was significantly increased prior to UCC in PBCC lambs (26±6 mL/ kg/min, P<0.0005). At UCC PBF was significantly greater in PBCC lambs compared with UCMwoPR (P=0.012), UCMwPR (P=0.019) and immediate UCC (P=0.017) lambs. PBF was similar in UCMwoPR, UCMwPR and immediate UCC lambs (figure 3).

With each milk, MBP increased by  $12\%\pm1\%$  and  $8\%\pm1\%$  in UCMwoPR and UCMwPR lambs, respectively, and decreased by  $10\%\pm1\%$  and  $5\%\pm0.5\%$ , respectively, between milks during UC release. In immediate UCC lambs, MBP increased by  $46\%\pm11\%$  (P<0.0005) over the first 70 heartbeats after UCC, whereas ventilation prior to UCC in PBCC lambs resulted in no change in MBP ( $5\%\pm6\%$ , P=0.46) after UCC. Indeed, MBP increased by  $16\%\pm3\%$  after eight milks in UCMwoPR lambs (P=0.008), by  $30\%\pm3\%$  in UCMwPR lambs (P=0.007) and by  $46\%\pm11\%$  immediate UCC lambs (P<0.0005) over

the first 70 heartbeats after UCM or UC clamping. Compared with PBCC, MBP was increased significantly in response to UCMwoPR (P=0.042), UCMwPR (P=0.021) and immediate UCC (P=0.049). There were no differences in MBP between UCMwoPR, UCMwPR and immediate UCC (figure 4).

With each milk, CABF increased by  $32\% \pm 2\%$  and  $15\% \pm 2\%$  in UCMwoPR and UCMwPR lambs, respectively, and decreased by  $23\% \pm 1\%$  and  $8\% \pm 1\%$ , respectively, between milks during UC release. During UCM, CABF increased by  $40\% \pm 8\%$  (P<0.0005) and  $40\% \pm 2\%$  (P=0.004) in UCMwoPR and UCMwPR, respectively, and by  $71\% \pm 11\%$  (P<0.0005) in response to immediate UCC. In PBCC lambs no significant change in CABF was observed ( $27\% \pm 10\%$ , P=0.1) following UCC (figure 4).

 $SctO_2\%$  decreased significantly after UCC in all groups, although the decrease was least in PBCC lambs (-17%, IQR 13-26) compared with UCMwoPR (-26%, IQR 23-25, P=0.03), UCMwPR (-35%, IQR 27-44, P=0.02) and imme-

diate UCC (-34%, IQR 28-41, P=0.02) lambs (figure 3). HR did not differ between groups in the 5 min after UCC.

# dISCuSSIOn

Although UCM is becoming increasingly popular at the delivery of preterm infants, little is known about the acute physiolog- ical effects of UCM and how it influences the cardiovascular



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**Figure 3** Pulmonary blood flow (PBF) and cerebral oxygenation (SctO<sub>2</sub>%). (A) PBF comparing umbilical cord milking without placenta refill (UCMwoPR) and umbilical cord milking with placental refill (UCMwPR) during umbilical cord milking (UCM). (B) PBF comparing physiologicalcord clamping (PBCC) and immediate cord clamping (ICC) immediately after cord clamping. (C) PBF in PBCC increased after ventilation. Prior to umbilical cord clamping (UCC), PBF increased by 26 mL/kg/min ±6 (P<0.0005). (D) SctO<sub>2</sub>% decreased significantly in all groups after UCC. The decrease was least in PBCC compared with UCMwoPR, UCMwPR and ICC.

transition after birth. We have now quantified the blood transfer achieved by two different UCM strategies and compared the physiological changes associated with UCM with immediate UCC and PBCC strategies. We found that both UCM strategies caused large fluctuations in mean arterial blood pressures and cerebral blood flows that are similar to clamping the UC multiple times. While it is commonly assumed that UCM enhances blood transfer to the fetus, we found that only UCMwPR produced a net blood volume transfer towards the fetus. The measured transfer of approximately 8.8 mL/kg (IQR 8-10 mL/kg) of blood during UCMwPR likely represents half the volume the lamb received, as we only measured blood flow in one umbilical vein and one umbilical artery. The volume transfused via UCMwPR is therefore consistent with the reported blood volume transferred during UCM in humans.<sup>16 31</sup> UCMwoPR caused large fluctuations in blood pressure and cerebral blood flow without any benefit of blood volume transfer. While PBCC greatly mitigated the large oscillations in blood pressure and cerebral blood flow, it did not result in net blood transfer to the lamb. Thus, neither UCM technique was found to be equivalent to PBCC in terms of physiological stability. Both UCM techniques caused major adverse changes in arterial blood pressure and cerebral blood flows and did not stimulate an increase in PBF. As such, neither UCM strategies would be expected to duplicate the benefit of

PBCC and prevent the large decrease in cardiac output that can occur with immediate UCC.

Several trials have examined UCC followed by a single UC milk.<sup>16–18–19–32</sup> We did not include a group representing this strategy because the UC is significantly shorter in sheep than humans, which would prevent a significant transfusion with this method, and the physiological consequences of immediate UCC in lambs are well known.<sup>2829</sup>

UCMwoPR did not achieve a net blood transfer to the lamb because blood rapidly refilled the umbilical vessels from the lamb between milks. It has been shown that multiple UCMs prior to UCC (intact-UCM) provide no benefit compared with one time UCM of a >20 cm length of cord after UCC (cut- UCM).  $\frac{16}{16}$  The most likely explanation for these observations is that during intact UCM, unless blood is only allowed to refill from the placental end, there is only a net transfer of blood to the fetus with the final milk. Nevertheless, the process of milking generated marked haemodynamic fluctuations, with no increase in PBF. We consider this finding to be of concern, as large swings in arterial pressure and cerebral flow caused by UCM are potentially injurious and increase the risk of cerebral vascular injury in preterm infants with an immature cerebral circulation. Similarly, holding the cord above the infant (by 20-30 cm) and milking blood down into the infant must generate pressures in



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Figure 4 Mean blood pressure (MBP) and mean carotid artery blood flow (CABF) measured heartbeat to heartbeat. (A) MBP comparing umbilical cord milking without placenta refill (UCMwoPR) and umbilical cord milking with placental refill (UCMwPR) during umbilical cord milking. (B) MBP comparing physiological-based cord clamping (PBCC) and immediate cord clamping (ICC) immediately after umbilical cord clamping. (C) CABF comparing UCMwoPR and UCMwPR. (D) CABF comparing PBCC and ICC.

the umbilical vein that are in excess of 20–30  $\text{cmH}_2$ O, which must dissipate into the central venous/liver portal systems. This is potentially hazardous for the infant and requires further investigation.

Previous animal studies have shown that PBCC, which involves initiating ventilation prior to UCC, greatly mitigates the decrease in cardiac output and the large swings in arterial blood pressure and cerebral blood flow associated with immediate UCC.<sup>28 29</sup> The same large increases in arterial blood pressure and cerebral blood flow associated with immediate UCC were also observed in UCM (figures 1, 5 and 6), and occurred repeatedly with each milk. As we were able to exactly duplicate these arterial flow and pressure changes by simply occluding and releasing the UC without milking it (data not shown), these changes may simply result from the physiological response to umbilical artery occlusion. While immediate UCC results in a single step-like increase in arterial pressure and flows, UCM results in large oscillating swings in arterial pressures and flows with each milk. As such, depending on the number of milks, UCM has the potential to be considerably more injurious than immediate UCC.

In contrast to UCM and immediate UCC, PBCC produced minimal fluctuations in cerebral blood flow and arterial blood pressure, which is consistent with our previous findings.<sup>28,29</sup> In addition, as ventilation had commenced prior to UCC, PBCC lambs also had much higher PBFs prior to UCC than all other groups. While it is often assumed that UCM increases PBF, our results clearly demonstrate that UCM does not increase PBF. Instead, PBF only increased following ventilation onset, irrespective of the timing of UCC, once again demonstrating the close relationship between lung aeration and the increase in PBF. In UCM, UCC prior to an increase of immediate UCC lambs. This

reduction in cardiac output is caused by a loss of left ventric- ular preload (no umbilical venous return) combined with an increase in afterload caused by UCC and persists until the lungs aerate and PBF increases. In contrast, PBCC lambs had already increased their PBF prior to UCC, maintaining preload through pulmonary venous return.

We were unable to detect any placental transfusion using the PBCC strategy, which is consistent with our previous findings.<sup>33</sup> Recent studies in twins (both monochorionic and dichorionic) have shown that when born vaginally, first-born twins have significantly lower haemoglobin levels (at 2 hours and 48 hours after birth) than their second-born twin.  $\frac{34.35}{No}$  No differences were observed between first-born and second-born twins delivered by caesarean section, providing compelling evidence that placental transfusion may not occur in infants born by caesarean section. As all our lambs were delivered by caesarean section, this likely explains why we were unable to detect placental transfusion during PBCC. Furthermore, our inability to detect placental transfusion, despite a 10-fold increase in PBF in PBCC lambs, demonstrates that an increase in pulmonary blood volume associated with an increase in PBF is not responsible for placental transfusion. This is consistent with the previous finding that the increase in pulmonary blood volume, associated with pulmonary vascular dilation, is small and can only account for a total increase in blood volume of  $\sim 2\%$ .<sup>36</sup> Mathematically, this can be explained by the fact that the volume of a tube increases with the increasing radius squared, whereas flow increases with increasing radius to the fourth power.

The lambs in our study had significant respiratory distress and required aggressive ventilation because they were not exposed to antenatal steroids, did not receive surfactant and did not breathe spontaneously. In newborns with exposure to antenatal steroids

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and who begin to breathe spontaneously before UCC, we would expect a larger increase in PBF and more benefit to the PBCC strategy. However, the most appropriate physiological targets for a PBCC-based strategy are unknown.

Our findings are not congruent with the reported clin- ical studies in that we have failed to demonstrate a significant 'placental transfusion' employing the widely described UCM technique. Repeated clinical studies have inferred a placental transfusion with either delayed UCC or UCM, which is commonly assessed indirectly by measuring haemoglobin levels or weight changes, and attributed improved clinical outcomes to this transfusion<sup>1 10 13 14 37</sup> In our newborn lamb studies, we have repeatedly demonstrated significant haemodynamic fluctu- ations with cord clamping that are negated if the lamb is venti- lated prior to UCC (PBCC). This series of experiments further demonstrates that a 'placental transfusion' from an efficient cord milking technique further compounds the degree of haemodynamic disturbance during transition, suggesting that prior ventilation and not transfusion might be the main advantage of any delav in UCC.

Although this animal model offers considerable advantages in clearly eliciting the physiological changes that are associated with interventions during transition, it has some limitations. All our lambs are delivered by caesarean section in which both the ewe and fetus/lamb are anaesthetised. The majority of prema- ture infants will initiate breathing in the delivery room, which may positively affect PBF and may affect umbilical blood flow. However, our analysis focuses on physiological changes that would occur prior to the initiation of breathing in the majority of preterm infants.<sup>11 13 38</sup>Another limitation of this study is that the fetal lamb undergoes instrumentation prior to the start of the experiment. All groups had similar baseline measurements and normal blood gas values that demonstrated stability. The UC is considerably shorter in sheep than humans and is not as accessible for milking. Lambs have a pair of umbilical veins; thus, we are assuming that blood flows in the measured and unmea- sured vessels are similar. Lastly, these animal experiments are conducted only over the transition period and do not provide any information on longer term outcomes, as seen in clinical trials.

# **COnCluSIOnS**

We have performed the first physiological investigation of UCM, using preterm lambs to test two different UCM techniques. We demonstrated that UCM creates large oscillating swings in arterial pressures and cerebral blood flows with each milk and did not increase PBF. UCMwoPR did not result in a placental transfusion, whereas UCMwPR did. During PBCC, PBF increased prior to UCC and greatly mitigated the large increase in arterial pressures and cerebral blood flows associated with UCC, but did not result in a placental transfusion. Further rigorous evaluation of UCM is required prior to routine clinical use.

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12 The Baby-Directed Umbilical Cord Clamping Feasibility Study (Baby-DUCC)<sup>7</sup>

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# Clinical paper

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Introduction: Over five percent of infants born worldwide will need help breathing after birth. Delayed cord clamping (DCC) has become the standard of care for vigorous infants. DCC in non-vigorous infants is uncommon because of logistical difficulties in providing effective resuscitation during DCC. In Baby-Directed Umbilical Cord Clamping (Baby-DUCC), the umbilical cord remains patent until the infant's lungs are exchanging gases. We conducted a feasibility study of the Baby-DUCC technique. Methods: We obtained antenatal consent from pregnant women to enroll infants born at $\geq$ 3 2 weeks. Vigorous infants received $\geq$ 2 min of DCC. If the infant received respiratory support, the umbilical cord was clamped $\geq$ 6 0 s after the colorimetric carbon dioxide detector turned yellow. Maternal uterotonic medication was ad- ministered after umbilical cord clamping. A paediatrician and researcher entered the sterile field to provide respiratory support during a cesarean birth. Maternal and infant outcomes in the delivery room and prior to hospital discharge were analysed. <i>Results:</i> Forty-four infants were enrolled, 23 delivered via cesarean section (8 unplanned) and 15 delivered vaginally (6 via instrumentation). Twelve infants were non-vigorous. ECG was the preferred method for re- cording HR. Two infants had a HR < 100 BPM. All HR values were > 100 BPM by 80 s after birth. Median time to umbilical cord clamping was 150 and 138 s in vigorous and non-vigorous infants, respectively. Median ma- ternal blood loss was 300 ml. <i>Conclusions:</i> It is feasible to provide resuscitation to term and near-term infants during DCC, after both vaginal and cesarean births, clamping the umbilical cord only when the infant is physiologically ready.

# Introduction

Over five percent of infants born worldwide will need help breathing after birth [1,2]. Failure to establish breathing after birth claims the lives of more than 800,000 infants every year [1,3-6]. Re- cently, delayed cord clamping (DCC) has been incorporated into the care of vigorous newborns. DCC is believed to provide a time-based, increase in blood flow from the placenta to the newborn, resulting in a transfusion [7-9]. To date, most clinical studies of DCC have only

included infants who were all vigorous and spontaneously breathing [10–13]. Currently, consensus guidelines for neonatal resuscitation state that if an infant is not vigorous or breathing after birth, the um-bilical cord should be cut so that the infant can be moved to a re- suscitation platform where cardiorespiratory support can be provided [14-16]. Ideally, effective ventilation should be established within 1 min after birth, but effective ventilation often takes longer to achieve, during which time oxygenation is inadequate and cardiac output is compromised [17,18].

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Animal studies suggest that delayed cord clamping improves hemodynamic stability [19–22]. The crucial factor determining benefit is establishing lung aeration and increasing pulmonary blood flow prior to umbilical cord clamping. Before the establishment of pulmonary gas exchange and pulmonary venous return to the heart, the infant is dependent on the placental circulation to provide cardiac preload, oxygenation, and elimination of carbon dioxide. Establishing pulmonary venous return and pulmonary gas exchange prior to umbilical cord clamping takes advantage of the placental circulation to maintain neonatal homeostasis until the infant is ready [19–22]. Early umbilical cord clamping in the non-vigorous infant may further exacerbate hypoxia and inadequate perfusion.

The purpose of this study was to determine the feasibility of the Baby-Directed Umbilical Cord Clamping intervention (Baby-DUCC) in infants born  $\geq 32$  weeks' gestation. In the Baby-DUCC intervention, the umbilical cord is clamped after pulmonary gas exchange is established, and may be applied to infants who require respiratory support after birth. Specifically, we wanted to evaluate adherence to and safety of the study protocol, develop methods for monitoring the infant prior to umbilical cord clamping, and collect physiologic data during the Baby-DUCC intervention whether the infant did or did not receive resuscitation after birth.

#### Methods

This was a single centre, non-randomized, feasibility cohort study in infants born at a gestational age  $\geq 3.2$  weeks at the Royal Women's Hospital in Melbourne, Australia, a perinatal referral centre with > 7500 births annually. The study was approved by The Royal Women's Hospital Human Ethics Research Committee and registered with the Australian New Zealand Clinical Trials Registry (ACTRN12617000610336).

# Baby-directed umbilical cord clamping intervention

The goal of the Baby-DUCC intervention is to ensure that neonatal adaption to ex-utero life begins prior to umbilical cord clamping. We devised strategies to provide respiratory support in accordance with Australian Neonatal Resuscitation Guidelines during a 2–5 min period of delayed cord clamping [23]. We supported the newborn in the "maternal space" which was dictated by the length of the umbilical cord, anticipated to be 30-70 cm [24]. A colorimetric exhaled carbon dioxide ( $CO_2$ ) detector (PediCap® Nellcor, USA or Neostat® Mercury Medical, USA, based on availability) or end tidal  $CO_2$  monitor (NM3 Respiratory Profile Monitor Phillips Healthcare, USA) was placed be- tween the T-Piece and facemask prior to birth and used as a physiologic target [25]. Exhaled carbon dioxide was used to determine that pul- monary gas exchange had begun. If the infant received respiratory support, the umbilical cord was clamped  $\geq 60$  s after the colorimetric detector turned yellow (or the end tidal CO2 was  $\geq 15$  mmHg) or

≥ 2 min after birth, whichever occurred later. Indications for umbilical cord clamping and moving the infant to the resuscitation platform included: if the infant was still receiving respiratory support at 5 min after birth, if the maternal or neonatal care team believed that the Baby- DUCC intervention was interfering with the care of the infant or the mother, and if intubation or chest compressions were indicated. If the infant was vigorous and did not require respiratory support after birth, the umbilical cord was clamped ≥ 2 min after birth. Maternal utero- tonic medications to prevent post-partum haemorrhage were adminis- tered after the umbilical cord was clamped because ovine studies sug- gest uterine contractions reduced umbilical cord venous blood flow [26].

# Eligibility

Infants were eligible if they were  $\geq 32$  weeks' gestation at birth.

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Verbal permission was obtained from the maternal care team (midwife, obstetrician, and anaesthesiologist where relevant) prior to approaching families for written informed consent. The maternal care team was asked to approve delaying maternal administration of a uterotonic agent until the umbilical cord was clamped. Pregnant women of monochorionic twins, multiples of > 2, fetuses with known congenital anomalies compromising cardiorespiratory transition after birth (including congenital diaphragmatic hernia, hydrops fetalis, cya- notic congenital heart defects, and airway anomalies that may com- promise the ability to provide face mask PPV), and with a diagnosis of HIV were not approached. Written, informed, antenatal consent was obtained prior to birth. A convenience sample of 44 infants was re- cruited.

#### Monitoring and respiratory support in the maternal space

To deliver respiratory support in the maternal space, we used a portable resuscitator (Giraffe Stand Alone Resuscitation System, GE Healthcare, USA) to provide PPV, continuous positive airway pressure (CPAP), blended oxygen, and suction. Heart rate (HR) was monitored (Intellivue X<sub>2</sub>, Phillips Healthcare, USA) via electrocardiographic electrodes (ECG), peripheral oxygen saturation monitor (SpO<sub>2</sub> NM3 Respiratory Profile Monitor Phillips Healthcare, USA), or portable ultrasound machine (GE Healthcare Venue 50, USA). The monitor was attached to the portable resuscitator and visible to the neonatal clinical care team. We used a GoPro Hero3 (GoPro, USA) focused on the monitor to record heart rate data and timing of events, including time of birth, first cry, and cord clamping. Our goal was to obtain an accu- rate HR by 1 min after birth.

# Cesarean birth

Asepsis was maintained by sterilising T-pieces, facemasks, ECG electrodes, and pulse oximetry (PO) sensors via either ethylene oxide sterilisation (Steritech Pty Ltd, Australia) or autoclave. Items that could not be sterilised, like reusable ECG and PO cables, ultrasound probes, and a firm cot mattress were placed in sterile sheathing. A paediatrician and a researcher wore sterile gowns and gloves and entered the sterile field prior to the birth, standing next to the surgeon, similar to the strategy reported by Winter et al. [22] Once the infant was born, the infant was placed on the mattress and dried with sterile towels. The researcher assessed the HR using either ECG electrodes applied the chest, PO positioned on the right hand or wrist, or ultrasound to visualize the HR, while the paediatrician assessed the condition of the newborn. A midwife stood next to the portable resuscitator to change the pressures or oxygen, if requested (Supplemental video 1).

# Vaginal birth

Following a vaginal birth, the infant was placed on the mother's chest or abdomen and assessed by the attending paediatrician or midwife. ECG electrodes were applied to the chest or back, depending on the infant's position. If the infant required respiratory support, the infant was moved from the mother's chest to a firm mattress placed at the end of the mother's bed and the paediatrician managed the airway at the infant's head. If the mother was placed in stirrups for the birth, the infant was placed in an open cot positioned between the mother's legs. If the infant needed respiratory support, the delivering obstetrician or midwife was asked to move out of the maternal space to allow space for the paediatrician with the mother's bed height adjusted to ensure the umbilical cord was not under tension.

# Data collected and statistical analysis

Our primary objective was to determine the feasibility of monitoring the newborn and potentially providing support prior to

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# Table 1

Baseline characteristics, infant and maternal outcomes. Emergency births defined as instrumental vaginal births (forceps or vacuum) or unplanned cesarean sections. Completed study protocol was defined as umbilical cord clamping  $\geq 2$  min after birth for vigorous infants or  $\geq 60$  s after end-tidal CO2 detector was  $\geq 15$  mmHg (yellow colour change if using disposable, colourimetric CO<sub>2</sub> detectors) in infants receiving positive pressure ventilation and maternal uterotonic medication administered after end-point reached. mmHg = millimetres of mercury, BPM = beats per minute, C° = degrees Celsius, ml = millilitres. Post-partum haemorrhage defined as  $\geq 500$  ml maternal blood loss.

	All subjects (N = 44)	Vigorous (N = 32)	Non-vigorous (N= 12)	p-value
Baseline Characteristics				
Gestational age at birth (weeks <sup>days</sup> )	39 <sup>1/7</sup> (38 <sup>5/7</sup> -40 <sup>1/7</sup> )	391/7 (384/7-405/7)	39 <sup>5/7</sup> (39 <sup>0/7</sup> -40 <sup>0/7</sup> )	0.71
Emergency births	14/44, 32%	7/32, 22%	7/12, 58%	0.02
Male sex	21/44, 48%	14/32, 44%	7/12, 58%	0.39
Time to umbilical cord clamping, seconds	140 (122-260)	150 (124-270)	162 (158-174)	0.37
Completed study protocol	42/44, 95%	31/32, 97%	11/12, 92%	0.52
Neonatal outcomes				
Weight, grams	3307 (2973-3710)	3307 (2913-3619)	3650 (3064-4028)	0.36
Apgar at 1 min	8 (8-8.25)	8 (8-9)	8 (5-8)	0.1
Apgar at 5 min	9 (9-9)	9 (9-9)	9 (9-9)	0.44
pH, umbilical venous blood	7.26 (7.21-7.30)	7.29 (7.22-7.33)	7.25 (7.18-7.26)	0.18
PCO2 mmHg, umbilical venous blood	46 (42-53)	44 (40-52)	48 (47-53)	0.12
PO2 mmHg, umbilical venous blood	25 (20-31)	23 (19-25)	26 (25-32)	0.23
pH, umbilical arterial blood	7.20 (7.15-7.26)	7.24 (7.18-7.27)	7.14 (7.08-7.15)	0.03
PCO2, mmHg, umbilical arterial blood	52 (45-57)	45 (43-55)	63 (59-66)	0.05
PO2, mmHg, umbilical arterial blood	29 (24-43)	25 (22-37)	47 (38-50)	0.15
Antenatal HR, BPM	140 (135-150)	141 (138-150)	138 (107-150)	0.4
Time to first cry, seconds	12 (1-43)	1 (0-14)	57 (50-105)	< 0.001
First temperature, C°	36.7 (36.5-36.9)	36.7 (36.6-37)	36.6 (36.4-36.8)	0.28
Admitted to the nursery	7/44, 16%	4/32, 13%	3/12, 25%	0.32
Maternal outcomes				
Maternal blood loss, ml	300 (300-500)	325 (300-563)	300 (288-448)	0.44
Post-partum haemorrhage	12/44, 27%	9/32, 28%	3/12, 25%	0.88

Bold values indicate a significant difference (p < 0.05) between vigorous and non-vigorous infants.

umbilical cord clamping, until the physiology end-point was reached (Baby-DUCC). We collected and analysed demographic characteristics and short-term outcomes, including infant HR, method and success of determining the HR, time of umbilical cord clamping, time of uterotonic medication administration, maternal blood loss, rates of postpartum haemorrhage (PPH), infant's first recorded temperature after birth, and infant and maternal complications (infections, ICU admission, blood transfusions, etc). Medians (IQR) are depicted for nonnormally distributed variables. A Friedman's test with Bonferroni correction for multiple comparisons was used for non-parametric data to analyse the changes in continuous variables over time. Measurements were analysed using IBM SPSS Statistics 24 (SPSS, Inc, Chicago, IL), and statistical significance was considered as p < 0.05.

# Results

Patient characteristics, infant and maternal outcomes are described in Table 1. Completion of the Baby-DUCC protocol was 95% (42/44), with the two protocol violations occurring after cesarean births. The first violation was cord clamping at 90 s after an unplanned cesarean because of concern for excessive maternal bleeding. The infant was vigorous at birth and the maternal blood loss was 750 ml. The second protocol violation was also early cord clamping at 85 s because of complete placental delivery after a planned cesarean birth. Twelve out of 44 enrolled infants (27%) received resuscitation during DCC. Eight infants received vigorous stimulation to initiate spontaneous breathing. One infant appeared dusky after a routine vaginal birth and received CPAP with supplemental oxygen for hypoxia starting at 4 min, using SpO<sub>2</sub> monitoring to guide therapy. Three infants received PPV for ap- noea prior to umbilical cord clamping. There was minimal time be- tween deciding to provide ventilation and applying a face mask. The first infant to receive PPV was born via elective caesarean section under spinal anaesthesia. Ventilation was started at 60 s, and  $ECO_2 > 15$  mmHg by 80 s (see Supplementary video). The second in- fant was born via emergency caesarean under general anaesthesia for

fetal bradycardia. PPV was initiated at 50 s with ECO<sub>2</sub> detected at 70 s via pedicap colour change. The third infant had fetal compromise and was born vaginally with instrumentation and the mother was in stirrups. By 41 s, the infant was placed on an open cot in the maternal space and the pediatrician was evaluating the infant. Ventilation was initiated at 50 s with ECO<sub>2</sub> detected by 65 s.

Overall, 15 infants (34%) were delivered by planned cesarean section, 8 (18%) via unplanned cesarean section, 15 (34%) via unassisted vaginal birth, and 6 (14%) via instrumental vaginal birth. A higher proportion of non-vigorous infants were delivered under emergent conditions, had a longer time to initiate crying after birth, and had a lower pH in umbilical artery cord blood compared to the vigorous infants.

We had more success obtaining a continuous HR by 60 s using ECG electrodes than by using PO alone or PO in combination with ultrasound. We attempted ECG recording in 33 infants and found that ECG accurately provided data by 40, 50, and 60 s in 60%, 67%, and 91% of these infants, respectively. We attempted to use ultrasound only or in combination with PO in 7 infants. Ultrasound allowed HR early assessment in 86% of these newborns, but did not provide a continuous numerical value. Use of ultrasound was challenging as it involved bringing the machine into the maternal space in addition to the portable resuscitator, required that the probe be continually on the infant's chest, and necessitated clinician estimation of the HR, in real time, by looking at the ultrasound screen. Seven infants (16%) were admitted to the neonatal unit; 4 for prematurity, one for a cyanotic episode on day 2 after birth, one for suspected sepsis, and one for bilious emesis. No infant was admitted for respiratory distress.

The HR in the Baby-DUCC cohort remained consistent after birth (p = 0.423). Fig. 1 shows infant HR in Baby-DUCC compared with established normative values measured with PO in infants receiving immediate cord clamping [28]. We observed a HR < 100 in only 2 infants. One infant with HR < 100 was non-vigorous and responded to vigorous stimulation. All infants had a HR > 100 by 80 s after birth.

Twenty-seven percent of the mothers had blood loss  $\geq$  500 mL. Only

# Douglas Blank, MD: PhD Thesis Submission, December 2018 PhD title: Umbilical cord management during neonatal resuscitation



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Maternal Blood Loss with Baby-DUCC (Oxytocin Delay), N=44



Fig. 2. Scatter plot of maternal blood loss, each dot represents a delivering mother.

1 mother had > 1000 ml blood loss, a vaginal birth complicated by a perineal tear. Fig. 2 shows peripartum maternal blood loss, with each data point representing the 44 delivering mothers. No mother was admitted to the ICU or received a blood transfusion. These rates of PPH are consistent with our institution's historical averages.

# Discussion

This is the first study using a physiologic target to direct umbilical cord clamping regardless of the need for neonatal resuscitation, and the first study to delay uterotonic medications until after delayed clamping of the umbilical cord. This study adds to the growing literature reporting the logistics and potential advantages of providing respiratory support prior to umbilical cord clamping [27,29–31]. We also describe novel methods for safely monitoring and providing respiratory support after a cesarean birth without disrupting the aseptic field.

Human and animal studies of immediate cord clamping (ICC) demonstrate that the key to regaining physiologic stability in the compromised newborn is to establish lung aeration and increase pulmonary blood flow [16–18.32–34]. Hemodynamic instability after birth may cause significant morbidity (i.e. intraventricular hemorrhage, hypoxic ischemic encephalopathy), increased rates of invasive procedures in the delivery room, and mortality [35–41]. Establishing pulmonary venous return and pulmonary gas exchange prior to umbilical cord clamping takes advantage of the placental circulation to maintain neonatal homeostasis until adaption after birth has begun, potentially decreasing neonatal instability and reducing the need for invasive procedures in the delivery room. The most appropriate physiologic targets for a Baby-DUCC based strategy are unknown. Animal and human studies suggest that an increase in ECO<sub>2</sub> is a good candidate for indicating successful neonatal adaption after birth. ECO<sub>2</sub> predicts the return of an adequate heart rate in infants with bradycardia, accurately correlates with lung aeration, and can be used to time readiness for umbilical cord clamping [17,18,33].

We endeavored to minimize and simplify the logistical component of the Baby-DUCC intervention because we envision the application of this technique to be most valuable after an unplanned birth, when a quick set up is mandatory. We also thought it important that the equipment needed for Baby-DUCC to be inexpensive and readily available in most hospitals. We did not provide an external source of heat because the infants were more mature and the continuity of placental circulation may provide adequate heat during the minutes of DCC. Future studies with larger cohorts are needed to determine the risk of hypothermia during DCC in term and late preterm infants. We were limited to one method for HR monitoring after a cesarean birth

because the pediatrician and the researcher who scrubbed in had to

carry the sterile items into the surgical field and hold the items prior to birth. We did not prepare a table to place sterile items for supporting the infant, which may be an alternative approach that offers multiple monitoring options (i.e. pulse oximetry and ECG) and potential intubation in the maternal space. ECG was the preferred method of early HR monitoring during this study and has demonstrated advantages over pulse oximetry, palpation, and ausculation [42–44]. Techniques for using ultrasound to detect HR and using ultrasound in the sterile field after a cesarean birth have been reported [45,46]. However, the ECG monitor was small enough to be attached to the portable resuscitator, the HR value could be verified by looking at the QRS complex, and videotaping the ECG tracing provided a continuously recorded HR. Ethylene oxide sterilisation of ECG and PO sensors had no effect on adhesive or sensory performance and the devices functioned as expected.

We observed a HR < 100 in only two infants and only within 80 s of birth. One infant was delivered via planned cesarean for breech presentation and required a degree of physical manipulation for birth. The second infant was delivered via unplanned cesarean for non-reassuring fetal cardiotocographic tracing. Both infants received stimulation to initiate breathing but did not cry until more than 40 s after birth. It is possible that the umbilical cord blood flow was compromised during both births. Our hypothesis is that apnoeic, non-vigorous infants with intact placental circulation after birth maintain the fetal homeostasis of oxygenation and cardiac preload. Therefore, we theorise that bradycardia would be observed less often in the non-vigorous infant with delayed cord clamping than immediate cord clamping. Despite significant advances in the development and implementation of the Helping Babies Breathe program, morbidity and mortality from birth asphyxia in resource limited settings remains far greater than resource rich settings. [4,6]. Placental support prior to umbilical cord clamping is theoretically available after the vast majority of births and may be beneficial where the burden of birth asphyxia is highest. Fourteen umbilical cord venous blood gases and 12 umbilical cord arterial gases were available (9 paired samples). Studies have previously reported higher PaO2 after DCC versus immediate cord clamping [47,48]. The median value of the arterial PaO2 was higher than the venous PaO2. Although not statistically significant, the increase in arterial PaO2 may indicate that the neonatal lungs contributed oxygen to the placental circulation prior to umbilical cord clamping.

This was a small feasibility study that does not provide evidence of clinical benefit from the Baby-DUCC intervention. No infants with a diagnosis of hypoxic ischemic encephalopathy and no infants compromised enough to be emergently intubated or receive chest compressions. The difference in early HR between the Baby-DUCC cohort and the historical cohort with immediate cord clamping may be due to a difference in monitoring technique (ECG versus PO), as there is evidence that HR recorded by PO shows lower values for the first minute after birth [28,49]. Blood flow through the umbilical cord during Baby-DUCC was not directly monitored. A published study suggests that umbilical cord pulsations are not an accurate method to monitor umbilical cord blood flow and flow is likely affected by spontaneous breathing [50]. We often needed to reposition infants because the umbilical cord was either stretched or kinked, which may compromise placental-infant circulation and should be assessed in future delayed cord clamping studies. Uterotonic medications were given after the umbilical cord was clamped. The exact time to administer uterotonic medications for the management of the third stage of labour is not established [51,52]. However, it is possible that the delay of uterotonic medications to the mother places her at a higher risk for PPH and the theoretical benefit of delaying the medication to avoid disruption of umbilical blood flow to the infant is not worth the added risk to the mother. It is somewhat reassuring we have not detected a rate of PPH that differs from our institutional experience, but this study is not powered to address this question.

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# Conclusions

It is feasible to provide resuscitation and monitor term and nearterm infants during delayed cord clamping, using physiologic targets to indicate when the infant is ready for umbilical cord clamping. Baby-Directed Umbilical Cord Clamping takes advantage of the low resistance placental circulation to continue to provide cardiac preload and gas exchange until the infant's lungs are exchanging gases and providing pulmonary venous return. Appropriately designed and pow- ered randomized controlled trials to determine the efficacy of this ap- proach are needed.

# Conflicts of interest

The authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and there are no other persons who satisfy the criteria for authorship but are not listed. All authors agree to the order of au- thors listed. We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

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# Ethics

The protocol was performed in accordance with guidelines established by the National Health and Medical Research Council of Australia. The study was approved by The Royal Women's Hospital Human Ethics Research Committee and registered with the Australian New Zealand Clinical Trials Registry (ACTRN12617000610336).

# Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<u>https://doi.org/10.1016/j.resuscitation.2018.07.020</u>.

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# 13 Discussion and Future Directions

# Summary of the problem

Birth is a dangerous event, likely the most dangerous moments of anyone's life. Up to 10% of infants born worldwide will require assistance breathing in the first minutes after birth and failure to breathe after birth claims the lives of over 800,000 infants annually.<sup>8-14</sup> Over 95% of these infants are born in poor countries, most are born at term and would otherwise be completely healthy. (3-Minute Thesis Presentation by Doug Blank, Monash University, 2016, <u>https://www.youtube.com/watch?reload=9&v=V4NbMJrbiA0</u>). Recently, there has been a resurgence in interest of delayed cord clamping (DCC), during which clamping of the umbilical cord is delayed until pre-specified time. Studies show that preterm infants receiving DCC have less transfusions, lower rates of intraventricular haemorrhage, and lower rates of necrotizing enterocolitis, while term infants have higher iron stores and likely lower mortality. <sup>15,16</sup> In these studies, all infants receiving DCC were breathing spontaneously.

The ideal conditions to clamp the umbilical cord in infants who need help initiating breathing after birth is unknown. For months, the placenta supports the fetal circulation and provides oxygen and nutrients ideal for growth and development. If infants do not breathe at birth, current guidelines recommend immediate cord clamping (ICC) so the infant can be moved to a resuscitation platform for the provision of respiratory support.<sup>8,12,14</sup> The hemodynamic instability and poorer condition at birth is a root cause of intraventricular haemorrhage, hypoxic ischemic encephalopathy, and mortality.<sup>18-24</sup> It is widely recognized that the key to regaining physiologic stability during neonatal resuscitation is to achieve lung aeration and pulmonary blood flow.<sup>8,12,14,25-30</sup> Therefore, achieving lung aeration, pulmonary gas exchange, and pulmonary venous return to the heart prior to umbilical cord clamping would likely retain the physiologic stability present prior to birth. With very few exceptions, continuation of placenta circulation immediately after birth is a free resource available to every infant worldwide. It is logical to assume that the placental circulation is available to support the newborn for few minutes after birth, supplying oxygen and refilling the heart, until the newborn is ready to support herself.

# Major findings

-Chapter 7: Lung ultrasound can distinguish pulmonary pathologies, including atelectasis and pneumothorax, immediately after birth.

-Chapters 8 and 9: Neonatal adaption after birth can be monitored using lung ultrasound to describe lung aeration and lung liquid clearance.

-Chapter 10: Exhaled carbon dioxide monitoring detects changes in lung aeration, pulmonary blood flow, and provides a direct measurement of gas exchange as newborns initiate breathing after birth.

-Chapter 11: Umbilical cord milking and immediate cord clamping in preterm lambs causes dangerous fluctuations in cerebral blood flow and blood pressure. Ventilation prior to umbilical cord clamping, physiological based cord clamping (PBCC) or baby-directed umbilical cord clamping (Baby-DUCC), preserves cerebral oxygenation and minimises fluctuations in cerebral blood flow or blood pressure. Umbilical cord milking can provide an increase in blood volume into the lamb if using the placental refill technique. PBCC did not result in an increase in blood volume.

-Chapter 12: Resuscitation of term and late preterm infants using a Baby-DUCC approach is feasible and safe. Baby-DUCC may offer physiologic advantages over ICC because continuity of placental circulation ensures cardiac preload and pulmonary gas exchange until lung aeration, pulmonary gas exchange, and pulmonary venous return to the left ventricle is established.

The findings of this thesis have sparked two distinct directions of research:

We have launched a randomised controlled trial to test Baby-DUCC versus ICC in term and late preterm infants. I hypothesise that Baby-DUCC will reduce bradycardia in newborns with inadequate respiratory effort in the first minutes after birth, which may reduce the risk of mortality and significant morbidity in infants following asphyxia. If a physiological advantage of Baby-DUCC is demonstrated, a second randomised controlled trial will be conducted to determine whether this technique reduces the risk of hypoxic ischemic encephalopathy and/or death, specifically in resource limited settings.

An observational study has commenced to test if lung ultrasound immediately after birth can independently predict the level of support that extremely preterm infants will receive after birth. If LUS accurately predicts which infants will require administration of surfactant and which infants will be successful on CPAP, earlier, life-saving treatments can be administered, and invasive, dangerous procedures can be avoided.

# Future direction of study for lung ultrasound

In **Chapters 7, 8, and 9**, I explored the use of lung ultrasound (LUS) to describe neonatal adaption after birth and to describe pulmonary pathology. In **Chapter 7**, LUS accurately detected pneumothorax, verified by post-mortem examination in premature, ventilated, newborn lambs without any false positive diagnoses.<sup>2</sup> In this study, I described a novel, gold-standard technique to diagnosis air leak syndromes on post-mortem examination. In **Chapter 8** and **Chapter 9**, LUS was used to describe respiratory changes with the initiation of breathing.<sup>3,4</sup> LUS images prior to breathing were reported for the first time, definitively describing the appearance of liquid filled airways. As a result, the LUS grading scheme was modified. The study adds to the understanding of how to interpret LUS images. Establishment of the pleural line, indicating lung aeration, and substantial clearance of liquid is seen during the first few breaths after birth and complete liquid clearance was typically achieved within the first 4 hours after birth.

Rates of mortality and morbidity are extremely high in our tiniest, most premature patients. We know that over half of the infants born ≤28 weeks will receive mechanical ventilation as a life-saving therapy. Non-invasive ventilation has replaced mechanical ventilation and surfactant delivery as the primary respiratory strategy for extremely premature infants, reducing death and bronchopulmonary dysplasia.<sup>38</sup> However, over 50% of these infants started on non-invasive ventilation will later require mechanical ventilation.<sup>23,38,39</sup> Furthermore, delaying surfactant delivery in extremely premature infants with severe respiratory distress syndrome increases the risk of death and significant pulmonary injury.<sup>40</sup> LUS has been reported to predict which preterm babies (median 32 weeks gestational age) on CPAP at 2 hours after birth will fail non-invasive ventilation and receive surfactant.<sup>41</sup> In **Chapters 8 and 9**, I showed that healthy infants >35 weeks gestational age all had LUS images indicating successful lung aeration and liquid clearance by 10 minutes after birth.<sup>3,4</sup> If LUS changes occur in the delivery room in infants >35 weeks and LUS can accurately predict non-invasive ventilation failure in preterm babies on CPAP at 2 hours after birth, then LUS in the delivery room may predict the need for ventilation and surfactant administration in babies ≤28 weeks.

With a continued collaboration between the Ritchie Centre and the Royal Women's Hospital, we have started the DOLFIN Jr study (ACTRN 12617001256369) with funding from GE and the Emergency Medicine Foundation (https://gex.brightidea.com/ct/h.bix?c=296E6C9F-01AC-4F68-BA11-7E16F472BE03). In this study, I will test the hypothesis that LUS obtained in the delivery room in 50 extremely preterm infants, in the first 20 minutes after birth, can predict the level of respiratory support these infants will receive in the first 72 hours after birth. In the extremely premature infant, time is critical, making LUS the ideal diagnostic tool as it is safe, simple, quick, and can be used immediately after birth. If the hypothesis is correct, LUS will become a valuable diagnostic tool in the delivery room and subsequent studies can test if clinical decision making using LUS reduces the risk of significant morbidities in extremely preterm infants.

# Future direction for Baby-Directed Umbilical Cord Clamping (Baby-DUCC)

The work presented in this thesis was intended to develop an understanding of the physiologic changes that occur in the newborn during delivery and to find a suitable physiologic target that indicated the

newborn is ready to be separated from the placenta and the mother. In the Baby-DUCC feasibility study (Chapter 12), I prove that term and near-term newborns can be safely and effectively resuscitated prior to umbilical cord clamping and provide data that Baby-DUCC may confer advantages over immediate cord clamping. Several studies performed at the Ritchie Centre, including **Chapter 11**, show that the umbilical cord should not be clamped at a specific time after delivery. The umbilical cord should be clamped when the infant is ready. As indicated in **Chapter 10**, rising exhaled carbon dioxide (ECO<sub>2</sub>) levels can indicate infant readiness for umbilical cord clamping. ECO<sub>2</sub> is simple to monitor after birth and is the correct marker for Baby-Directed Umbilical Cord Clamping.

The primary goal of the first breaths after birth is to clear liquid from the distal gas exchange regions of the lung to allow the entry of air. Pulmonary gas exchange can only commence once these regions have aerated. Therefore, there is no ECO<sub>2</sub> when these regions are liquid filled, but as the infant gradually aerates its lungs, the exchange of oxygen for carbon dioxide in the lungs can commence and carbon dioxide first appears in the exhaled gas.<sup>27</sup> Rising exhaled carbon dioxide levels indicate airway patency, lung aeration, pulmonary gas exchange, and pulmonary venous return to the heart.<sup>25,30</sup> In other words, exhaled carbon dioxide starts at zero after birth and rises once the lungs aerate and commence gas exchange. If the newborn still has access to the placental circulation prior to the onset of pulmonary gas exchange, I hypothesise that the newborn will maintain physiologic stability in the form of a steady, normal heart rate, higher oxygen saturation, and higher cerebral oxygen delivery as is apparent in newborn lambs.<sup>6,32,36,37</sup>

The feasibility and safety of Baby-DUCC was demonstrated in **Chapter 12**. The heart rate values of the enrolled infants provide confidence that Baby-DUCC may indeed offer haemodynamic stability in comparison to immediate cord clamping. In a collaboration between Monash Medical Centre and the Royal Women's Hospital, we have initiated a randomised controlled trial to test Baby-DUCC versus ICC (ACTRN 12618000621213). We hypothesise that establishing effective ventilation, either via PPV or effective spontaneous breathing, prior to umbilical cord clamping decreases the incidence of bradycardia in infants born at ≥32 weeks gestational age compared with management. We have chosen to perform a randomised controlled trial in 120 infants who need help breathing at birth to test if Baby-DUCC provides a physiologic advantage. The results of this trial will help us design future RCTs with clinically important outcomes.

We believe that compromised infants who have achieved effective gas exchange prior to umbilical cord clamping will have improved haemodynamic stability during resuscitation because the placental circulation will continue to provide cardiac preload and gas exchange until pulmonary gas exchange is established. Improved haemodynamic stability in the first minutes of life may decrease the need for intensive resuscitation interventions after birth (e.g. PPV, emergent intubation, and chest compressions) and decrease the risk of significant morbidities, including hypoxic ischaemic encephalopathy and death.<sup>15,17,31</sup>

To reiterate, 5 to 10% of infants born worldwide will require assistance breathing in the first minutes after birth and failure to breathe after birth claims the lives of over 800,000 infants annually.<sup>8-14</sup> Over 95% of these infants are born in poor countries, with most of them born at term and would otherwise be completely healthy. Baby-DUCC is a resource currently available for free to virtually all infants born worldwide, if the placental-fetal circulation is adequate. In academic centres, the interest in resuscitation and stabilisation during delayed cord clamping after birth is mostly in premature infants and term infants with known cardio-pulmonary pathologies, like congenital diaphragmatic hernia. There is typically sufficient time to plan for a predicted high-risk delivery. However, up to 1/1000 infants in Australia suffer from significant hypoxic ischaemic encephalopathy, with significant risks for morbidity and mortality. Often these births occur unexpectedly and with similar incidence inside and outside academic centres.<sup>21</sup>

Understanding the typical course of placental circulation in the minutes after birth is crucial to ensuring placental circulation can be adequate. For example, there is a paucity of studies describing umbilical blood flow in newborns during delayed cord clamping and reporting umbilical blood gas values during delayed cord clamping.<sup>42,43</sup> How does birth change placental circulation from the in-utero homeostasis that promoted healthy fetal growth? How do surgical delivery and uterotonic medications affect placental circulation? Are there antenatal conditions that predict compromise of the placental circulation after birth?

Umbilical cord management relies on understanding the dynamic changes occurring after birth and with active management of the third stage of labour.

The heart rate and SpO<sub>2</sub> of vigorous infants  $\geq$ 32 weeks receiving delayed cord clamping may be different to established accepted normal values.<sup>27,7,28</sup> We anticipate that only 10-20% of the infants in this study will receive resuscitation. We intend to initiate HR and SpO2 monitoring immediately after birth, while the clinical team determines if the infant needs resuscitation. If the infants are vigorous after delivery, they will not be randomised and will receive at least two minutes delayed cord clamping. We will collect heart rate and SpO<sub>2</sub> values in the first 10 minutes after delivery in vigorous infants who receive at least two minutes of delayed cord clamping after birth. We will analyse the observational data of the vigorous infants separately as an observational study, anticipating data in over 500 infants.

The ultimate benefit of PBCC and the goal of the Baby-DUCC studies is to reduce mortality and morbidity in infants worldwide. If there is a physiological advantage to Baby-DUCC, the next study will investigate whether infants who are supported with Baby-DUCC have less hypoxic ischemic encephalopathy and death than infants supported with ICC.

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