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Physiological measurements of the effect of cord clamping strategies

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PHYSIOLOGICAL MEASUREMENTS OF THE EFFECT OF CORD CLAMPING STRATEGIES



Emma Brouwer

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PHYSIOLOGICAL MEASUREMENTS OF THE EFFECT OF CORD CLAMPING STRATEGIES

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PART ONE

Preface and General introduction



P

PREFACE

PREFACE

Wednesday, 5:30pm. You are a neonatal resident and your evening shift has just started. During the handover, your colleague informed you about a woman, 26 weeks pregnant, who had been admitted to the obstetrical ward earlier in the day because of premature contractions with 6 centimetres cervix dilation. She is currently receiving tocolytic medication to suppress premature labour, and corticosteroids have been given to accelerate fetal lung maturation.

It's 10:15pm; the obstetric resident calls. Despite the tocolytic medication the woman has been receiving, the premature contractions have persisted and premature delivery is now imminent. While you are on your way to the obstetrical ward you call the attending neonatologist and nurse to inform them about the delivery. They will join you at the obstetrical ward as soon as they can.

SCENARIO 1

It's 10:18pm. You arrive at the room adjacent to the delivery room and start preparing the resuscitation table by switching on the heater and setting up the ventilation device according to local protocol. Both the neonatologist and nurse arrive, and together you finish preparing the resuscitation table, all equipment, and the medication as well as switching on and setting up a respiratory function monitor (RFM). This monitor allows for continuous measurements of ventilatory settings and neonatal parameters to help guide any decisions about interventions during resuscitation. All data gathered during resuscitation, such as ventilator settings, neonatal heart rate and oxygen saturation measurements, are automatically stored and can be used for auditing or for research purposes. Once everything is prepared, you step into the delivery room to ensure the baby can be transferred to the resuscitation table as soon as possible.

A baby boy is born at 10:38pm. The baby is reactive but has a blueish colour; he briefly makes some crying sounds but stops after a few seconds. The obstetrician clamps and cuts the umbilical cord immediately, as instructed, and hands the baby over to you. To keep the baby warm, he is placed in a plastic wrap after which he is transferred to the resuscitation table in the adjacent room. The father of the baby follows into the adjacent room, leaving the obstetrician and his wife in the delivery room.

The baby is placed on the resuscitation table at 10:39pm. The nurse has already started both the Apgar clock and the RFM recording and a pulse-oximeter sensor is placed around the right hand to measure heart rate and oxygen saturation. Respiratory support is started by placing a mask over the baby's mouth and nose.

There are no visible chest wall rises, which would indicate spontaneous breathing, and ventilation with positive airway pressure is therefore initiated. After 1 minute the RFM displays that both heart rate and oxygen saturation are too low and it shows no spontaneous breathing during the evaluation. Ventilation is continued while both heart rate and oxygen saturation increase. After administering ventilation for several minutes, during which time the administered oxygen is set to 100% (air contains 21% oxygen), the baby starts breathing spontaneously. The baby continues breathing and is further stabilised using continuous positive airway pressure (CPAP) to make spontaneous breathing easier, while the administered oxygen can gradually be decreased to 30%. **It's now 10:48pm.**

The nurse prepares the incubator for transport, while you explain to the father what has happened over the past couple of minutes. Afterwards you ensure that the obstetrician informs the mother about the status of the baby, as she has not seen or held him yet. Fifteen minutes after the birth, the baby is transferred to the incubator. Before transporting him to the neonatal intensive care unit (NICU) the incubator is briefly placed in the delivery room next to the mother, so she can see her baby boy. The nurse opens one of the apertures so the mother can touch her baby for a short moment.

SCENARIO 2

10:18pm: you've arrived at the obstetrics ward and transfer a mobile resuscitation table with an adjustable platform to the delivery room, next to the mother in labour, and start preparations. While setting up the equipment, both the neonatologist and nurse arrive, and together you finish preparing the mobile resuscitation table, the respiratory function monitor (RFM), and all the equipment and medication. The briefing of the obstetric and neonatal team takes place, tasks are allocated, and use of the table is clarified. After the procedure has been discussed, you walk into the adjacent room and wait for the delivery to progress further.

At 10:30pm the obstetrician asks you to step back into the delivery room as the baby is about to be born. A baby boy is born at 10:38pm. The baby is reactive and has a blueish colour; he briefly makes some crying sounds and continues breathing. The obstetrician closes a plastic wrap around the baby while the adjustable platform of the mobile resuscitation table and the over-head heater are placed above the pelvis of the mother. The baby is placed on the adjustable platform while he remains attached to the umbilical cord.

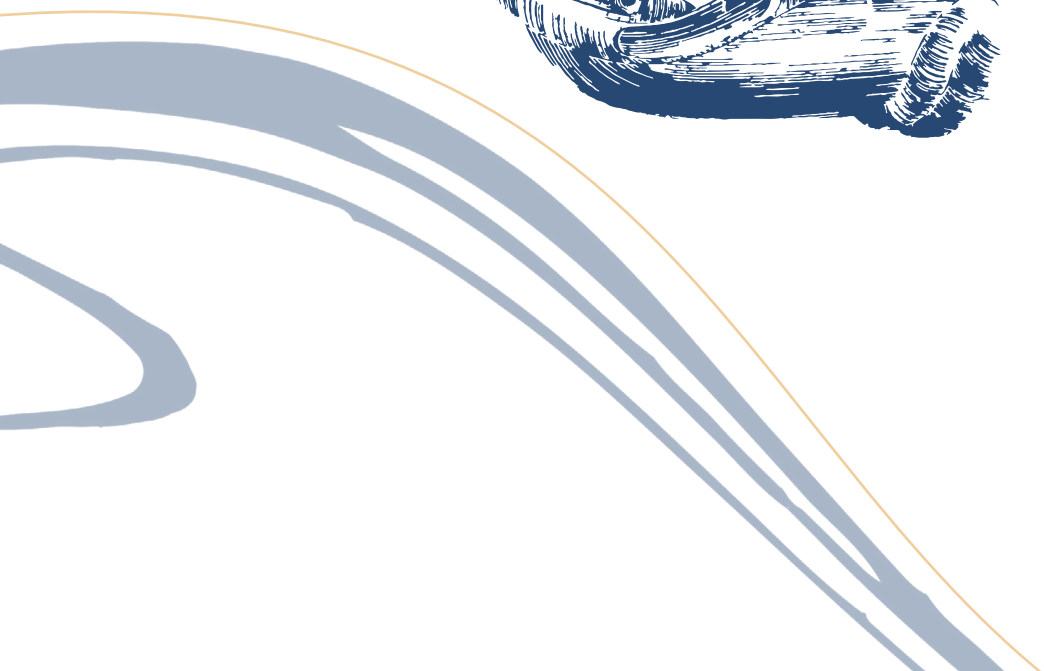
The nurse has already started both the Apgar clock and the RFM recording, and a pulse-oximeter sensor is placed around the right hand. A mask is placed over the baby's nose and mouth and breathing efforts are visible on the RFM. The baby is

only supported with some positive airway pressure to support the breathing, and no ventilation is necessary. The first pulse-oximeter measurements are displayed after 1 minute, heart rate is good and stable, and oxygen saturation is acceptable.

Breathing support is continued while the heart rate remains stable and oxygen saturation increases. The mother is invited to touch her baby and gently rub the sole of his foot, which will stimulate him to breathe with even more effort. The baby's breathing is regular and stable, and the oxygen saturation gradually increases while the administered oxygen can be decreased back to 30%. This means that the baby is stable and breathing, and the neonatologist gives the obstetrician a sign that the umbilical cord can be clamped. **It is now 10:44pm.**

The nurse has been continuously updating the parents on everything that has happened during the resuscitation. After the obstetrician has clamped the umbilical cord, the father is allowed to cut the cord. The baby remains on the mobile resuscitation table, close to the mother, while the nurse prepares the transport incubator. Fifteen minutes after he was born, the baby is transferred to the incubator and transported to the NICU.

These two scenarios, whilst they could realistically occur at our clinical wards, are fictive. Two different cord clamping strategies are presented, including the logistics and clinical implications. In this thesis, we have focusses on both these cord clamping strategies, to obtain more knowledge on the underlying physiology, and to compare both strategies.





GENERAL INTRODUCTION

GENERAL INTRODUCTION

UMBILICAL CORD CLAMPING: PAST, PRESENT, FUTURE

Clamping and cutting of the umbilical cord after birth is a normal and necessary procedure during the third stage of labour, yet the exact timing has been debated for millennia, at least back to the time of Aristotle in 350BC.⁽¹⁾ However, the timing and method of umbilical cord clamping and cutting has changed over time: ranging from how to tie the umbilical cord in the Trotula (a medieval compendium of women's medicine) to only cutting the umbilical cord after the placenta had been delivered in primitive cultures.^(2, 3) It is still unclear when the timing of umbilical cord clamping shifted from after placental delivery to before it, although the first description of this change dates from the 17th century.⁽³⁾

Of note: umbilical cord clamping and cutting are used interchangeably in the literature, but as cord clamping is an internationally accepted term to describe both simultaneously this will henceforth be the term used in this thesis, indicating clamping and cutting.

Another important change in the timing of cord clamping occurred after the 'active management of the third stage of labour' was implemented into clinical practice in the 1960s.⁽⁴⁾ Active management of the third stage of labour was aimed at reducing post-partum haemorrhage (PPH), although there was no scientific evidence suggesting delaying cord clamping was related to excessive maternal blood loss. Nevertheless, early cord clamping was included in the active management trias together with the use of prophylactic uterotonics upon delivery of the anterior shoulder and controlled cord traction to deliver the placenta. A later review of the data showed the positive effect of implementing this new strategy was solely due to the use of uterotonics and not related to early cord clamping.⁽⁵⁾

Postponing (delaying) the moment of cord clamping has been the subject of numerous research projects over the last decades, demonstrating that both term and preterm infants could benefit from this.^(6, 7) The beneficial effects of delayed cord clamping (DCC) led to changes in both obstetrical and neonatal resuscitation guidelines⁽⁸⁻¹⁰⁾ that currently recommend delaying umbilical cord clamping for at least 1 minute, but only when the infant is uncompromised.^(9, 10) However, infants who were compromised after birth, i.e. who were in need of respiratory support, were excluded from most trials on DCC. Consequently, due to absence of evidence, when infants are compromised guidelines either suggest immediately clamping the cord, to establish effective ventilation, or no recommendations are given.^(9, 10)

Most preterm infants are in need of respiratory support at birth and immediate clamping is still recommended. Prematurity markedly increases the risk of neonatal morbidities and remains the main cause of neonatal mortality. These morbidities entail, but are not limited to, respiratory distress syndrome, intraventricular haemorrhage (IVH), necrotizing enterocolitis (NEC), sepsis, and retinopathy of prematurity, influencing both short- and long-term outcomes. As the risks of morbidity and mortality are directly related with gestational age (GA) at birth, infants born <28 weeks GA (extremely preterm) are at the highest risk for adverse outcomes.⁽¹¹⁾ Additionally, as they often fail to achieve lung aeration and sufficient gas exchange due to the immaturity of their respiratory system, these infants require respiratory support. Currently, the cord needs to be clamped immediately or within 30-60 seconds to provide effective respiratory support and monitor the clinical condition of the infants, denying these infants the benefits of DCC.

However, new resuscitation tables have been developed that enable effective neonatal resuscitation at the maternal bedside, while the umbilical cord remains intact. Implementing this new approach in clinical practice will allow preterm infants to receive DCC and, thereby, also receive the accompanying benefits. Additionally, as both maternal and neonatal care can be assured, the moment of umbilical cord clamping no longer has to remain time-based. Instead, cord clamping can be delayed until after the infant has been stabilised, as is done during physiological based cord clamping (PBCC; detailed explanation below).

THE BENEFITS OF DELAYED CORD CLAMPING

The beneficial effects seen after DCC have been the basis for current guideline recommendations. Studies have shown that DCC for term-born infants can lead to an increase in haemoglobin in the first hours after birth, a decrease in the risk of developing iron-deficiency in the first months of life, and improvements in neurodevelopmental outcomes.^(6, 12) For preterm-born infants, DCC is associated with a reduction in the risk of hospital mortality and the need for blood transfusions, and with improved blood pressures.^(7, 13) These beneficial effects as a result of performing DCC have all been attributed to placental transfusion.

Placental transfusion

Placental transfusion, or the net shift in blood volume from placenta to neonate during DCC, is a widely accepted phenomenon and has been the subject of several studies. To demonstrate placental transfusion, either neonatal blood volume was calculated using ¹²⁵I-tagged human serum albumin, remaining placental blood volumes were measured, or neonatal weight was used.⁽¹⁴⁻¹⁶⁾ These studies demonstrated that placental transfusion occurred at an average rate of 2-3ml/kg/

min, with the majority of blood being transfused in the first minute and transfusion likely to be completed within 3 minutes of birth. Based on these results, guidelines recommend DCC for 1-3 minutes if no resuscitation is needed; in other words, DCC has become time-based.⁽⁹⁾

Although placental transfusion is an accepted effect of DCC, the physiology causing the net shift in blood volume remains unclear. Early studies suggested both gravity and uterine contractions as the driving factors for placental transfusion. The gravity theory was later refuted by showing a similar placental transfusion for infants that were held higher than the introitus compared to infants held at the level of the introitus, using gain in weight as a measure of placental transfusion.⁽¹⁷⁾ Additionally, the hypothesis on uterine contractions was deemed unlikely based on a study showing that uterine contractions cause reductions in uterine or fetal placental blood flow rather than increasing it.⁽¹⁸⁻²⁰⁾

Uterine contractions, however, might still affect placental transfusion in a different way. A new hypothesis suggests that uterine contractions during labour might increase pressure within the fetus, leading to a shift in blood volume from the neonate into the placenta. This creates an accumulation of blood within the placenta, whereas after birth, this accumulated blood volume shifts back into the infant to rebalance the blood volume when the pressures are relaxed. This increase in blood flow towards the infant after birth is known as placental transfusion.

It is also possible that spontaneous breathing provides the driving force for placental transfusion by generating a sub-atmospheric (negative) intrathoracic pressure during inspiration. It is proposed that this negative intrathoracic pressure creates a greater influx of umbilical venous blood flow, while restricting arterial outflow, resulting in a net increase in blood volume.⁽¹⁹⁾ This theory could explain why experimental studies have consistently failed to simulate placental transfusion, as those studies have employed positive pressure ventilation strategies to aerate the lungs, which generates positive intrathoracic pressures.

PHYSIOLOGICAL-BASED CORD CLAMPING

To understand the association between the timing of cord clamping and transitional changes at birth, we first need to explain the physiology of the fetal-to-neonatal transition.

Prior to birth

Before birth, the placenta is the primary site of both nutrient and respiratory gas exchange for the fetus. Deoxygenated blood leaves the fetus through the umbilical arteries to the placenta. After passing through the placenta blood is oxygenated and flows back to the fetus through the umbilical vein and ductus venosus towards the right atrium. Due to the funnel-shaped form of the ductus venosus and the Eustachian valve/ridge in the right atrium, the majority of oxygenated blood flows via the open foramen ovale to the left atrium and left ventricle to guarantee oxygenation of the myocardium and brain.⁽²¹⁾ Approximately 30-50% of left ventricular preload is provided by the placenta, i.e. cardiac output is dependent on placental venous return.^(22, 23) Most of the deoxygenated blood of the fetus coming from the inferior and superior vena cava is directed via the right ventricle towards the common pulmonary trunk. As the pulmonary vascular resistance (PVR) is high, most of the blood is then directed to the systemic circulation (aorta) via the ductus arteriosus (R to L shunt)(figure 1).⁽²²⁾ As the placenta has a very low vascular resistance, most of this blood then flows to the placenta where oxygenation will take place.

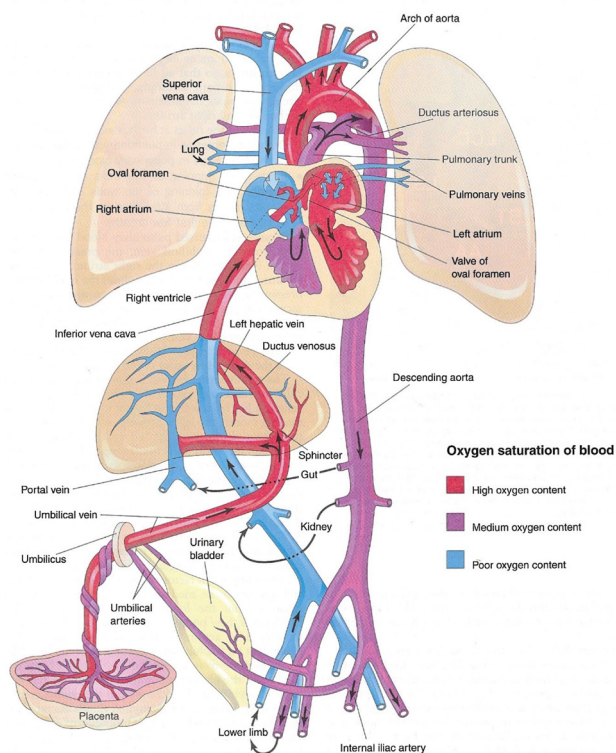


Figure 1 | Fetal circulation (prior to birth)

"This figure was published in *The Developing Human - Clinically Oriented Embryology*, 8th edition, KL Moore & TVN Persaud, Page 328-329, Copyright 2008 by Saunders, an imprint of Elsevier Inc."

After birth

According to international guidelines, the umbilical cord is clamped 1-3 minutes after birth in healthy term-born infants.⁽⁹⁾ As the infant starts breathing or crying spontaneously, lung liquid is cleared across the distal airway walls due to the pressures gradients generated by breathing. As a result, the lungs aerate, which decreases PVR and increases pulmonary blood flow (PBF). Once PBF has increased, cardiac output is no longer dependent on placental venous return for preload, which is now provided by pulmonary venous return. When the umbilical cord is clamped after the lungs are aerated, cardiac output can therefore remain stable as preload is now provided by pulmonary return, while the lungs also function as the primary source of gas exchange (figure 2). However, when the umbilical cord is clamped prior to lung aeration, which is often the case in preterm infants, cardiac output is compromised as PBF remains low and placental venous return is absent, thus compromising preload to the left ventricle.

The cardiovascular instability resulting from cord clamping prior to lung aeration entails great fluctuations in cardiac output, blood pressures, and blood flow.⁽²³⁾ Consequently, these fluctuations may increase the risks for adverse outcomes in preterm infants such as IVH, NEC, and death. These detrimental outcomes could potentially be avoided if cord clamping is delayed until after lung aeration has been established.

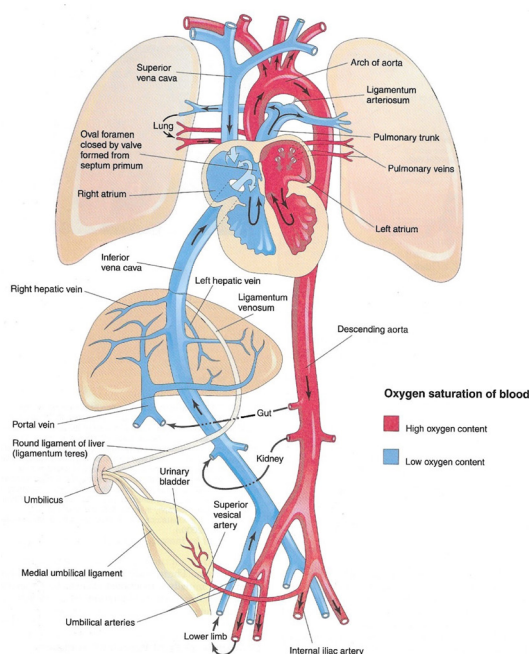


Figure 2 | Neonatal circulation (after birth)

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Physiological-based cord clamping

Physiological-based cord clamping (PBCC) is defined as delaying umbilical cord clamping until lung aeration has commenced. Instead of delaying cord clamping based on a specific amount of time, as with DCC, the umbilical cord is now clamped based on the infant's clinical and physiological status. The cardiopulmonary stability (described above) that results from PBCC has been demonstrated in experimental studies, but while the benefits are clear in theory^(20, 23) and look promising for improving neonatal outcomes, neither the benefits nor physiology have yet been demonstrated in a clinical setting.

As performing PBCC is not feasible using the standard resuscitation tables, a new resuscitation table was constructed: the Concord birth trolley. This mobile resuscitation table is designed to provide the highest standard of care for preterm infants while the umbilical cord remains intact (figure 3). All equipment needed for stabilisation and/or resuscitation is built in to ensure that even infants in need of immediate resuscitation are able to receive the potential beneficial effects of PBCC. In addition to the potential cardiovascular benefits, the PBCC approach allows for both parents to remain close to their baby in the first minutes of life.



Figure 3 | Physiological-based cord clamping approach using the Concord resuscitation table

Image by Sophie Cramer

As mentioned before, the PBCC approach has so far only been investigated in experimental settings and clinical data is lacking. When translating this to the clinical setting it is important to confirm that similar beneficial physiological effects can be observed before performing large trials investigating the important clinical outcomes. The Concord resuscitation table allows us to investigate PBCC in a clinical setting, while closely monitoring neonatal parameters such as heart rate, oxygen saturation, and need for additional oxygen. Monitoring these parameters is pivotal to investigating the physiological changes during PBCC and to confirm the cardiovascular results seen in experimental studies.

AIM AND OUTLINE OF THIS THESIS

The general aim of this thesis is to investigate the physiological changes that occur with physiological and time based cord clamping strategies, to obtain a better understanding of the optimal cord clamping moment and factors of influence. More specifically, this thesis will focus on the effect of spontaneous breathing on placental transfusion (part two) as well as the effects of PBCC on preterm neonates during and directly after resuscitation (part three). This thesis comprises experimental and observational studies and a randomised controlled trial.

In part one of this thesis a **general introduction** of umbilical cord clamping strategies are presented over time as well as the physiological and (beneficial) clinical effects that accompany these strategies.

Part two of this thesis focuses on placental transfusion and includes studies assessing the mechanisms that are responsible for this net blood volume shift as well as the effects of spontaneous breathing. While various theories have been put forward to explain the phenomena of placental transfusion, most of these theories have been refuted. However, there seems merit to the theory of spontaneous breathing as the driving force underpinning placental transfusion. To further investigate this, we collaborated with Professor Hooper's research group and investigated the effects of spontaneous breathing on umbilical venous blood flow in preterm born lambs, which is described in **chapter 1**. In this study blood flow measurements, intrapleural pressure and body weight were continuously measured to obtain a better understanding of the effect of spontaneous breathing on umbilical blood flow and placental transfusion. In addition, to investigate the effect of breathing during DCC an observational study was performed in healthy term born infants, described in **chapter 2**. Echocardiographic ultrasound measurements of the inferior vena cava (IVC), hepatic vein (HV) and ductus venosus (DV) were obtained while simultaneously visualizing diaphragm movement in order to correlate flow patterns to the related respiratory phase. Both HV and DV flow were used as a derivative of umbilical blood flow and therefore placental

transfusion. In **chapter 3** an observational study is described in which both preductal and umbilical pulse oximetry measurements were obtained in infants who were stabilised after birth. Cardiac generated pressure pulses continue to reach the cord even after cord clamping and umbilical pulsatility can be palpated. Less motion artefacts and less vasoconstriction potentially make the umbilical cord a favourable location to quickly obtain heart rate (HR) measurements.

Part three of this thesis focusses on the implementation of PBCC in a clinical setting and the physiological changes during fetal-to-neonatal transition that occur with PBCC. In **chapter 4** an observational study is described in which the feasibility and safety of the PBCC approach are assessed when using the Concord resuscitation trolley. Both neonatal and obstetric teams were extensively trained and both briefing and debriefings were completed in order to improve this approach. During neonatal stabilisation vital parameters such as HR and oxygen saturation (SpO_2) were continuously monitored which enabled us to observe physiological changes in neonatal transition during PBCC and potentially improve hemodynamic stability. **Chapter 5** describes a non-inferiority randomised controlled trial on the effectiveness of the PBCC approach. The aim of this approach is to establish lung aeration prior to cord clamping and to provide full standard of care in neonatal stabilisation. This study compares the time needed to complete stabilisation to determine whether the PBCC approach was non-inferior to the time-based cord clamping (TBCC) approach. While transition is currently observed and measured during neonatal stabilisation, the infant's transitional status after birth could potentially be used as a predictor for (adverse) neonatal outcome. In **chapter 6** an observational echocardiographic ultrasound study is described focused on measuring the infant's transitional status after birth. DA flow measurements were obtained at 1 hour after birth in infants who received PBCC or TBCC and correlated with oxygenation parameters, which can currently be used to assess transitional status. Additionally, as infants received either PBCC or TBCC, measurements could be compared to observe if the increased hemodynamic stability of PBCC would still be present.

In part four, we provide an overall discussion on the main findings of the studies performed in this thesis, in regard to the current literature (**General discussion**). Future perspectives are contemplated with suggestions for further research. This thesis concludes with a summary of the discussed studies, which is provided in both English and Dutch.

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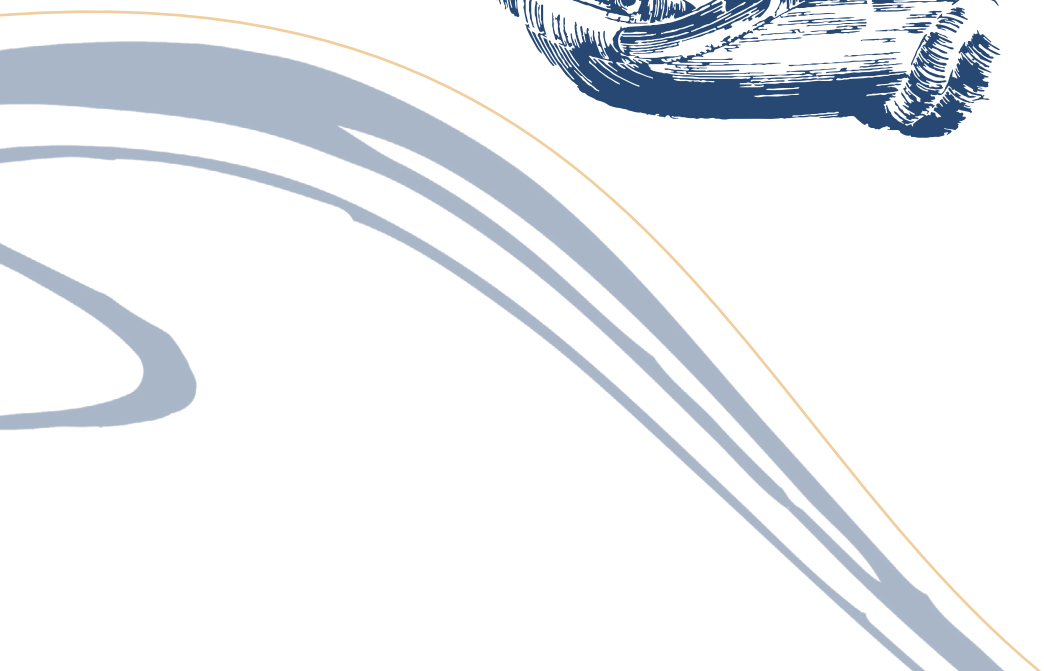
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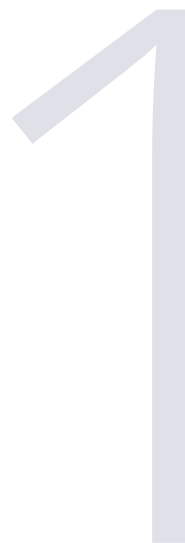
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PART TWO

Placental transfusion and the effect of
spontaneous breathing





CHAPTER 1

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

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AB te Pas
GR Polglase
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KJ Crossley
K Rodgers
D Blank
S Yamaoka
AW Gill
M Kluckow
SB Hooper

Arch Dis Child Fetal Neonatal Ed 2020;105:F26-F32

ABSTRACT

INTRODUCTION

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

OBJECTIVE

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

METHODS

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

RESULTS

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

CONCLUSION

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

INTRODUCTION AND RATIONALE

Placental to infant blood transfusion during delayed umbilical cord clamping (DCC) after birth⁽¹⁾ is evidenced by an increase in birth weight and a decrease in placental blood volume⁽²⁻⁴⁾ and is thought to occur at 2-3 mL/kg/min over the first 3 min after delivery.⁽⁵⁾ Animal studies have also shown that DCC until after ventilation onset prevents the reduction in cardiac output caused by immediate cord clamping (ICC).⁽⁶⁾ This finding has led to the concept that cord clamping should be based on the infant's physiology (i.e. whether it is breathing) rather than on 'time after birth', which can be unrelated to the infant's physiological state.⁽⁷⁻¹⁰⁾ This concept is termed 'physiological based cord clamping (PBCC)' and highlights the importance of pulmonary ventilation in mediating the benefits of DCC.⁽¹¹⁻¹³⁾

In term newborns, DCC increases haemoglobin and ferritin levels, reduces rates of iron deficiency and anaemia in the first year of life.⁽¹⁴⁾ In preterm infants, small randomised trials have shown that DCC is associated with less intraventricular haemorrhage, necrotizing enterocolitis, sepsis and need for blood transfusions, which are common complications associated with preterm birth.⁽¹⁵⁻¹⁷⁾ As a result, the WHO now recommends DCC for >60 s in infants not requiring resuscitation.

While placental transfusion is an accepted feature of DCC, little is known about the physiology causing the net movement of blood from the placenta into the infant during DCC. Most commentaries suggest that placental transfusion results from gravity (i.e. when the infant is placed below the mother) or from uterine contractions squeezing blood out of the placenta into the infant.^(1, 18) However, recent clinical trials in humans and animal experiments have failed to demonstrate an effect of gravity or uterine contractions on placental transfusion.^(4, 19) In a unique observational study in normal term infants, Doppler ultrasound was used to investigate blood flow patterns in both the umbilical artery and vein during DCC.⁽²⁰⁾ This study showed that umbilical venous (UV) flow was heavily influenced by breathing and crying. During inspiration, umbilical venous flow appeared to increase, whereas between breaths it appeared to cease. Similarly, umbilical artery flow was influenced by breathing and crying and at times was bidirectional, which was thought to result from uterine contractions.⁽²⁰⁾

Animal experiments investigating the mechanisms of placental transfusion have consistently failed to detect a shift in blood volume from the placenta into the newborn.^(9, 19) Possible explanations for this relate to the animal models used, which have mostly involved lambs delivered by caesarean section, followed by mechanical ventilation.^(9, 19) In this situation, intermittent increases in intrathoracic pressure combined with end-expiratory pressures may restrict net placenta-to-infant blood transfusion. In contrast, all human studies showing placental

transfusion have been in vaginally delivered spontaneously breathing infants.⁽²¹⁾ In this study, we hypothesised that the sub atmospheric (negative) intrathoracic pressure created during inspiration would enhance UV flow into the newborn, leading to a net increase in weight.

METHODS

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

Fetal surgery

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

Experimental protocol

Prior to delivery, electronic recordings of blood flows in the left pulmonary artery, umbilical vein and right carotid artery as well as pressures in the carotid artery and IP space commenced using a data acquisition system (Powerlab ADI, Sydney, Australia). As general anaesthesia is known to inhibit spontaneous breathing activity, lambs were delivered under spinal anaesthesia at 133-134 days of

gestations. Ewes were sedated with 10 mL propofol 1% (10 mg/mL) to administer spinal anaesthesia (lignocain 2%, 0.1 mL/kg) via the sacral lumbar space. Following successful induction of spinal anaesthesia, mild sedation of the ewe continued using midazolam (1.0 mg/kg/hour at 20-25 mL/hour). The fetal head and neck were exteriorised via caesarean section and the lambs were intubated using an endotracheal tube (4.5 mm cuffed endotracheal tube). Lambs were intubated as the application of facemask without leak is problematic and, in addition intubation ensured opening of the larynx. A transcutaneous oxygen saturation probe (Masimo, Radical 7, California, USA) was placed around the right foreleg, a near infrared spectroscopy (NIRS) optode (Casmed Foresight, CAS Medical Systems, Branford, Connecticut, USA) was placed over the left frontal cortex, and a rectal temperature probe was inserted and used to correct blood gas measurements. The lamb was dried and placed on electronic scales (Bilanciai D70, Campogalliano, Italy) immediately beside the ewe, taking care to avoid stretching or mechanically interfering with the umbilical cord; the lamb's weight was recorded continuously. Lambs were given naloxone (400 µg intravenous) and anexate (0.04 mg bolus intravenous) to counter the inhibitory effects of fentanyl (postoperative analgesia of the ewe) and midazolam (maternal sedation), respectively, on respiration. They were also given caffeine (20 mg/kg loading dose and 10 mg/kg/hour) and doxapram (5 mg/kg loading dose and 2.5 mg/kg/hour) to stimulate breathing.

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

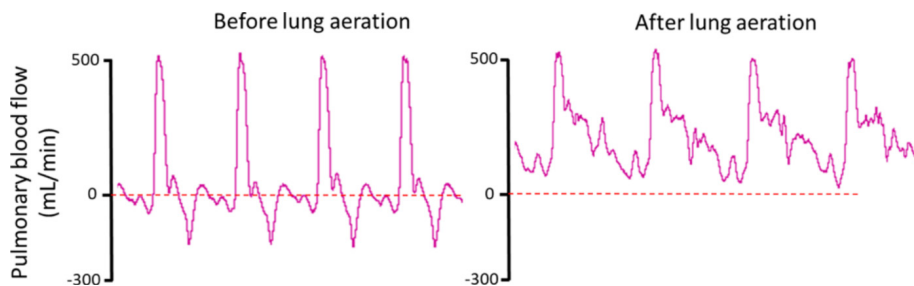


Figure 1 | Pulmonary blood flow (PBF) wave form before and after lung aeration

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

The ewe was euthanised following cord clamping, using an overdose of pentobarbitone (100 mg/kg intravenous). Lambs were euthanised after completion of the ventilation period using an overdose of pentobarbitone administered intravenously. Lamb body and organ weights were recorded post mortem.

Analytical methods

Recordings of physiological measurements made prior to, during, and after delivery were analysed using LabChart 8 (ADInstruments). A breath-by-breath analysis, which involved analysing every individual spontaneous breath in all animals, was performed to observe the effect of breathing on weight and UV flow. All breathing events were annotated manually by the researcher (EB) to determine change in lamb weight, IP pressure and UV blood flow with each spontaneous breath. To make IP pressure comparable across animals, measurements were standardised per individual (mean was subtracted and values were divided by the SD). The basal IP pressure is a relative measure that varies between animals, whereas the change in IP pressure from baseline is a precise measurement. Standardised values per animal allow interpretation of the data in terms of quantiles, for example, a value of 2 corresponds to the 97.5% quantile of IP measurements within this animal. Analysis of carotid arterial (CA) pressure, CA blood flow and PBF, involved averaging values over five consecutive heartbeats for each time point.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics V.24.0. Data on CA pressure, CA blood flow, PBF, UV flow and temperature are presented as median and IQR. Paired t-test was used to compare weight measurements at delivery with weight measurements prior to cord clamping. A linear regression analysis was used to determine the effect of breathing on UV flow and weight in separate analyses. Animal ID were added as fixed factor to each model to account for clustering of measurements within animals. A p-value ≤ 0.05 was considered to be statistically significant. A sensitivity analysis was performed for the relationships between the reduction in IP pressure and both UV flow and weight by excluding the animal with the largest number of measurements. These analyses (data not shown) were performed to exclude the possibility that measurements from a single animal dominated the results. As results were very similar to the analysis with the complete data, the outcomes were not influenced by the number of measurements from any one animal.

RESULTS

Fetal characteristics and blood gas status at the beginning of experiments are displayed in table 1. One out of the six lambs was male.

Table 1 | Fetal characteristics

N=6	Fetal	5 min prior to cord clamping	5 min after cord clamping	10 min after cord clamping
Gestational age [days]	134 [134-134]	-	-	-
pH	7.39 [7.37-7.42]	7.27 [7.27-]	7.13 [7.12-7.22]	7.12 [7.07-7.24]
pCO ₂	45.6 [44.6-49.0]	54.4 [44.9-]	70.2 [64.4-73.3]	72.1 [52.5-85.8]
pO ₂	23.6 [21.2-27.1]	35.1 [32.9-]	42.4 [22.7-51.7]	74.0 [47.0-96.1]
Saturation [%]	69.4 [58.9-72.3]	82.0 [74.9-]	84.4 [50.5-90.9]	96.8 [74.5-98.1]

Characteristics displayed as median [IQR]

Effect of spontaneous breaths on lamb weight

The breath-by-breath analysis evaluated 1117 breaths, which resulted in an average reduction of IP pressure of 8.6 ± 0.3 mmHg (range 2.2 to 72.2 mmHg); this equates to a mean reduction in pressure of 11.2 ± 0.6 cmH₂O (range 2.9 to 93.8 cmH₂O). Of the 1117 breaths analysed, 626 breaths were excluded from weight analysis due to major changes caused by artefacts such as body movement. Of the 491 breaths analysed for weight, 46.6% of breaths were associated with an

increase in weight, 5.9% were associated with no change in weight and 47.5% of breaths were associated with a decrease in weight. Overall, the mean increase in lamb weight per breath was $0.02 \pm 2.5\text{g}$ (figure 2A). The increase in weight was positively correlated with the standardised reduction in IP pressure. The regression coefficient was 0.839 meaning that for an IP pressure that is 1 SD above the mean, weight increased by 0.839 g. Median [IQR] lamb body weight was 3969 [3635-4131] g at delivery, 3924 [3668-4080] g at 10 minutes before cord clamping and 4038 [3610-4201] g at time of cord clamping. No net placental transfusion was measured or observed during spontaneous breathing prior to cord clamping as measured by body weight change ($p=0.418$).

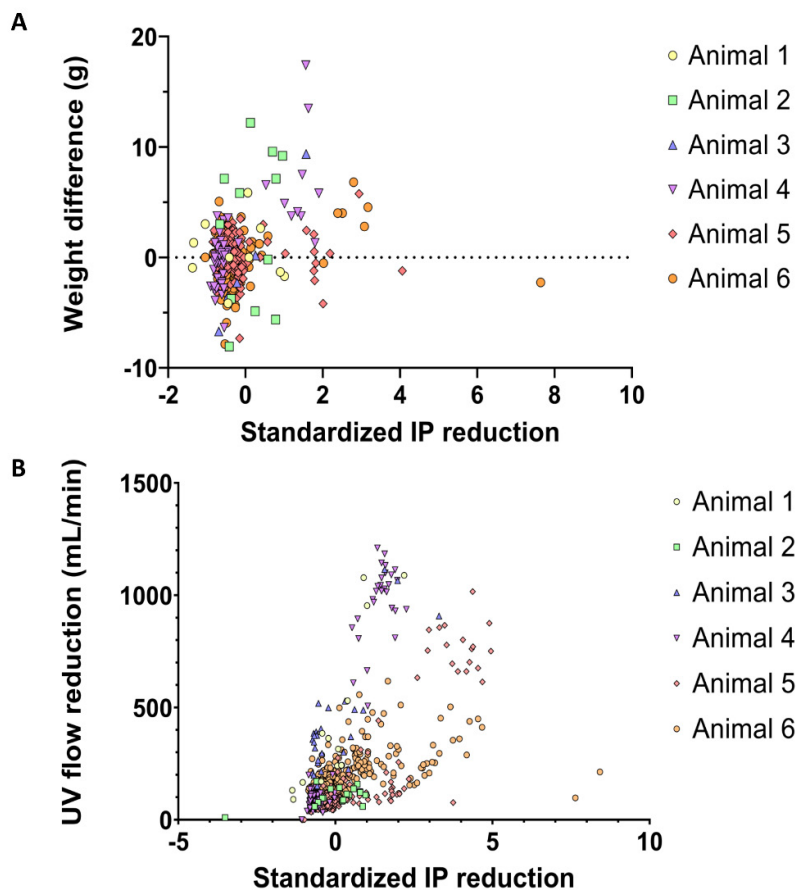


Figure 2 | Relationship between intra-pleural pressure and body weight change and common umbilical venous (UV) blood flow per breath

Common Umbilical Venous flow

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

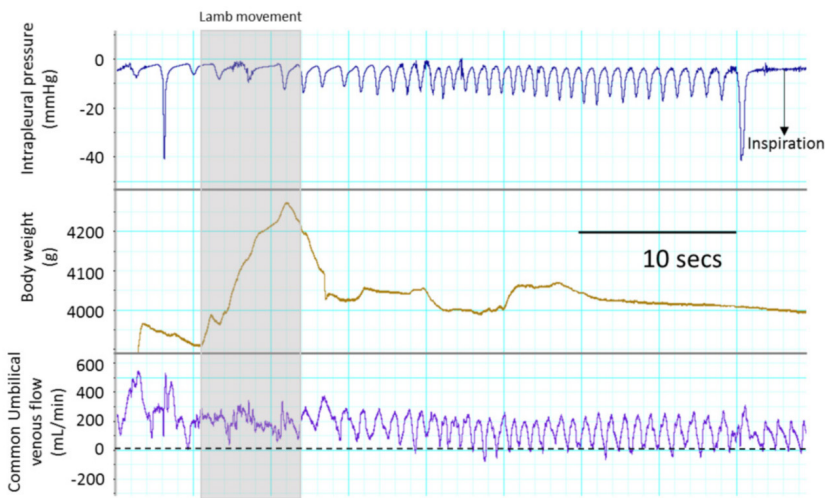


Figure 3 | Physiological recordings of intra-pleural (IP) pressure, body weight and common umbilical venous (UV) blood flow

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

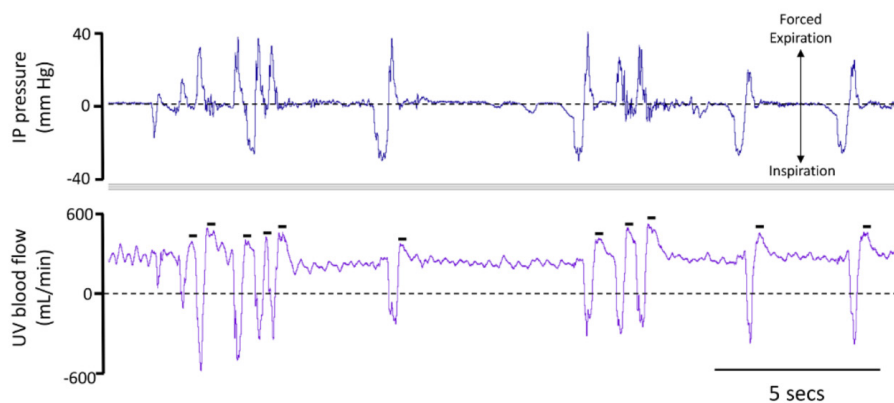


Figure 4 | Physiological recordings of intra-pleural (IP) pressure and common umbilical venous (UV) blood flow

Haemodynamics

Carotid arterial pressure and flow

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

Pulmonary artery blood flow

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

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

Time	CA pressure (mmHg)	CA flow (mL/min)	PA flow (mL/min)	Body Temperature
-10 min	48.73 [40.37 - 56.11]	60.35 [43.10 - 83.63]	137.82 [62.22 - 254.00]	39.5 [39.0 - 39.8]
-5 min	50.32 [46.94 - 58.39]	43.32 [27.25 - 61.75]	240.53 [138.00 - 298.67]	38.6 [37.7 - 39.5]
-2 min	48.02 [41.16 - 55.40]	42.33 [32.94 - 57.20]	312.64 [179.14 - 403.90]	38.2 [37.4 - 38.9]
Cord clamped	54.45 [49.03 - 61.00]	49.27 [40.37 - 62.33]	354.40 [224.28 - 457.53]	38.2 [37.8 - 38.8]
1 min	54.98 [50.06 - 62.14]	52.15 [46.46 - 73.45]	255.87 [218.88 - 422.99]	38.2 [37.8 - 38.7]
2 min	58.96 [47.83 - 65.98]	58.14 [50.32 - 86.52]	314.78 [218.00 - 462.60]	38.1 [37.2 - 38.4]
3 min	55.51 [50.16 - 64.59]	62.65 [47.64 - 92.08]	281.72 [224.34 - 446.64]	38.1 [37.8 - 38.4]
4 min	58.83 [49.43 - 70.06]	68.41 [61.64 - 105.60]	313.45 [239.18 - 541.80]	38.0 [37.2 - 38.3]
5 min	59.97 [48.41 - 69.04]	73.61 [61.91 - 92.85]	319.15 [217.48 - 449.91]	38.0 [37.5 - 38.6]
6 min	55.28 [48.74 - 70.17]	74.36 [53.45 - 93.81]	260.98 [200.38 - 320.13]	37.9 [37.0 - 38.6]
7 min	58.27 [49.60 - 74.29]	82.93 [69.51 - 126.51]	389.07 [268.02 - 463.93]	36.7 [35.7 - 38.5]
8 min	55.30 [47.03 - 79.75]	85.66 [64.12 - 106.34]	343.40 [280.24 - 437.31]	37.8 [37.4 - 38.6]
9 min	71.65 [48.77 - 75.96]	71.93 [59.60 - 102.05]	356.79 [286.36 - 600.84]	38.1 [37.2 - 38.7]
10 min	64.26 [46.97 - 71.41]	78.96 [48.34 - 97.13]	355.93 [275.70 - 567.71]	38.0 [37.3 - 38.7]

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

CA, carotid arterial; PA, pulmonary artery.

DISCUSSION

International resuscitation guidelines currently recommend DCC for 60 s in infants who do not need resuscitation, largely based on the concept of placental to infant blood transfusion.^(23, 24) While previous studies have shown a beneficial effect of DCC on neonatal outcomes,^(14, 21) the physiological mechanisms underpinning the net shift of blood from the placenta into the infant during DCC remains unknown. We consider that a better understanding of this physiology and the factors influencing placental transfusion could help optimise the practice of DCC in the delivery room. We hypothesised that the reduction in intrathoracic pressure associated with inspiration would provide a pressure gradient favouring blood flow from the placenta into the infant and restrict flow in the opposite direction. However, contrary to our hypothesis, we found that UV flow into newborn lambs transiently reduced, ceased or even reversed during individual breaths, depending upon the respiratory pattern, and that the reduction in flow was positively correlated with the depth of the inspiration. It is not surprising, therefore, we also did not observe a relationship between spontaneous breathing and weight gain of the lamb.

Evidence for placental transfusion in humans is well established⁽⁵⁾ and includes an increase in infant weight during DCC, an increase in neonatal blood volume and a decrease in blood volume remaining within the placenta after DCC.^(1, 2, 5, 25, 26) However, our previous animal studies have been unable to provide any evidence of placental transfusion, which we considered was due to the absence of spontaneous breathing; instead all lambs were mechanically ventilated.^(9, 19) As such, in this study we aimed to investigate whether sub atmospheric pressures generated during inspiration resulted in placental transfusion. To measure placental transfusion, lamb body weights and UV flows were measured continuously throughout a period of DCC. However, the body weight measurements proved to be challenging as any slight movement or touching of the lamb caused large rapid changes in measured weight that were artefactual and unrelated to body weight changes; these sections of physiological recordings were manually detected and excluded from analysis. To counter the inhibitory effects of maternal sedation on spontaneous breathing, all lambs received drugs to reverse these effects and therefore body movements were common during the experiment, resulting in some unreliable weight measurements (figure 3). Nevertheless, even when the lambs were not moving and the weights were stable, we could not identify a relationship between breathing (IP pressure) and weight change over an entire breathing episode (figure 2A). Nevertheless, the linear regression analysis detected a relationship between the standardised IP reduction and a very small increase in weight. Considering that the UV flow was markedly reduced with each breath (see below), it is difficult to explain how this might occur. However, the effect of individual breaths on weight change are transient, reversed at the end of the breath and may involve a transient discrepancy between UV flow into and umbilical arterial (UA) flow out of the lamb.

Although we found that UV flow was influenced by spontaneous breathing and the associated decrease in IP pressure, the relationship was opposite to what we hypothesised. We hypothesised that the decrease in intrathoracic pressure associated with inspiration would provide a pressure gradient to increase UV flow into the lamb. However, we found that UV blood flow transiently decreased during inspiration, commonly decreasing to zero at end-inspiration. Furthermore, we found a strong positive relationship between the degree of decrease in UV flow and the size of the reduction in intrathoracic pressure generated by inspiration (figure 2B). While we were initially surprised by this finding, it is consistent with previously described findings measured by ultrasound in human fetuses during periods of fetal breathing movements (FBM).⁽²⁷⁾ This study showed that during inspiration, contraction of the diaphragm causes narrowing and closure of the inferior vena cava (IVC) as it passes through the diaphragm, causing IVC flow to cease. Blood returning from the UV, either via the ductus venosus or hepatic veins, also has to pass through the diaphragm via the same orifice as the IVC. As such, it is likely

that the ductus venosus constricts along with the IVC as the diaphragm contracts, causing flow in the UV to cease during inspiration. Due to the large compliance of the low pressure venous system, it is highly likely that these reductions in flow are slightly delayed in relation to the timing of inspiration. We found the delay to be 120-300ms in the intra-abdominal common UV, but this delay is likely to be larger when measured further upstream, closer to the placenta. As a result, when measured upstream in the cord, depending on the breathing rate, the reductions in UV flow may be completely out of synchrony with breathing. This finding explains why increases in UV flow appeared to be related to inspiration, whereas cessations in UV flow appeared to be related to expiration, in our previous study in human infants; in that study UV flows were measured upstream in the cord.⁽²⁰⁾ Nevertheless, the findings convincingly demonstrate that large sub atmospheric reductions in intrathoracic pressure cannot account for placental transfusion.

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

Ventilating the lung before clamping the umbilical cord, also known as PBCC, is known to promote haemodynamic stability immediately after birth.^(6,28) In particular, it avoids the large rapid increase in blood pressure caused by increasing systemic vascular resistance when the cord is clamped and the reduction in cardiac output caused by the loss of umbilical venous return and ventricular preload.⁽¹⁰⁾ Avoiding these significant cardiovascular events is likely to be a major contributing factor to the reduction in neonatal morbidities, such as intraventricular haemorrhage, associated with DCC.⁽⁶⁾ We found that CA pressure increased slightly following umbilical cord clamping (by ≈ 0.5 mmHg) but this increase is substantially less than

the increase that occurs with cord clamping before ventilation/breathing onset (≈ 20 mmHg).⁽⁶⁾ While the CA pressure tended to increase over the subsequent 9 min (by ≈ 15 mmHg), this increase appeared more to do with the lamb's activity levels than to cord clamping. Similarly, CA blood flow increased over the same time frame (by 30 mL/min), which indicates that cardiac output was sustained throughout the cord clamping period. PBF doubled in the minutes leading up to cord clamping, which was expected as it is well established that the increase in PBF after birth is triggered by lung aeration resulting from breathing onset.^(29, 30) Although PBF tended to increase in response to cord clamping, before cord clamping the majority of right ventricular output was mainly directed through the lungs rather than across the ductus arteriosus. This is clearly indicated by the PBF wave form (figure 1), whereby after transition, forward flow into the lung occurs throughout the cardiac cycle. Forward flow into the lung during diastole results from a reversal of flow through the ductus arteriosus due to the reduction in PVR causing a reversal in the pressure gradient between systemic and pulmonary circulations.⁽²²⁾ As clamping of the umbilical cord increases systemic arterial pressure, the increase in PBF following cord clamping likely results from increased left-to-right shunting of blood through the ductus arteriosus during diastole. These results are consistent with our previous findings in ventilated lambs.⁽⁶⁾ Taken together, these results clearly demonstrate a haemodynamically stable transition when lung aeration precedes cord clamping, irrespective of whether the lambs were ventilated or breathed spontaneously.

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

CONCLUSION

In summary, contrary to our hypothesis, we found that during inspiration, UV flow was either reduced or ceased depending upon the size of the inspiration. This finding was likely caused by narrowing or closure of the IVC during contraction of the diaphragm during inspiration as previously described in human fetuses during FBM *in utero*.⁽²⁷⁾ The reduction in UV flow was positively correlated with the depth of the inspiration. Increases in IP pressure, likely caused by abdominal contractions, also reduced UV flow and caused it to transiently reverse, resulting in blood flowing out of the lamb and pooling in the cord or placenta. This study confirms the previous results of Bhatt *et al*, showing haemodynamic stability when ventilation onset precedes cord clamping and demonstrates that the mode of ventilation (spontaneous breathing vs mechanical ventilation) does not impact on this stability or on placental transfusion.⁽⁶⁾ Instead, the haemodynamic stability is reliant on the increase in PBF. Clearly further research is required to understand the mechanisms responsible for placental transfusion.

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2

CHAPTER 2

The effect of breathing on venous return in infants at birth: an observational study

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ABSTRACT

OBJECTIVE

To investigate the effect of spontaneous breathing on venous return in term infants during delayed cord clamping at birth.

METHODS

Echocardiographic ultrasound recordings were obtained directly after birth in healthy term-born infants. A subcostal view was used to obtain an optimal view of the inferior vena cava (IVC) entering the right atrium, including both the ductus venosus (DV) and the hepatic vein (HV). Colour Doppler was used to assess flow direction and flow velocity. Recordings continued until the umbilical cord was clamped and were stored in digital format for offline analyses.

RESULTS

Ultrasound recordings were obtained in 15 infants, with a median [IQR] gestational age of 39.6 [39.0-40.9] weeks and a birth weight of 3560 [3195-4205] g. Flow was observed to be antegrade in the DV and HV in 98% and 82% of inspirations, respectively, with flow velocity increasing in 74% of inspirations. Retrograde flow in the DV was observed sporadically and only occurred during expiration. Collapse of the IVC occurred during 58% of inspirations and all occurred caudal to the DV inlet (100%).

CONCLUSION

Spontaneous breathing was associated with collapse of the IVC and increased antegrade DV and HV flow velocity during inspiration. Therefore, inspiration appears to preferentially direct blood flow from the DV into the right atrium. This indicates that inspiration could be a factor driving placental transfusion in infants.

INTRODUCTION AND RATIONALE

It is well established that delayed cord clamping (DCC) is beneficial to preterm and term infants⁽¹⁻³⁾ and it is therefore currently recommended for all infants not needing immediate resuscitation.⁽⁴⁾ However, the underlying mechanisms behind these benefits are unclear. Studies have demonstrated that delaying cord clamping until after breathing onset, maintains cardiac output throughout the fetal-to-neonatal transition by ensuring continued umbilical venous return to the heart. Similarly, DCC enables the net movement of blood volume from the placenta to the neonate (placental transfusion), which is also dependent on sustaining umbilical venous return into the infant.⁽¹⁻³⁾ However, despite placental transfusion being verified in several clinical studies, the underlying physiological mechanisms remain elusive.⁽⁵⁻⁷⁾ Possible mechanisms for placental transfusion include the effects of gravity and uterine contractions.^(8, 9) However, the effect of gravity has recently been refuted in experimental and human studies,^(10, 11) and experimental studies have shown that uterine contractions markedly reduce (rather than increase) umbilical venous blood flow (UVBF).^(12, 13)

It has also been suggested that spontaneous breathing during DCC enhances umbilical venous return by generating sub atmospheric intrathoracic pressures that increase umbilical venous inflow and reduce umbilical arterial outflow from the infant. This could explain the maintenance of cardiac output and net increase in neonatal blood volume when DCC occurs after breathing onset.^(13, 14) Indeed, studies using residual placental blood volumes as a measure for placental transfusion have shown that the onset of respiration prior to cord clamping reduces residual placental blood volume.^(15, 16) Ultrasound studies of umbilical blood flow in human fetuses have also shown that fetal breathing movements (FBM) are associated with distention of the umbilical vein and an increase in UVBF. In contrast, the inspiratory phase was associated with a concentric reduction in the cross section of the inferior vena cava (IVC), resulting in significantly reduced flow.^(17, 18) We previously used ultrasonography during DCC after birth to show that umbilical blood flow continues much longer than assumed prior to that study and that spontaneous breathing influences UVBF. However, the exact relationship between inspiration/expiration and umbilical blood flow could not be defined since inspiration and expiration were not specifically recorded.⁽¹⁴⁾

We recently examined the effect of spontaneous breathing on UVBF in newborn lambs during DCC and surprisingly found that inspiration causes UVBF to decrease or cease.⁽¹⁹⁾ This was attributed to constriction of the IVC at the level of the diaphragm during inspiration, as previously observed in human fetuses with antenatal ultrasound.⁽¹⁸⁾ In fetal sheep, the umbilical venous blood traverses the ductus venosus (DV) and passes into the IVC before entering the thorax. It then

passes along a 3-4cm segment of the intrathoracic IVC before entering the right atrium (RA) or foramen ovale. As such, constriction of the IVC at the diaphragm was thought to also reduce UVBF through the DV.^(20, 21) However, in humans the abdominal IVC ends in a funnel-like venous structure, which also contains the orifices of the hepatic vein (HV) and the DV. This subdiaphragmatic venous vestibulum is located directly below and at the level of the diaphragm, before entering the RA (figure 1).⁽²²⁾ It is unclear whether inspiration interferes with UVBF in humans as it does in sheep based on this anatomical difference. We hypothesise that in humans, inspiration causes an increase in UVBF, as occurs antenatally, at the expense of flow through the IVC, thereby prioritising umbilical venous return to the heart directly after birth. Our aim was to investigate the effect of spontaneous breathing on umbilical venous return by measuring both blood flow in the DV, as a measure of UVBF, and the diameter of the IVC.

METHODS

A prospective observational study was performed from February 2018 until September 2019 at Leiden University Medical Centre.

Study population

Infants born vaginally between 37 and 42 weeks of gestational age were eligible if they were born after an uncomplicated and low-risk pregnancy to a multiparous woman. Infants were excluded if they were known or suspected to have congenital heart or pulmonary conditions, if the infant needed respiratory support or if additional oxygen during transition was required. Written parental consent was obtained prior to birth.

Equipment and procedures

Directly after birth, infants were dried and placed on the woman's chest with an intact umbilical cord, as per standard of care. Infants were placed in a supine position, before ECG leads were attached and ultrasound measurements commenced. If placement of the infant in a supine position was determined to be undesirable by the midwife for any reason, if the umbilical cord was too short, or if one of the parents felt uncomfortable with repositioning the baby, the study procedure was discontinued and the infant excluded from the study.

Recordings were performed using a Vivid-S6 or Vivid-S60 (GE Healthcare) echocardiographic ultrasound machine with a neonatal/paediatric 6S probe. The probe was placed gently over the subcostal region and slowly rotated and tilted until an optimal view of the IVC entering the RA was obtained, which also included both the DV and the HV (figure 1). Recordings continued until the umbilical cord was clamped, at the discretion of the midwife, and recordings were stored in digital format for offline analyses. In the event that parents withdrew consent, the woman or the neonate needed medical support, or for any other reason the umbilical cord needed to be clamped prior to finishing the ultrasound examination, measurements were discontinued immediately.

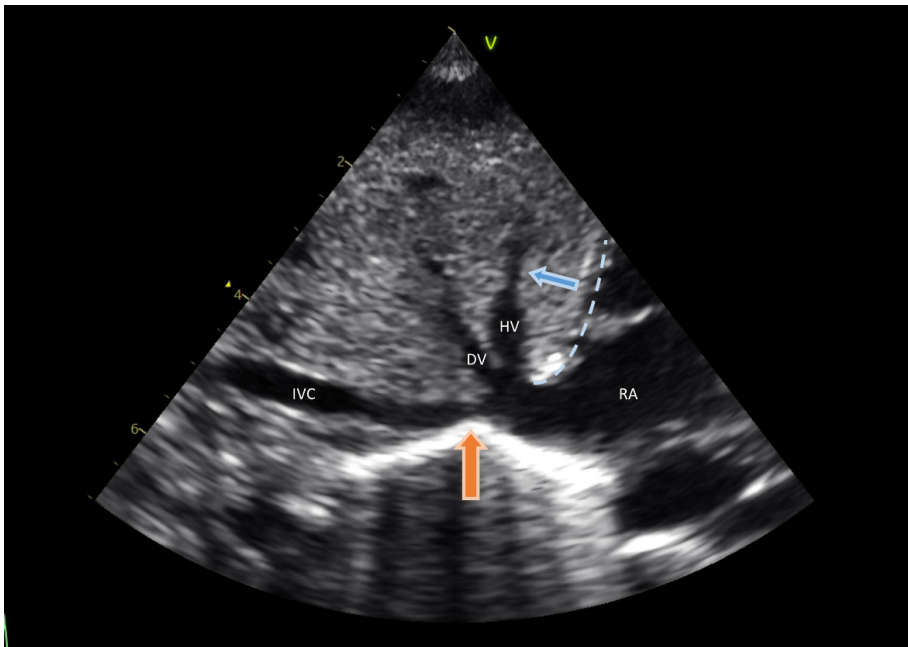


Figure 1 | Subcostal view of the inferior vena cava (IVC) entering the right atrium (RA) with both the ductus venosus (DV) and hepatic vein (HV) visible Dashed line: location of the diaphragm. Blue arrow: direction of diaphragm movement with inspiration. Orange arrow: location of IVC collapse, directly caudal of DV inlet.

Measurements

Respiration

Spontaneous breathing was observed by diaphragm movements visible in all recordings. Caudal or caudal-anterior movement of the diaphragm was defined as inspiration, whereas cranial or cranial-posterior movement was defined as expiration (figure 1). Both inspiration and expiration were measured separately and as part of a complete respiration cycle including inspiration and expiration within one recording. If no diaphragm movement was visible, this was considered a 'respiratory pause', which may occur after both inspiration (expiratory hold; helps maintain functional residual capacity (FRC) and clear lung liquid) and expiration (postexpiratory hold).⁽²³⁾

Ductus venosus

A subcostal view was used to visualise both the DV and the HV. Colour Doppler was then used to visualise flow direction in both vessels during inspiration, expiration and respiratory pauses. Flow velocity in the DV and HV was assessed with colour Doppler using M-mode. Increase of flow was evaluated as a visual increase of colour in the respective vessel (figure 2). Flow direction was defined as antegrade (towards the infant) if the majority of flow during a respiratory phase was antegrade, which was assessed with colour Doppler using M-mode. Flow was defined as retrograde, if the majority of flow during a respiratory phase was retrograde (away from the infant).

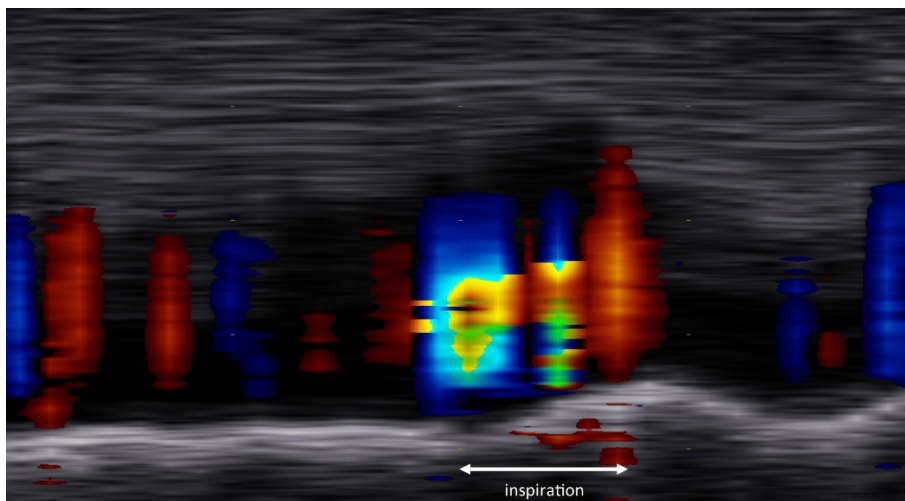


Figure 2 | Increasing antegrade flow (blue) in the hepatic vein during inspiration

Inferior vena cava

Colour Doppler flow measurements in the IVC were unavailable due to the 90° angle of the subcostal view. IVC diameter (IVC-Dia) was measured using standard two-dimensional grey scale images of the subcostal view. Change in IVC-Dia was measured for every recorded inspiration movement, when the IVC was visible over the entire length. If a change in IVC-Dia was present during inspiration, the location of this diameter change was noted as either cranial or caudal to the DV inlet into the subdiaphragmatic venous vestibulum or at the DV.

Statistical analysis

Data are presented as mean ($\pm$ SD), median [IQR] or number (%) where appropriate. SPSS V.24.0 software for Windows was used for the database and statistics.

RESULTS

A total of 17 infants were included in the study, although ultrasound measurements could not be performed in 2 infants due to complications at birth, that is, shoulder dystocia and postpartum haemorrhage. Thus, ultrasound recordings were obtained in 15 infants, with a median [IQR] gestational age of 39.6 [39.0 - 40.9] weeks and a birth weight of 3560 [3195 - 4205]g (table 1). Measurements were obtained over a median time of 06:35 [05:12-07:56]min.

Table 1 | Baseline characteristics

Maternal characteristics	
Maternal age	33 [30 - 35]
Gravidity	3 [2 - 4]
Parity	3 [2 - 3]
Neonatal characteristics	
Gestational age, weeks	39.6 [39.0 - 40.9]
Female	7 (46.7)
Birthweight, grams	3560 [3195 - 4205]
Apgar 1 min	9 [9 - 9]
Apgar 5 min	10 [10 - 10]
Apgar 10 min	10 [10 - 10]

Data presented as median [IQR] or n (%)

Using direct assessment of diaphragm movement, we were able to observe 129 inspirations and 118 expirations, of which 104 respiratory cycles were complete and thereby included both inspiration and expiration, with or without a respiratory pause, within one recording. The median recorded respiratory cycles per infant were 5 [3-11]. However, due to the short duration of the recordings, it was not possible to distinguish between respiratory pauses that occurred after inspiration or after expiration. Therefore, all observations of ‘respiratory pauses’ were combined.

Flow in DV and HV

DV and HV flow was observed in all infants. Antegrade flow in the DV was observed in 56 of 57 (98%) inspirations, 27 of 46 (59%) expirations and 33 of 41 (81%) respiratory pauses. Retrograde flow occurred sporadically in the DV and was only observed in 2 of 46 (4%) expirations (table 2).

Table 2 | Flow direction in the DV and HV during respiration

DV	Antegrade flow	Retrograde flow	High frequency bidirectional flow
Inspiration (N=57)	98%	0%	2%
Expiration (N=46)	59%	4%	37%
Respiratory pause (n=41)	81%	0%	19%
HV			
Inspiration (n=61)	82%	0%	18%
Expiration (n=72)	25%	13%	62%
Respiratory pause (n=52)	17%	0%	83%

Data presented as (%)

Antegrade flow in the HV was observed in 50 of 61 (82%) inspirations, whereas in 45 of 72 (62%) expirations and 43 of 52 (83%) respiratory pauses an alternating bidirectional (antegrade/retrograde) flow pattern occurred at a high frequency, which was approximately twice the heart rate (figure 3). Retrograde flow in the HV also occurred sporadically and was only observed in 9 of 72 (13%) expirations. Evaluation of flow increase in either the DV, the HV or in both vessels shows that antegrade flow velocity increased during 56 of 76 (74%) inspirations and during 12 of 50 (24%) respiratory pauses. Increases in retrograde flow velocity occurred in 9% (7 of 79) expirations.

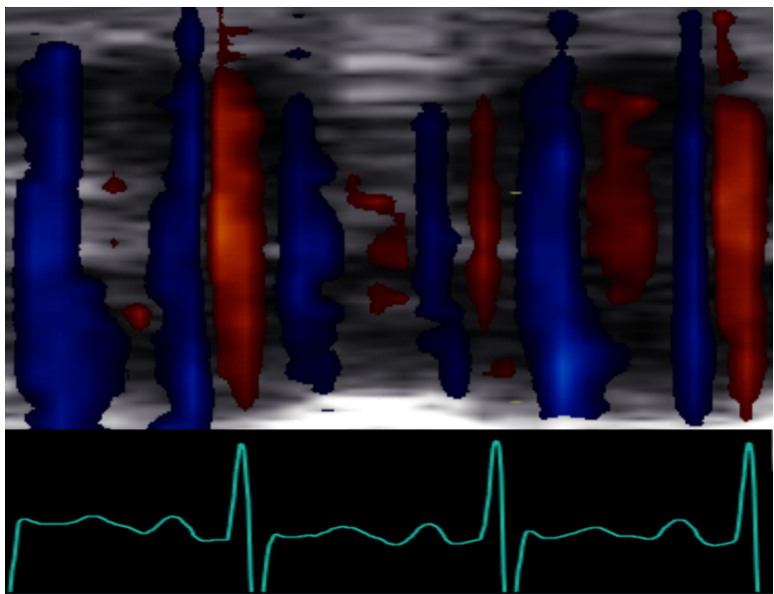


Figure 3 | Alternating antegrade (blue) and retrograde (red) blood flow in the hepatic vein with corresponding ECG signal

Diameter of IVC

IVC-Dia could be reliably measured in all infants. The IVC collapsed in 66 of 101 inspirations and respiratory pauses, but in only 7 of 67 (10%) expirations (table 3). This collapse always (72 of 72, 100%) occurred caudal to the DV inlet into the subdiaphragmatic venous vestibulum. Of these constrictions, 64 of 72 (89%) occurred directly below the DV inlet (figure 1), with a median change in diameter of 2.6 [1.9-3.5]mm (from 3.6 [2.7-4.5]mm to 0.0 [0.0-2.1]mm) whereas in 11% the IVC constriction occurred further upstream. On these occasions, the collapse occurred 10 [9.3-10.2]mm caudal to the DV inlet into the vestibulum, with a median change in diameter of 2.2 [2.0-2.8]mm (from 4.1 [3.4-5.3]mm to 2.1 [1.6-2.5]mm).

Table 3 | Collapse of the IVC during respiration

	Inspiration (n=69)	Expiration (n=67)	Respiratory pause (n=32)
Collapse	40 (58)	7 (10)	26 (81)
Complete	23 (58)	5 (71)	5 (19)
Incomplete	17 (42)	2 (29)	21 (81)
No Collapse	29 (42)	60 (90)	6 (19)

Data are noted as n(%).

DISCUSSION

We used ultrasound to assess the effect of breathing on DV and HV blood flow in human infants at birth prior to umbilical cord clamping. We observed that breathing was associated with an increase in antegrade blood flow in both the DV and the HV during inspiration. While blood flow continued in antegrade direction in the DV during both expiration and respiratory pauses, blood flow in the HV became bidirectional and alternated at high frequency (approximately twice the heart rate). Furthermore, inspiration was associated with collapse of the IVC, resulting in an obstructed IVC flow as has previously been described in human fetuses.⁽¹⁸⁾ These observations indicate that inspiration facilitates and prioritises umbilical venous return from the placenta into the heart during DCC. Thus, breathing could contribute to placental transfusion during DCC, and as such the presence or absence of breathing may be a complicating factor in previous placental transfusion studies.⁽²⁴⁾

Our observations of the effect of inspiration on DV and HV in term infants are in line with previous antenatal ultrasound studies of breathing movements in humans. Kiserud *et al.*⁽²⁵⁾ demonstrated a 10-fold increase in pressure gradient between the DV and IVC during inspiration. Nyberg *et al.*⁽¹⁷⁾ showed that umbilical blood flow increased by 42% during inspiration, when measured at the placental end of the umbilical cord.⁽¹⁷⁾ Our current findings confirm those of a previous study in which we observed increases and decreases in umbilical blood flow that appeared to be related to breathing.⁽¹⁴⁾ However, when we examined the underlying physiology using a spontaneously breathing lamb model, we were surprised to find the opposite effect, whereby UVBF decreased during inspiration.⁽¹⁹⁾ In contrast to humans, lambs have a lengthy (3-4cm) intrathoracic segment of the IVC and so it is possible that this difference in anatomy is responsible for the opposite effects on UVBF during breathing. That is, as the DV joins the IVC before it enters the chest in fetal sheep, the effect of diaphragmatic contraction on IVC blood flow also reduces DV flow. If this is correct, it is likely that the highly significant benefits of physiological-based cord clamping (lung aeration and breathing onset before cord clamping) that have been demonstrated in sheep are even greater in humans.

Presumably, a complete collapse of the IVC directly below the DV inlet during inspiration, causes a cessation of IVC flow. When combined with an increase in antegrade flow in both the DV and the HV, this would result in preferential blood flow streaming from the DV into the RA. This could explain the increase in neonatal blood volume during DCC in spontaneously breathing infants. It would also explain why infants who breathe prior to cord clamping, have less residual placental blood volume, that is, more placental transfusion.⁽¹⁶⁾

The IVC was observed to collapse caudal to the DV inlet during inspiration, which is a similar location to the IVC collapse observed previously during FBM, which was associated with a concentric reduction in IVC-Dia. It is possible, however, that our results represent an underestimation of IVC collapse. Namely, we excluded recordings in which the IVC was not visible over the entire length to correct for the potential influence of partial volume effect/movement of the IVC out of the imaging plane and to ensure an optimal and accurate assessment of the IVC collapse. However, excluding these recordings could have influenced our results and led to an underestimation of the true relationship between IVC collapse and inspiration.

Interestingly, we found a high-frequency bidirectional flow pattern in the HV that is unlikely to be respiratory in origin as it was most commonly observed during respiratory pauses or expiration. However, as the measured frequency was approximately twice the heart rate, we speculate that it is due to atrial pressure changes during the cardiac cycle resulting in retrograde pressure waves within the HV. This is evidenced by aligning the individual components of the ECG to the observed flow patterns in the HV (figure 3). Atrial pressures increase twice per cardiac cycle, once during atrial contraction and again at maximum atrial filling immediately before the atrioventricular valves open.⁽²⁶⁾ Indeed, retrograde flow in the HV has been described during atrial contraction (a-wave), but also during maximal atrial filling (v-wave).⁽²⁷⁾ It is possible that the immaturity (and therefore stiffness) of the preterm heart increases this effect, resulting in retrograde flow in the HV twice per cardiac cycle. As this high frequency bidirectional flow pattern was most commonly seen during respiratory pause and expiration, it is likely that this cardiac effect on HV blood flow is diminished by the negative intrathoracic pressure created during inspiration.

Retrograde flow in either the DV or the HV was observed sporadically and only during expiration. While retrograde flow in the HV has been described during atrial contraction and atrial filling,^(26, 27) antenatal presence of retrograde DV flow is often an indication of placental insufficiency and cardiac compromise in infants with severe growth restriction.⁽²⁸⁾ Unfortunately, antenatal DV Doppler measurements were unavailable in this study. However, as we only included healthy infants of low-risk pregnancies, it is highly unlikely that the retrograde flow observed in this study was an extension of antenatal flow patterns based on placental insufficiency and/or cardiac compromise.

Obtaining these measurements directly at birth has proven to be challenging and we were only able to collect data from a small group of healthy term infants. The infant was placed on the mother's chest and measurements may have been influenced by movement from the infant itself, or by repositioning or stimulation of the infant by the parents and caregivers, which limited the number of reliable

measurements. Although a larger study would be needed to confirm our findings, the observations made in this small group of infants were quite consistent, making generalisation of our results plausible.

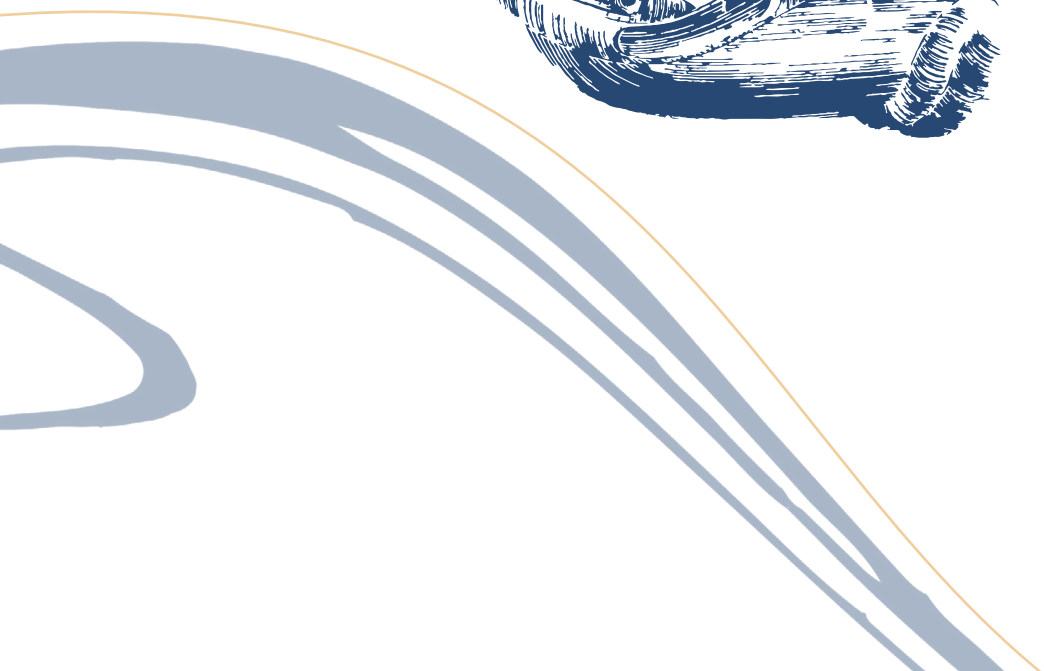
CONCLUSION

Blood flow in both the DV and HV is predominantly antegrade and increases during inspiration in healthy term-born infants. Interestingly, collapse of the IVC during inspiration consistently occurred caudal to the DV inlet and therefore had minimal impact on umbilical venous return, unlike in sheep. Instead, breathing during DCC tends to preferentially direct umbilical blood flow through the DV and into the RA. As such spontaneous breathing may partially explain the net increase in neonatal blood volume by providing the driving force for placental transfusion.

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3

CHAPTER 3

Umbilical cord pulse oximetry for measuring heart rate in neonates at birth: a feasibility study

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ABSTRACT

OBJECTIVE

To determine the feasibility of measuring heartrate (HR) by umbilical pulse oximetry (PO) after cord clamping and whether reliable HR signals can be obtained faster when compared to preductal PO in infants needing stabilisation at birth.

METHODS

Predictal and umbilical HR measurements were obtained in infants >25 weeks gestational age. During stabilisation and after cord clamping, a PO sensor was placed around the umbilical cord in addition to the standard preductal PO measurements. Umbilical PO measurements were not visible to caregivers. Video of the infant was recorded as part of standard care. HR data of the first ten minutes after birth were reviewed and compared. HR signal was considered reliable when signal identification and quality >30% and a stable plethysmograph pulse wave was observed.

RESULTS

In total, 18 infants needing respiratory support at birth were included (median [IQR] gestational age 32 [31-33] weeks, birthweight 1723 [1019-2130] grams). Reliable HRs from umbilical PO were obtained in all infants, but the time between sensor application and obtaining a reliable HR signal was longer than with preductal PO (19 [16-55] seconds vs. 15 [11-17] seconds; $p=0.01$). Umbilical HR was consistently lower than preductal HR (mean($\pm$ SD) difference 36($\pm$ 22) bpm; Intraclass Correlation Coefficient (95% CI): 0.1 (0.03-0.22)).

CONCLUSION

In infants needing stabilisation at birth, it is feasible to obtain reliable HRs when using umbilical PO, but takes longer when compared to preductal PO. Although both PO signals were reliable, umbilical cord measurement produced lower HRs than preductal PO, which warrants further investigation.

INTRODUCTION AND RATIONALE

Heart rate (HR) is considered the most important parameter to evaluate the infant's condition and the effect of interventions during transition at birth.⁽¹⁻³⁾ Although auscultation of the heart and palpation of umbilical cord pulses are still practiced, these methods are reportedly inaccurate and often lead to underestimation of HR.^(4, 5) International guidelines recommend either electrocardiogram (ECG) or pulse oximetry (PO) of the right hand (preductal) if available.⁽²⁾ However, there is still uncertainty whether evaluating HR using electrical activity (ECG) or pulsatility following contraction of the heart (PO) is more indicative of cardiac output. In addition, ECG has some disadvantages as electrodes can injure the fragile skin, cause pain or distress, and cleaning the skin can prolong electrodes placement as they do not adhere well to a wet or vernix covered skin.⁽⁶⁻⁸⁾

Although preductal PO has been recommended, it is recognised that obtaining a reliable HR can be difficult.⁽²⁾ Several studies have shown a delay in obtaining a reliable HR signal after placing a preductal PO sensor on the right hand,⁽⁸⁻¹⁶⁾ which takes from 12(9-30) to 87.28(±12.11) seconds.^(11-13, 17) Factors contributing to the delay in HR assessment of preductal PO include; time needed to dry neonates after delivery, skin fragility in preterm neonates and presence of vernix or edema within the right hand.^(2, 9, 18) Motion artefacts and peripheral vasoconstriction can also reduce preductal PO accuracy.⁽⁹⁾

The delay in obtaining a reliable HR signal can influence the steps taken during resuscitation, which prompted researchers to develop alternative methods for obtaining a faster HR signal.⁽¹⁹⁾ While umbilical pulsatility can be palpated, this method leads to underestimation of the HR which has been attributed to caregiver miscalculation.^(4, 5) Measuring pulsatility by placing the PO sensor around the umbilical cord could provide a more objective assessment. Additionally, umbilical arteries are less subjected to peripheral vasoconstriction, the umbilical cords central position could decrease sensitivity for motion artefacts and less vernix or edema is present around the cord than the hand. Cardiac generated pressure pulses continue to reach the umbilical cord whether or not arterial blood flow has ceased and are therefore still detectable after umbilical cord clamping.⁽²⁰⁾ For these reasons, we hypothesised it is feasible to measure HR using umbilical PO and that a reliable signal can be obtained faster when compared to preductal PO. In this study we aimed to investigate whether it is feasible to measure HR by umbilical PO in infants needing stabilisation and determine whether a reliable signal can be obtained faster when compared to the standard preductal PO.

METHODS

A prospective observational study was performed at the Leiden University Medical Center (LUMC, Netherlands) and was approved by the accredited Medical Research Ethics Committee of Leiden-Den Haag-Delft (P19.108) and registered in the Dutch Trial Register (NL8316). Infants of >25 weeks gestational age who needed stabilisation at birth were enrolled from January 2020 until July 2020. Infants were eligible if stabilisation was anticipated, if they were placed on the standard resuscitation table and if preductal PO measurements were obtained. Parents were approached for study participation and written informed consent was obtained antenatally.

A Masimo SET PO (Masimo Radical-7, Masimo Corporation, Irvine, California, USA) was used to feature continuous numerical values of HR, SpO₂, signal identification and quality (SIQ) and plethysmograph signals. The Masimo PO contains a multisite low noise cabled Neonatal PO sensor which was placed on the infants right hand.⁽²¹⁾ A (non-)adhesive sensor was used for preductal HR measurements depending on birthweight: RD SET NeoPt if <1kg and RD SET Neo if ≥1kg to <3kg. For umbilical PO measurements a non-adhesive sensor for birth weight <1kg (RD SET NeoPt-500) was used. Sensors did not differ in accuracy and used the same light sensor.⁽²¹⁾ Both Masimo's were set to acquire data with maximum sensitivity.

Preductal PO measurements and a video recording of the infant were digitised using the NewLifeBox-E physiological recording system (Advanced Life Diagnostics, Weener, Germany) and recorded by the NewLifeBox Neo-RSD computer system (Advanced Life Diagnostics) using Polybench physiological software (Applied Biosignals, Weener, Germany). Umbilical PO measurements were obtained using a designated laptop containing the same Polybench physiological software.

Recordings of both PO signals were started as soon as the infant was born. All infants were placed on the resuscitation table after cord clamping, then dried or covered in a wrap. The umbilical PO sensor was placed by the investigator (on the base of the umbilical cord, where it was covered with skin) and the preductal PO sensor by a neonatal nurse. After placing the sensors, they were simultaneously connected to the devices. Umbilical PO measurements were shielded and alarms were muted, so caregivers were not informed or distracted by umbilical measurements. HR measurements of the umbilical PO were continued until 10 minutes postpartum. Preductal HR measurements could continue for standard of care. Study procedures did not interfere with standard of care at any time.

The recording on the resuscitation monitor was started at the time of birth, conform our departments standard procedure during neonatal stabilisation at birth. To

synchronise HR measures from both devices, time of recording on the designated laptop was corrected using the recording time on the resuscitation monitor. A reliable HR signal was defined as a SIQ >30% and/or a stable plethysmograph pulse wave, which is consistent with previous studies.^(12, 22, 23) The Masimo PO was set to average HR measurements over 8-12 seconds and the plethysmograph pulse waveform was displayed over 10 second intervals for analysis. This waveform was considered stable when a regular waveform was visible for at least 2/3 of each 10 second period that was displayed (figure 1).

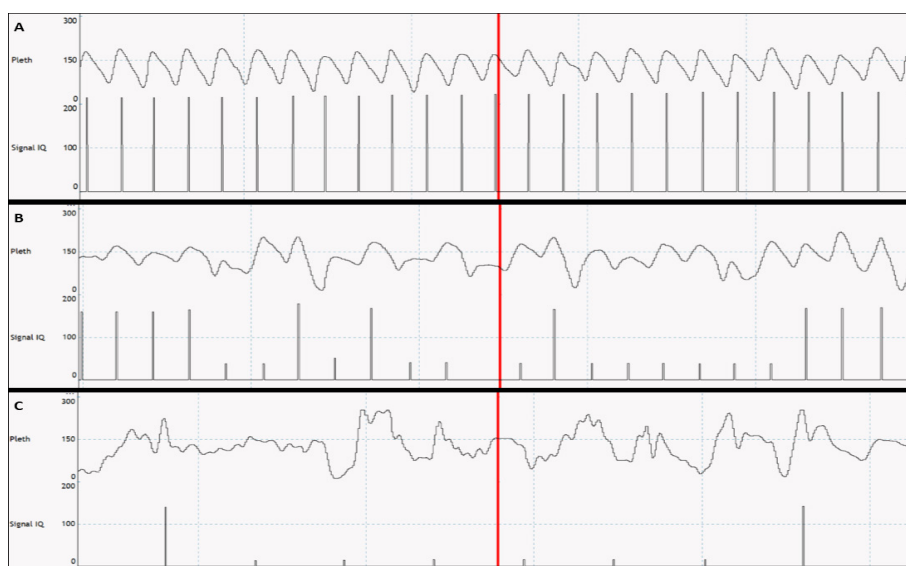


Figure 1 | plethysmograph wave patterns and the corresponding SIQ peaks

- 1a. Reliable signal with regular plethysmograph wave patterns and corresponding SIQ peaks.
 1b. A reliable signal, despite the low SIQ peaks, as a regular plethysmograph wave pattern is shown.
 1c. An unreliable signal is shown with low SIQ peaks and an irregular plethysmograph wave pattern.

HR data were obtained at 0.5 second-intervals during the first 10 minutes after birth and analysed for stability at each 10-second interval. HR measurements were considered valid for analysis if both pulse rates (umbilical and preductal) were present at that specific time interval. Furthermore, the time between touching the right hand or the umbilical cord for sensor placement and complete sensor placement was recorded. The total time of HR signal loss and the percentage of reliable HR signal after the first reliable HR signal appeared were calculated. The occurrence of replacing and reattaching the sensors were noted.

The primary outcome was feasibility; the proportion of infants with a reliable HR signal obtained from the umbilical cord. Secondary outcomes were the time needed to obtain an reliable umbilical HR signal after sensor placement and the proportion of infants where reliable umbilical PO signals were obtained for at least 20 seconds earlier than the preductal PO.

We calculated a sample size for the difference in time to obtain a reliable signal. Previous studies have shown that the time needed to display reliable HR measurements after complete preductal PO sensor placement varies between 12 (9-30) and 87.28 (± 12.11) seconds.^(11-13, 17) To identify a 20-second difference between the two methods (preductal versus umbilical), using a standard deviation of 20 seconds with a 2-sided α error of 5% and power of 80%, a total sample size of 32 paired measurements (16 infants) were needed. Assuming a 10% loss of patient data due to failure of measuring a HR signal, we decided to include a total of 18 infants. We considered 18 infants to be sufficient to test whether it is feasible to measure reliable umbilical HR.

Data analysis was performed using SPSS Statistics for Windows (IBM SPSS Statistics 25, Chicago, Illinois). Descriptive statistics were used for all baseline variables and secondary outcomes of umbilical PO. Normally distributed data are presented as mean ($\pm$ standard deviation; SD), whereas not normally distributed data are presented as median [interquartile range; IQR]. Categorical variables are presented as numbers and percentages. A Bland Altman plot was computed to assess agreement between umbilical and preductal HR measurements. Limits of agreement were calculated by two standard deviations ($SD \pm 1.96$) around the mean difference. A p-value of <0.05 was considered statistically significant. Continuous data were analysed using the Student's paired t test for data with normal distribution and a Wilcoxon signed-rank test for skewed continuous data. An intraclass correlation coefficient was calculated to assess the agreement between the two methods.

RESULTS

A total of 120 parent couples were screened for eligibility of which 60 parent couples could not be approached, mostly due to restrictions imposed by the COVID-19 pandemic. Sixty eligible parent couples were approached for study participation. In total, 36 parent couples consented, but for various reasons 18 measurements were not obtained (figure 2). Thus, the study was conducted in 18 infants with a median gestational age of 32 [31-33] weeks and a birth weight of 1723 [1019-2130] grams (table 1). Due to malfunctions of the recording software we were able to record umbilical HR measurements in 17 of 18 infants, whereas preductal measurements were recorded in 14 infants. In all 18 infants (100%), the umbilical sensor was applied successfully and in all 17 infants with umbilical HR recordings a reliable HR could be measured.

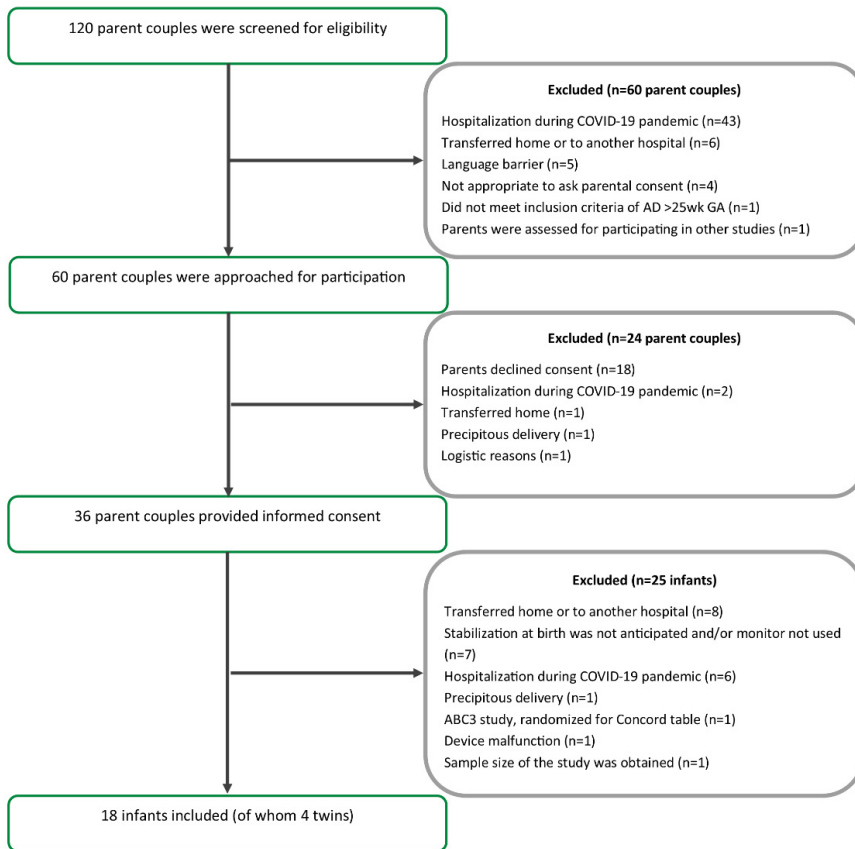


Figure 2 | Consort flowchart of patients in the study

Table 1 | Patient characteristics

Characteristics	N=18
Gestational age (weeks)	32 [31-33]
Birth weight (grams)	1723 [1019-2130]
Male sex	9 (50%)
Caesarean section	14 (78%)
Antenatal use of steroids	17 (94%)
Completed course*	14 (78%)
Incomplete course	3 (17%)
Gravidity	
1	5 (28%)
2	12 (67%)
3	1 (6%)
Parity	
0	8 (44%)
1	10 (56%)
Apgar score after 1 minute [#]	7 [6-9]
Apgar score after 5 minutes [#]	9 [8-10]
Apgar score after 10 minutes [#]	9 [9-10]
Respiratory support	
Tactile stimulation	18 (100%)
CPAP	14 (78%)
5 inflations given	9 (50%)
PPV ventilation	3 (17%)
Intubation	0 (0%)
Maximum FiO ₂ (%)	75 [38-100]

Characteristics displayed as median [IQR] or as n (%).

* Completed course described as 2 doses of steroids completed 48 hours prior to birth, within 2 weeks before delivery.

[#] Data available from 17/18 infants

The median time required for sensor placement was not different between PO's (umbilical vs. preductal: 18 [11-21] seconds vs. 14 [9-27] seconds; $p=0.5$). The median time needed to obtain a reliable HR after the sensor was connected to the PO device was a few seconds longer for umbilical PO (19 [16-55] seconds vs. 15 [11-17] seconds; $p=0.01$). Total time to acquire a reliable HR signal from birth was not different between PO's (183 [150-306] seconds vs. 179 [150-238] seconds; $p=0.1$). When using umbilical PO, no infant attained a reliable HR at least 20 seconds earlier (birth to reliable HR signal) than with preductal PO. After the first reliable signal, HR remained reliable in 96% [83-100] versus 99% [96-100] of the first 10 minutes ($p=0.2$), for umbilical and preductal PO respectively.

The umbilical sensor needed reattachment in five infants, while the preductal sensor was reattached in one infant ($p=0.3$). The umbilical sensor did not need to be replaced, whereas the preductal sensor needed to be replaced once.

When comparing all reliable HR measurements (11924 umbilical HR and 10409 preductal HR; 8902 paired (both present)), umbilical HR was lower when compared to preductal HR (125 [73-146] bpm vs. 147 [136-155] bpm; $p=0.001$). Although umbilical HR was consistently lower, the difference became smaller in time mostly due to increase in umbilical HR (figure 3). Reliable HRs of both PO's were compared in a Bland-Altman plot. The mean (± 1.96 SD) difference between all HR data pairs (preductal PO-umbilical PO) was $36(\pm 22)$ bpm with a 95% limit of agreement between -7 up to 79 bpm (figure 4). The intraclass correlation coefficient (95% CI) was 0.1 (0.03-0.22) which indicates a poor agreement between the two PO measurements.

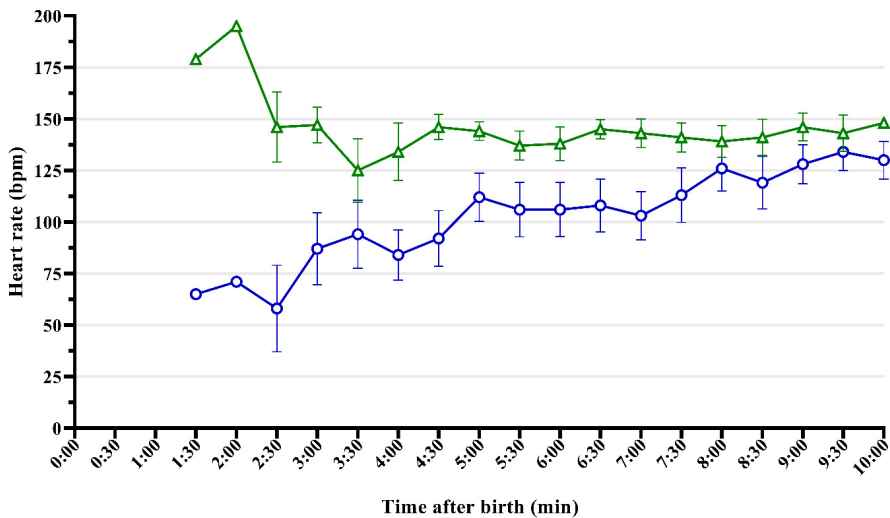


Figure 3 | Mean HR (SEM) in the first 10 minutes after birth measured by umbilical PO (blue) and preductal PO (green)

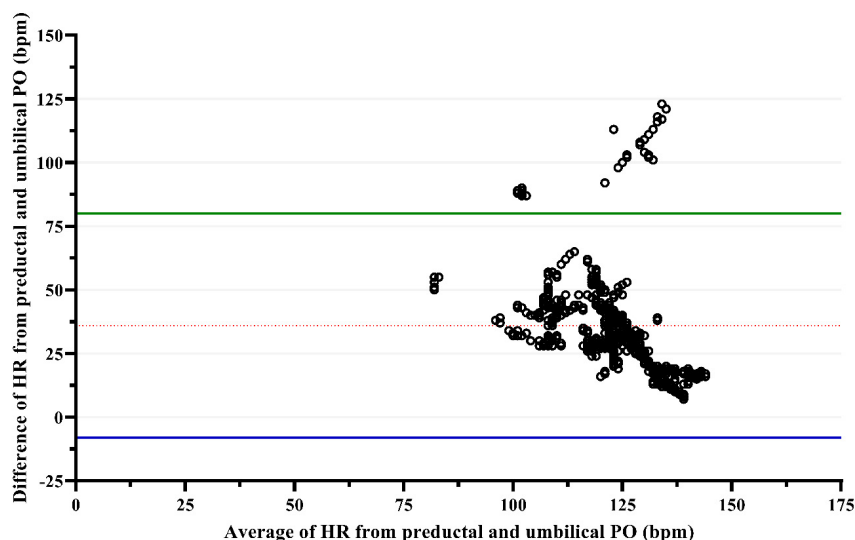


Figure 4 | Bland-Altman plot showing 95% limit of agreement in preductal and umbilical HR measurements (bpm)

DISCUSSION

In this study we observed it was feasible to obtain reliable HR measurements in infants at birth using PO with the sensor attached around the umbilical cord. We were able to obtain a reliable umbilical HR rapidly (on average within 20 seconds of sensor placement), but this was not faster than the standard preductal PO. This is largely because we were able to obtain preductal HR faster than in previous studies,^(11, 13, 17) which questions the necessity for an alternative method. Although it was possible to obtain reliable HRs from both locations, the umbilical sensor measured a consistently lower HR than the preductal sensor. There is consensus that when ECG is not possible, HR of infants can be evaluated using PO. Our findings demonstrate that HR can be estimated by both umbilical and preductal PO, but given the discrepancy between umbilical and preductal measured HR, it raises the question as to which method best reflects the infants clinical condition. We could measure reliable HR preductally faster (from sensor attached to first reliable HR) than described in previous studies.^(10, 16, 17) This can be explained by a different method in the use of PO that was recently implemented in our unit. In a recent observational study we observed that motion artefact is the predominant reason for a delay in obtaining a reliable pulse wave (unpublished data). When the hand is gently contained and motion is prevented after sensor application, a reliable HR is obtained earlier. Additionally, once a reliable HR signal is obtained it

remains reliable, even when the hand is released and motion occurs. This suggests that once a reliable signal has been detected, the Masimo PO algorithm is able to filter out motion artefacts. In contrast to our initial assumption, we observed that the umbilical cord is still subject to motion, which is not caused by movements of the infant, but by handling of the caregiver.

The consistent discrepancy in HR, with a lower HR measured at the umbilical cord, was a surprising finding. Since we only used HR data obtained from reliable signals and the HR from the umbilical cord and hand were synchronised in time, it is unlikely the difference is caused by the measuring methods. Previous studies demonstrated that counting HR by palpation of the cord led to underestimation of the HR.^(4, 5) Kamlin *et al.* compared calculating HR by manual palpation of the cord with ECG and observed that caregivers counted a similar lower HR (-21 (21) bpm difference) than measured by ECG.⁽⁴⁾ Since then it has been assumed that caregivers have difficulty counting HR in such a short time frame. This was supported by simulation studies where they observed that HR assessment by palpation was inaccurate when HR was above 100 bpm, but more accurate when HR was lower.⁽²⁴⁻²⁶⁾ However, based on our findings it is possible that the observed difference in clinical studies was a true physiological phenomenon and that less pulse waves are transmitted to the umbilical cord.

Studies have shown that lung aeration at birth is the primary trigger for the decrease in pulmonary vascular resistance leading to an increase in pulmonary blood flow.⁽²⁷⁻²⁹⁾ We recently demonstrated that this leads to a change in ductal shunting, from a right to left shunt in the fetus to a bidirectional shunt following lung aeration.^(30, 31) While a left to right shunt through the ductus arteriosus predominates during diastole, a right to left shunt is briefly restored during systole.⁽³¹⁾ Thus, during systole the right ventricle contributes to the pulse wave passing down the descending aorta, whereas during diastole, the left to right shunt diminishes the blood flow down the descending the aorta. Indeed, a difference in pulse waves between pre- and post-ductal blood vessels has been observed in experimental studies, with less pulse waves in the aorta.⁽³²⁾ All infants included in our study were breathing spontaneously with or without the need for CPAP at the time of measurements. We speculate that breathing effort at birth disrupts blood flow to the lower body when the ductus arteriosus is still open, with the sub-atmospheric pressure during inspiration increasing the left to right shunt.⁽³³⁾ This would then lead to a reduced volume change associated with the beat that is not detected by the plethysmograph algorithm. While this is a matter of speculation, this would probably explain why we observed difference in plethysmography waveforms between the preductal and umbilical oximetry measurements.

This is a small observational study and findings are not conclusive but create rationale for further investigation. The sensor and algorithm of the oximeter are not designed to measure HR at the umbilical cord and this could have influenced our findings. The feasibility of HR signal acquisition through the Wharton jelly was unknown until this study. Wharton jelly is considered to be a mucoid polysaccharide predominantly comprised of hyaluronin and chondroitin sulfate which are highly hygroscopic, providing vascular support that prevents kinking of the vessels during fetal movements.⁽²⁹⁾ However, the PO algorithm might have difficulties detecting a signal through other tissues than skin and subcutaneous fat of the hand and feet. Nevertheless, when using umbilical PO we were able to detect reliable HRs in all infants within a few seconds of preductal HR measurements. It is possible that a purpose built umbilical sensor and adjusted algorithm would lead to obtaining reliable signals much faster.

In conclusion, it is feasible to measure HR at the umbilical cord in preterm infants at birth using plethysmography of an oximeter, but this is currently not faster than the standard preductal measurement. The lower HR measured at the umbilical cord as compared to the preductal HR warrants further studies to confirm this but, if correct, evaluation of the infants clinical condition using HR by palpating the cord should be reconsidered.

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PART THREE

Physiological-based cord clamping



4

CHAPTER 4

Physiological-based cord clamping in preterm infants using a new purpose-built resuscitation table: a feasibility study

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ABSTRACT

OBJECTIVE

Physiological-Based Cord Clamping (PBCC) led to a more stable cardiovascular adaptation and better oxygenation in preterm lambs, but in preterm infants, this approach has been challenging. Our aim was to assess the feasibility of PBCC, including patterns of oxygen saturation (SpO_2) and heart rate (HR) during stabilisation in preterm infants using a new purpose-built resuscitation table.

DESIGN

Observational study.

SETTING

Tertiary referral centre, Leiden University Medical Centre, The Netherlands.

PATIENTS

Infants born below 35 weeks' gestational age.

INTERVENTIONS

Infants were stabilised on a new purpose-built resuscitation table (Concord), provided with standard equipment needed for stabilisation. Cord clamping was performed when the infant was stable (HR >100 bpm, spontaneous breathing on continuous positive airway pressure with tidal volumes > 4mL/kg, $\text{SpO}_2 \geq 25^{\text{th}}$ percentile and fraction of inspired oxygen (FiO_2) <0.4).

RESULTS

Thirty-seven preterm infants were included; mean (SD) gestational age of 30.9 (2.4) weeks, birth weight 1580 (519) g. PBCC was successful in 33 infants (89.2%) and resulted in median [IQR] cord clamping time of 4:23 [3:00–5:11] min after birth. There were no maternal or neonatal adverse events. In 26/37 infants, measurements were adequate for analysis. HR was 113 [81–143] and 144 [129–155] bpm at 1 min and 5 min after birth. SpO_2 levels were 58% [49%–60%] and 91% [80%–96%], while median FiO_2 given was 0.30 [0.30–0.31] and 0.31 [0.25–0.97], respectively.

CONCLUSIONS

PBCC in preterm infants using the Concord is feasible. HR remained stable, and SpO_2 quickly increased with low levels of oxygen supply.

INTRODUCTION AND RATIONALE

Multiple trials in preterm infants have shown that delayed umbilical cord clamping (DCC), as compared to immediate umbilical cord clamping (ICC), reduces the risk of mortality and the need for blood transfusions.^(1, 2) The mechanism of increased survival is not fully understood; however, the beneficial effect of DCC mainly was attributed to a larger circulating neonatal blood volume by increased placental transfusion.⁽³⁾ Preterm infants requiring stabilisation or resuscitation at birth were generally excluded from DCC and received ICC, because of the necessity to transfer these infants to a resuscitation table to provide respiratory support.

While it is recognised that the pulmonary and hemodynamic transitions at birth are intimately linked,^(4, 5) current clinical practice recommends cord clamping at a fixed time point, irrespective of the infant's transitional status. ICC can cause a sudden reduction in cardiac output, heart rate (HR) and blood pressure, whereas cord clamping after onset of ventilation (physiological-based cord clamping; PBCC) avoids both this and the large disturbances in systemic and cerebral haemodynamics in experimental settings.^(5, 6) These findings may explain the bradycardia and hypoxaemia that are often observed in preterm infants after cord clamping.⁽⁷⁻⁹⁾ Therefore, a more physiological approach to the timing of cord clamping is likely to be beneficial. Particularly preterm infants may benefit from this approach since large fluctuations in tissue oxygenation and perfusion increase the risk of mortality and morbidity.⁽¹⁰⁾

Recent studies investigating providing respiratory support in preterm infants with an intact umbilical cord deemed the procedure feasible.⁽¹¹⁻¹³⁾ Although these studies monitored respiratory effort, HR and oxygen saturation (SpO₂), these parameters were only measured after cord clamping. Moreover, the timing of cord clamping was always done at a fixed time point. We postulate that including the transitional status of the preterm infant as a key determinant for the timing of cord clamping may result in optimised cord clamping.

To enable PBCC in preterm infants, a new purpose-built resuscitation table (the Concord) was designed in a collaborative initiative of the Neonatal, Obstetric and Medical Engineering Departments of Leiden University Medical Centre (LUMC), according to the following criteria: (1) ability to provide full standard care for preterm stabilisation, (2) ability to monitor the infant, (3) prevention of kinking and stretching of the umbilical cord and (4) ability to proceed unhindered care for the mother. The aim of this study was to assess feasibility of the PBCC approach in preterm infants using this novel device.

METHODS

We performed a prospective observational feasibility study at the LUMC from October 2016 to November 2017. The study was registered in the Netherlands National Trial Register (NTR6095), and Institutional Review Board approval (P16.146) was obtained prior to starting the study. An external safety board was not installed.

Study population

Infants were included if they were born between 26 and 35 weeks of gestational age (GA), and no complications other than preterm birth were expected. Informed consent was requested of all pregnant women who had been admitted to the obstetric department in our institution for imminent preterm birth. Inclusion started with preterm infants born between 30 and 35 weeks of gestation. After including 8 infants without any problems, we proceeded to include infants born between 26 weeks' gestation and 35 weeks' gestation. Initially, only infants born vaginally could be included, as precautions concerning sterility of the procedure in the operating room were still in progress. After 6 months of gaining experience and taking the appropriate precautions, infants born after caesarean section were included as well. Exclusion criteria were signs of placental abruption or placenta praevia, signs of severe fetal distress determined by the clinician and the necessity for an emergency caesarean section ordered to be executed within 15 min. In this feasibility study, the predefined goal was to include a total minimum of 16 infants, a minimum of 8 infants in both GA groups and a minimum of 5 infants born after caesarean section. All infants of parents who had consented to participate were allowed to be included even though the convenience sample was reached, which is why the final number exceeds our predefined samples.

Equipment

A new custom-designed resuscitation table (the Concord, figure 1) was used to provide full standard of care for stabilisation of preterm infants at birth with an intact umbilical cord. The Concord is a mobile device, of which the platform can be adjusted to be positioned in very close proximity to the birth canal without stretching the umbilical cord. The platform contains a slit through which the umbilical cord can be placed. The table is provided with the same equipment for resuscitation as used in our standard resuscitation table, being a T-piece ventilator, radiant warmer, suctioning device, oxygen blender, heater and humidifier, and a heated resuscitation circuit. In addition, the Concord contains a respiratory function monitor (NewLifeBox Neo-RSD computer system; Advanced Life Diagnostics, Weener, Germany), depicting respiratory function, SpO₂, HR and fraction inspired

oxygen (FiO_2), as well as a video camera (Applied Biosignals, Weener, Germany). We obtained SpO_2 and HR measurements using a pulse oximeter (Masimo Radical, Masimo Corporation, Irvine, California, USA). All resuscitation procedures were video recorded as is customary at our institution. The signals were digitised at 200 Hz using the NewLifeBox -R physiological recording system (Advanced Life Diagnostics) supported by Polybench physiological software (Applied Biosignals).



Figure 1 | The Concord resuscitation table

Procedure

Prior to the start of the study all caregivers involved in labour care were trained in using the Concord, for which a standard operating procedure was written. All neonatal caregivers were trained and accredited for neonatal resuscitation. After each procedure details of birth and aspects of stabilisation were evaluated with the staff involved. Any safety issues or problems reported during the evaluation would be resolved before the next infant would be included.

The Concord was placed next to the woman's bed before progression to the second stage of labour or next to the theatre table prior to the start of caesarean section. All equipment was checked and set up in advance. The standard resuscitation table and equipment were also prepared as fall back option. If the attending neonatologist or obstetrician considered that PBCC should not be performed or interrupted, the infant would be taken to the standard resuscitation table for (further) stabilisation.

The time of birth was defined as the moment when the infant was completely out of the birth canal. At this moment, the timer was started. To enable PBCC the obstetrician held the infant after birth, and the woman was asked to lower her left leg. The platform of the Concord was then placed above the woman's pelvis as close as possible to the birth canal to ensure that the umbilical cord was not stretched. The infant was placed on the platform and received stabilisation and heat loss prevention according to standard guidelines by the neonatal team, while the obstetrician assessed maternal condition and checked that the umbilical cord was not stretched nor kinked. After a vaginal delivery, parents were encouraged to touch and stimulate the infant during stabilisation. Infants were stabilised following the institutional newborn resuscitation guideline, starting with heat loss prevention, adequate positioning and tactile stimulation. Face mask ventilation breaths or sustained inflations were applied to inadequately breathing infants (initial FiO_2 : 21%-30%), followed by continuous positive airway pressure (CPAP) or a period of positive pressure ventilation (PPV) if necessary. Surfactant was not administered in the delivery room. As soon as the infant was considered stable, defined as the establishment of adequate breathing (average tidal volume ≥ 4 mL/kg) on CPAP, HR above 100 bpm, and SpO_2 above 25th percentile using $\text{FiO}_2 < 0.4$, the cord was clamped. Uterotonic drugs were administered immediately after cord clamping. Subsequently, the platform was pulled back and placed next to the bed of the woman, enabling physical contact with her infant. The infant was then transferred to the transport incubator and transported to the neonatal intensive care unit (NICU).

In case of twin birth, clamping of the first infant was performed according to PBCC or earlier if the second infant was about to be born. After clamping, the first infant was transferred to a standard resuscitation table, so the second infant could be stabilised using the Concord. Only the second infant was stabilised using the Concord when twins were born after caesarean section, due to logistical reasons. The PBCC approach would be aborted immediately in case of: (1) the maternal condition requiring more work space for the obstetrical team, (2) full cardiac resuscitation of the infant or (3) excessive maternal blood loss as determined by the obstetrical team.

Outcome and safety parameters

The primary outcome was the ability to perform PBCC. Secondary outcomes were timing of cord clamping, HR and SpO₂ in the first 10 min of life and important safety parameters: maternal blood loss, infant temperature at NICU admission and the incidence of polycythaemia or severe hyperbilirubinemia requiring exchange transfusion. Maternal and infant baseline characteristics and short-term outcomes were collected.

Measurements of HR, SpO₂ and FiO₂ were obtained every 0.5 s and automatically averaged over a 2 s interval by pulse oximeter. Measurements were obtained until 10 min after delivery of the neonate. Data points were analysed if a good quality pulse oximeter signal was obtained without any alarm messages (low identification, low perfusion index and sensor off). HR and SpO₂ measurements are displayed per minute, presented as median [IQR], starting from the first minute after birth. All measurements were averaged over a 6 s period around every whole minute. Measurements on HR and SpO₂ are specifically reported for infants born <32 weeks' GA, as these infants are most in need of resuscitation, and this GA group is most commonly used in literature.

HR measurements obtained around the time of cord clamping were reviewed (from 10 s before to 30 s after umbilical cord clamping). Additionally, we calculated the occurrence and proportion of time SpO₂ measurements were below, within and above target ranges only for infants born <32 weeks' GA, as defined by White *et al.* More specifically, at 3-4 min, 4-5 min, and 5-10 min, the corresponding SpO₂ target ranges were 70%-90%, 75%-90% and 80%-90%, respectively.⁽⁷⁾

Statistical analysis

Data were analysed using IBM SPSS Statistic V.24.0 software. Normally distributed data are presented as mean $\pm$ SD, not-normally distributed data as median [IQR]. Pearson correlation test was used to test associations between continuous variables. Friedmans analysis was performed to test for differences between measurements over a time period. Graphs were designed using GraphPad Prism 7.

RESULTS

A total of 118 parent couples were approached for study participation of which 82 couples consented. Thirty-seven infants were included (figure 2) with a mean GA of 30.9 $\pm$ 2.4 weeks. Baseline characteristics are shown in table 1.

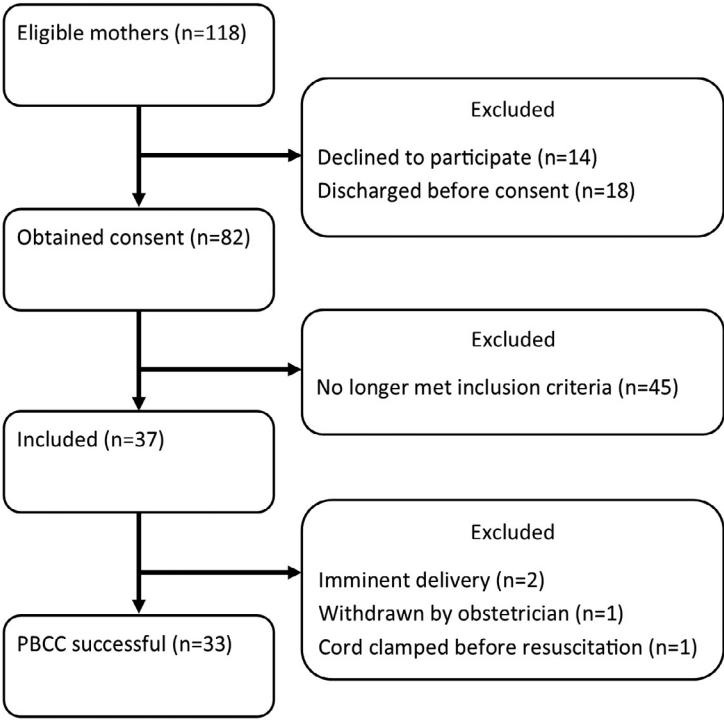


Figure 2 | Consort diagram

Table 1 | Baseline characteristics

Characteristics	All infants (n=37)
Gestational age, weeks	30.9 ± 2.4
Gestational age, range	26.3 - 35.9
Gestational age (weeks)	
32-35	11 (29.7)
30-32	15 (40.5)
26-30	11 (29.7)
Birth weight, g	1580 ± 519
Male	19 (51.4)
Twins (number of infants)	11 (29.7)
Maternal age, year	30.7 ± 3.9
Pre-eclampsia/HELLP	8 (21.6)
Chorioamnionitis	7 (18.9)
Antenatal steroids	
Complete (> 48 h)	19 (51.4)
Partly (0-48 h)	15 (40.5)
No	3 (8.1)
Caesarean section	8 (21.6)

Data are presented as mean ± SD and n (%).

HELLP, Haemolysis, Elevated Liver enzymes, Low Platelet syndrome.

Perinatal outcomes

PBCC was successful in 33 infants (89.2%, table 2), but was not performed in four infants for three reasons: (1) in two births the neonatal team arrived too late in the labour room to use the Concord and perform PBCC; (2) in one birth the umbilical cord was too short and had to be clamped immediately; and (3) in one birth the attending obstetrician had clinical concerns to perform PBCC (figure 2). We observed a median [IQR] cord clamping time of 4:23 [3:00 – 5:11] min after birth (vaginal birth 4:20 [2:55 – 5:11] min and caesarean section 4:49 [3:56 – 5:37] min). Most infants (94.6%) received respiratory support for stabilisation. No infant needed intubation or surfactant during stabilisation. Further details on perinatal outcomes are presented in table 2. There was no significant correlation between the timing of cord clamping and GA ($r=-0.161$; $p=0.37$), maternal blood loss ($r=-0.119$; $p=0.52$) or infant admission temperature ($r=-0.157$; $p=0.38$).

Table 2 | Perinatal outcomes

Perinatal outcome	All infants (n=37)
PBCC performed	33(89.2)
Cord clamping time, minutes	4:23 [3:00 – 5:11]
Apgar score at 1 min	7 [5 – 8]
Apgar score at 5 min	8 [8 – 9]
Umbilical cord – pH	7.25 ± 0.10
Respiratory support at birth	35 (94.6)
CPAP	35 (94.6)
PPV	11 (29.7)
Intubation	0
Maternal blood loss, ml	300 [200 – 475]
Temperature at NICU admission, °C	36.0 ± 0.70
Temperature 36.0-36.4°C	8 (21.6)
Temperature < 36.0 °C	18 (48.6)
Haemoglobin < 24 h, g/dL	18.01 ± 2.79
Haematocrit < 24 h, %	52.6 ± 7.63

Data are presented as mean ± SD; median [IQR] and n (%).

CPAP, continuous positive airway pressure; NICU, neonatal intensive care unit;

PBCC, physiological-based cord clamping; PPV, positive pressure ventilation.

Physiological parameters

Due to technical failure, measurements on HR, SpO₂ and FiO₂ were not stored in 7 out of 33 infants. Therefore, physiological parameters could be analysed in 26 infants. The first reliable measurements were obtained at a median [IQR] time of 81.3 [50.8-117.8] s after birth. Measurements are reported for infants born <32 weeks' GA (measurements available for 20 out of 26 infants) and are listed for all infants in table 3.

Table 3 | Physiological parameters

Minutes	HR <32 weeks median [IQR], n=20	SpO ₂ <32 weeks median [IQR], n=20	HR all infants median [IQR], n=26	SpO ₂ all infants median [IQR], n=26
1	123 [89-148]	54 [46-67]	113 [81-143]	58 [49-60]
2	127 [96-137]	58 [47-82]	126 [106-140]	59 [49-81]
3	134 [112-148]	79 [57-91]	135 [118-149]	72 [54-90]
4	141 [124-147]	93 [78-95]	141 [128-147]	90 [65-94]
5	144 [128-155]	92 [84-97]	144 [129-155]	91 [80-96]
6	143 [134-151]	91 [88-96]	143 [135-151]	91 [84-95]
7	146 [131-155]	91 [88-96]	148 [135-156]	91 [87-95]
8	145 [132-151]	93 [88-97]	145 [127-151]	93 [89-96]
9	144 [127-155]	93 [90-96]	145 [127-155]	93 [90-96]
10	138 [129-155]	92 [88-95]	141 [129-155]	93 [89-95]

Data are presented as median [IQR], for infants born <32 weeks of gestational age and all infants.

Median [IQR] HR for infants born <32 weeks' GA was 123 [89-148], 127 [96-137], 134 [112-148] and 144[128-155] at 1, 2, 3 and 5 min after birth (figure 3A, table 3). One infant experienced a HR < 60 bpm at the third minute, which occurred simultaneously with mask reposition and was thought to be the effect of the nasal trigeminal reflex. Median [IQR] HR at time of cord clamping was 139 [127-151] bpm, and Friedmans analysis showed no significant changes in HR before, during or after cord clamping, $\chi^2(2)=0.000$, $p=1.000$ (figure 3B).

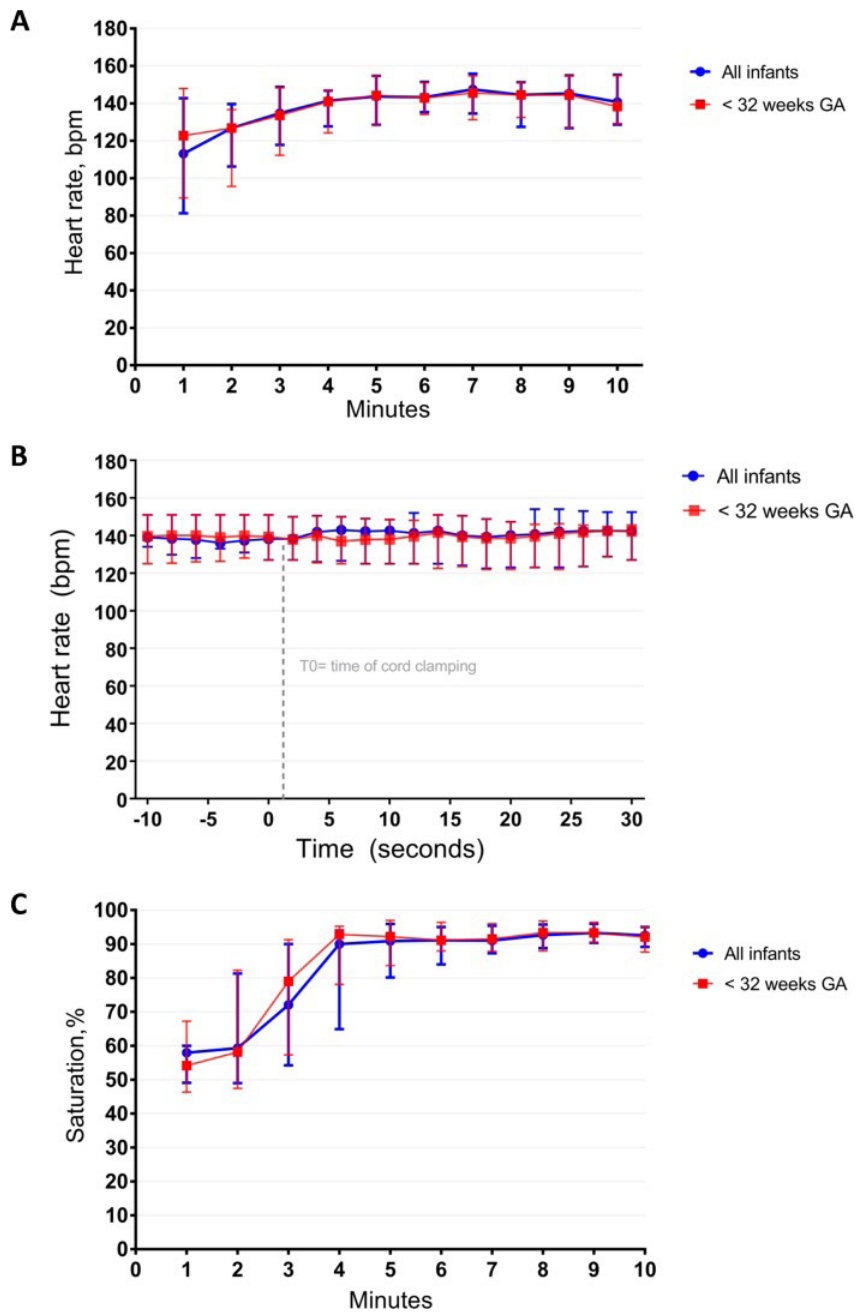


Figure 3 | Heart rate in the first 10 min after birth (A) and during cord clamping (B). Oxygen saturation in the first 10 min after birth (C)

Measurements for median [IQR] SpO₂ were 54% [46%-67%], 58% [47%-82%], 79% [57%-91%] and 92% [84%-97%] at 1, 2, 3 and 5 min (figure 3C, table 3), while median [IQR] FiO₂ given was 0.30 [0.30-0.31], 0.30 [0.30-0.31], 0.31 [0.29-0.51] and 0.31 [0.25-0.97], respectively. At periods 3-4 min, 4-5 min and 5-10 min proportions of time spent below the SpO₂ 'target range' for infants born <32 weeks' GA were 25.3%, 15.9% and 2.3% with administered median [IQR] FiO₂ of 0.32 [0.28-0.75], 0.31 [0.27-0.79] and 0.31 [0.26-0.48]. Proportion of time spent above SpO₂ value of 95% were 7.4%, 22.1% and 30.1%, respectively (table 4).

Table 4 | Proportion of time spent within, below or above SpO₂ target ranges

Time after birth	SpO ₂ target	Proportion of time below target	Proportion of time within target	Proportion of time above target	Missing values	SpO ₂ median [IQR]	FiO ₂ Median [IQR]
3-4 min	70-90	25.3%	34.3%	31.6%	8.8%	87 [67-92]	0.32 [0.28-0.75]
4-5 min	75-90	15.9%	21.8%	55.1%	7.2%	93 [84-95]	0.31 [0.27-0.97]
5-10 min	80-90	2.3%	28.9%	57.2%	10.7%	93 [88-96]	0.31 [0.26-0.48]

Proportions of time are listed as percentages. (all infants born <32 weeks GA n=20), proportion of time was calculated over 2280 measurements for 3-4 min and 4-5 min, and over 11400 measurements for 5-10min.

Clinical outcomes

Short term clinical outcomes are summarised in table 5. The majority of infants received phototherapy for hyperbilirubinemia; none of the neonates needed exchange transfusion. Three infants (8.1%) met the criteria of polycythaemia.

Table 5 | Short-term neonatal outcomes

Clinical outcomes	All infants (n=37)
IRDS	8 (21.6)
Surfactant therapy	7 (18.9)
Respiratory support < 72h	28 (75.7)
Nasal cannula	0
CPAP/High Flow	23 (62.2)
Invasive ventilation	5 (13.5)
Inotropes < 72h	2 (5.4)
PDA diagnosed	7 (18.9)
Treated	5 (13.5)
NEC ≥ stage 2	3 (8.1)
Late onset infection	11 (29.7)
Hyperbilirubinemia	
Phototherapy	35 (94.6)
Exchange transfusion	0
Erythrocyte transfusion	7 (18.9)
Polycythaemia	3 (8.1)
IVH	5 (13.5)
Grade 1	2 (5.4)
Grade 2	1 (2.7)
Grade 3	2 (5.4)
Venous infarction	1 (2.7)
Ventricular dilatation	1 (2.7)
ROP	4 (10.8)
Grade 1-2	4 (10.8)
Grade 3-4	0
BPD	3 (8.1)
Mild	0
Moderate	0
Severe	3 (8.1)

Data are presented as n (%).

BPD, bronchopulmonary dysplasia; CPAP, continuous positive airway pressure;

IRDS, infant respiratory distress syndrome; IVH, intraventricular haemorrhage; NEC, necrotizing enterocolitis; PDA, persistent ductus arteriosus; ROP, retinopathy of prematurity.

DISCUSSION

This is the first clinical study performing PBCC in preterm infants, using the infant's transitional status as the key determinant to direct the timing of cord clamping. Our results demonstrated that PBCC, when using the Concord, is feasible in a large majority of infants (89%). All failures occurred at the beginning of our project, suggesting a learning curve for involved caretakers in timely being prepared for the procedure and in adequately positioning the Concord. Feasibility was good when compared to recent studies (ranging from 59% to 100%) where respiratory support was started before cord clamping though using a different set-up.⁽¹¹⁻¹³⁾ International guidelines recommend delaying cord clamping for at least 30-60 s in stable preterm infants and immediate cord clamping for compromised infants.⁽¹⁴⁾ All recent studies providing respiratory support prior to cord clamping used time based cord clamping at 60-120 s.⁽¹¹⁻¹³⁾ Our PBCC approach led to considerably later cord clamping than reported in these studies using fixed time points, so infants potentially received a more complete placental transfusion. Yao *et al.* demonstrated this would take at least 3 min.⁽¹⁵⁾ Moreover, the Concord made it possible for infants needing respiratory support to benefit from delaying cord clamping.

This is the first study to record physiological parameters HR and SpO₂ continuously in the first minutes of life when performing PBCC in preterm infants. Previous studies have investigated the effects of ventilation prior to cord clamping,⁽¹¹⁻¹³⁾ but HR and SpO₂ were either not reported or were not recorded continuously, making it difficult to compare our findings with the literature. We observed that the HR of (very) preterm infants were higher and more stable than described so far for other methods of cord clamping.^(7, 16) In addition, no bradycardia was observed after umbilical cord clamping. Recent studies have described that bradycardia often occurs in the first minutes after birth when immediate or DCC is performed.^(7, 16, 17) Indeed, White *et al.* observed bradycardia in more than 50% of infants at 2 min after delivery, suggested by their median [IQR] HR of 88 [71-147] after immediate cord clamping. In our cohort, we rarely observed bradycardia (HR 123 [89-148]) and interestingly HR also remained stable around the moment of cord clamping.⁽⁷⁾ This suggests that cardiac output depended to a lesser extent on placental venous return, while pulmonary blood flow had successfully increased to compensate. It is striking that the HR was higher and the rate of increase was less steep in the first 2 min compared to the nomogram of preterm infants who needed no support and were <37 weeks of GA but received ICC.⁽¹⁶⁾ These observations correspond with the results of the experimental PBCC studies and imply that the preterm infants were haemodynamically stable, with a gradual transition and without adverse events.^(4, 5)

Median SpO₂ of the infants in our study increased quickly in the first 4 min after birth to a stable level above 90%, while there was very little change in the median FiO₂. Median SpO₂ increased at a faster pace than previously described for preterm infants in whom ICC was performed. Duration of hypoxaemia was shorter at lower levels of oxygen supply within the first 10 min.^(7, 8, 18) Avoiding hypoxaemia, especially in the first minutes after birth, is pivotal since this is associated with lower HR and increased risk of intraventricular haemorrhage and death.⁽¹⁹⁾ In addition, the occurrence of hypoxaemia and bradycardia would often lead to more vigorous PPV, which can be associated with increased morbidity. Mian *et al.* recently demonstrated that delivery of high tidal volume is associated with intraventricular haemorrhage.⁽²⁰⁾ Although our infants spent a greater proportion of time above the SpO₂ target ranges when compared to White *et al.*,⁽⁷⁾ only a small proportion had SpO₂ values > 95 % while lower levels of additional oxygen were given.

Clamping the umbilical cord without the occurrence of bradycardia and decreasing the duration of hypoxaemia is likely to be the effect of haemodynamic stability created by PBCC. Clamping the umbilical cord after lung aeration avoids decrease in left ventricular output, seen after immediate cord clamping, since the source of preload can easily switch from umbilical to pulmonary venous return.^(21, 22) Furthermore, the increase in afterload seen after ICC, due to loss of the placenta's low vascular resistance, is greatly mitigated during PBCC since the pulmonary circulation becomes an alternative route for systemic blood flow through the ductus arteriosus.^(23, 24) This results in the preservation of stable cardiac output and therefore a stable supply of oxygenated blood, which explains the higher SpO₂ values and absence of bradycardia after PBCC. Acknowledging that these observations are derived from experimental animal-based research, we think our results trigger further and ongoing collection of human data concerning primary physiological changes during newborn transition.⁽²⁵⁾

No serious adverse events regarding the Concord or the PBCC procedure were reported during the study. Maternal blood loss and the incidence of polycythaemia were within normal ranges and were not associated with duration of cord clamping.^(26, 27) All infants born before 32 weeks of gestation needed phototherapy, but no exchange transfusion was required. A potential draw back was the mean infant temperature at NICU admission of 36.0°C (range 34.5°C - 37.6°C). Although comparable to temperatures reported in some previous DCC studies,^(28, 29) heat loss prevention using the Concord was an important focus. During our project, extra effort was undertaken in additional education for involved caretakers to decrease the incidence of hypothermia, especially in infants delivered via caesarean section as they experienced more hypothermia. This was established by early activation of the radiant heater, thus adequately pre-warming the platform and adjusting room temperature in the operating theatre. Furthermore, the incidence of NEC in our cohort was higher than expected, mainly due to monochorionic twins of 32 weeks' GA both experiencing

NEC stage 2a, recovering without surgery. Interestingly, two out of the three infants experiencing NEC did not receive PBCC and were clamped early.

Although the study was not powered for physiological measurements, and measurements were available in only 26 out of 37 infants, IQR for most measurements were small indicating little variability between individuals; therefore, one could conclude that this represents a good estimation of HR and SpO₂ during PBCC. Although various studies have demonstrated electrocardiogram (ECG) to give a more rapid and accurate measure of HR,^(30, 31) the use of pulse oximetry in our study was for pragmatic reasons. Whether it is better to use ECG (electrical activity) or pulse oximetry (pulse wave, indication that electrical activity is followed by a pulse wave arriving in the peripheral circulation) remains open for debate. Variation between measurements was largest in the first 3 min after birth, which is consistent with current reference ranges.^(16, 32) This was a single-centre study, and a subsequent larger multicentre randomised controlled trial is planned. This will enable us to evaluate effectiveness of PBCC in other centres and compare data with a control group.

In summary, considering the success percentage in performing PBCC, a more stable HR and faster increase in oxygenation and the absence of reported serious adverse events, we consider the PBCC approach in preterm infants using the Concord feasible. This report is the first to describe real-time monitoring of HR and SpO₂ during PBCC in very preterm infants, and measurements provide human data that seems to support most of the experimental findings. Importantly, HR remained stable around cord clamping, which makes it likely that PBCC may result in optimal timing of cord clamping and in optimal pulmonary and cardiovascular transition. Further physiological research and larger randomised clinical studies are required to establish effectiveness of PBCC in improving clinical benefits in this challenging group of infants.

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5

CHAPTER 5

Physiological-based cord clamping in very preterm infants – randomised controlled trial on effectiveness of stabilisation

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ABSTRACT

AIM

To test whether stabilising very preterm infants while performing physiological-based cord clamping (PBCC) is at least as effective as the standard approach of time-based delayed cord clamping (DCC).

METHODS

A randomised controlled non-inferiority study was performed in two centres from May until November 2018, including preterm infants born below 32 weeks of gestational age. Infants were allocated to PBCC or standard DCC. Infants receiving PBCC were stabilised on a purpose-built resuscitation table with an intact umbilical cord. The cord was clamped when the infant had regular spontaneous breathing, heart rate ≥ 100 bpm and $\text{SpO}_2 > 90\%$ while using $\text{FiO}_2 < 0.40$. In infants receiving DCC, the cord was clamped at 30-60 seconds after birth before they were transferred to the standard resuscitation table for further treatment and stabilisation. Primary outcome was time to reach respiratory stability.

RESULTS

Thirty-seven infants (mean gestational age 29+0 weeks) were included. Mean cord clamping time was $5:49 \pm 2:37$ min in the PBCC ($n=20$) and $1:02 \pm 0:30$ min in the DCC group ($n=17$). Infants receiving PBCC needed less time to reach respiratory stability (PBCC $5:54 \pm 2:27$ min; DCC $7:07 \pm 2:54$ min; mean difference corrected for gestational age $-1:19$ min, 95% CI $(-3:04 - 0:27)$), showing non-inferiority with the pre-defined limit of 1:15 min. No significant differences between the groups were found for maternal blood loss, postpartum haemorrhage, infant temperature at admission or short-term neonatal outcomes.

CONCLUSION

Stabilisation of very preterm infants with physiological-based cord clamping is at least as effective as with standard DCC.

INTRODUCTION AND RATIONALE

Management of very preterm infants in the first minutes of life can have a major impact on neonatal morbidity and mortality.^(1, 2) During the transition to extra-uterine life, lung aeration is pivotal to initiate the major physiological changes in respiratory and cardiovascular function that are required for survival after birth.^(3, 4) Most preterm infants require respiratory support at birth, as they often fail to aerate their immature lungs.⁽⁵⁾ Respiratory support is commonly started only after umbilical cord clamping, which in experimental studies has been shown to compromise cardiovascular function.⁽⁶⁾

Preterm infants could benefit from placental transfusion (blood transfer from the placenta to the infant) when cord clamping is delayed. A recent meta-analysis comparing delayed cord clamping (DCC) with immediate cord clamping (ICC) in preterm infants, showed increased haematocrits, less blood transfusions, a decrease in neonatal mortality and a trend towards less intraventricular haemorrhages (IVH).⁽⁷⁾ However, in most studies DCC was performed using a fixed time-point of 30-60 s whereas placental transfusion may take up to 3 min to complete.⁽⁸⁾ In addition, preterm infants needing immediate interventions for stabilisation or resuscitation were generally clamped immediately and excluded from these series. These infants are however at the highest risk of complications and may receive the greatest benefit from DCC.

While the rationale of most DCC studies was based on increased placental transfusion, experiments in preterm lambs have shown that delaying cord clamping until after ventilation onset prevents a significant reduction in cardiac output.⁽⁶⁾ This approach of physiological-based cord clamping (PBCC) in lambs also prevented large fluctuations in systemic and cerebral blood pressures and flows.⁽⁹⁾ In preterm infants these detrimental effects may be avoided when infants are first stabilised whilst connected to the cord and only clamped when the infant has a stable breathing pattern, possibly resulting in decreased risk of mortality and (cerebral) morbidity.⁽¹⁰⁾

The aim of the PBCC approach in preterm infants is to establish lung aeration, adequate pulmonary blood flow and pulmonary gas exchange prior to cord clamping. To facilitate this approach, a new purpose-built resuscitation table (the Concord) has been developed at Leiden University Medical Centre (LUMC). This mobile resuscitation table is designed to provide full standard care in stabilisation of preterm infants at birth while the cord remains intact. All equipment that is needed for stabilisation and resuscitation is incorporated in the table allowing preterm infants needing immediate respiratory support to also receive the possible benefits of PBCC. This study is the second in our Aeration, Breathing, Clamping (ABC) research project determining the benefit of PBCC using the Concord. We

recently demonstrated feasibility of this approach with a median cord clamping time of almost four and half minutes (ABC1 study).⁽¹¹⁾

Aim of the study

The aim of the present study (ABC2 study) was to assess whether using the Concord to stabilise preterm infants with PBCC is at least as effective as using the standard approach of stabilising infants after a period of DCC (30-60s) using a standard resuscitation table.⁽¹²⁾ Our hypothesis was that stabilisation with PBCC was non-inferior to stabilisation after DCC.

METHODS

Trial design

The study was a randomised controlled non-inferiority trial in two tertiary centres (LUMC and Erasmus University Medical Centre) and performed in the labour room or operating theatre in case of caesarean section. Infants were randomised 1:1 to either stabilisation according to the PBCC approach using the Concord, or stabilisation according to the standard DCC approach using the standard resuscitation table. The study was approved by the LUMC Institutional Review Board (IRB, P18.025) and registered in the Netherlands Trial Register (NTR7194/NL7004). Infants were included in the study from May 9th until November 18th 2018. Details on study procedures, randomisation, investigational products and sample size are as previously described.⁽¹²⁾

Participants

Infants born vaginally or by caesarean section prior to 32 weeks and 0 days of gestational age (GA) were eligible. Exclusion criteria were significant congenital malformations influencing cardiopulmonary transition, placental abruption, placenta praevia and signs of severe fetal distress necessitating emergency caesarean section. Parental written informed consent was obtained prior to birth.

Investigational procedures

Prior to the start of the study, all health care providers involved in labour room care were trained in using the Concord for PBCC. A standard operating procedure was developed to optimise close collaboration between neonatologists and obstetricians. All neonatal caregivers involved were trained and accredited for neonatal resuscitation. Centres adhered to local resuscitation guidelines for stabilisation of preterm infants.

Preterm infants randomised to the intervention group were stabilised according to the PBCC approach (figure 1). This involved providing standard postnatal respiratory management and heat loss prevention while the infant was on the Concord close to its mother, with the cord still intact. Importantly, stabilisation started as soon as the infant was placed on the platform, with the cord still intact, which was clamped only after the infant was judged to be stable, as defined by the presence of regular spontaneous breathing, a heart rate (HR) > 100 bpm and oxygen saturation (SpO₂) above 90% while using supplemental oxygen (FiO₂) < 0.40. Uterotonic drugs were administered immediately after cord clamping.

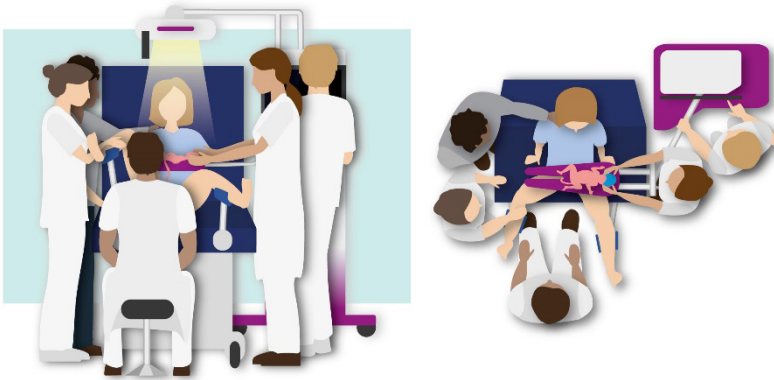


Figure 1 | Physiological-based cord clamping approach using the Concord

In case of twins and vaginal birth, a similar stabilisation protocol and definition of the moment of cord clamping was used. Clamping in the first infant was performed sooner if the second infant was about to be born. The first infant was then transferred to the standard resuscitation table, so that the second twin could be stabilised on the Concord.

PBCC could be abandoned at any time during the procedure, which involved clamping the cord and transferring the infant to the standard resuscitation table. This could result (1) from a clinical emergency in the woman or the second twin requiring additional working space for the obstetrician, (2) when full resuscitation was needed, and (3) when maternal blood loss was excessive and the obstetrician decided to clamp the cord and administer uterotonic drugs without delay.

Preterm infants randomised to the standard DCC group were transferred to the standard resuscitation table following cord clamping to administer any treatments or interventions required to stabilise the infant, according to local resuscitation

guidelines. Clamping was time-based and occurred 30-60 s after birth, depending on the clinical condition of the infant; early cord clamping was deemed necessary if the infant needed assistance. Uterotonic drugs were administered immediately after cord clamping.

Randomisation, blinding and treatment allocation

Randomisation was stratified by gestational age (24-27+6 and 28-31+6 weeks) and by treatment centre using variable block (4-8) sizes. Concealment of allocation was ensured by using the randomisation process of Castor EDC, an electronic data capture system. Blinding of the study was not possible.

In case of twins born vaginally, both infants were randomised to the same group. In case of twins born by caesarean section, it was technically not possible to perform PBCC for both infants. After consent, both infants were included; the first infant always received DCC and the second infant was randomised to either PBCC or DCC.

Primary outcome

Primary outcome was the time to stabilisation of the infant, starting from birth. A stable infant was defined by the presence of regular spontaneous breathing, a HR ≥ 100 bpm and SpO₂ above 90% while using FiO₂ < 0.40.

Secondary outcomes

In addition to patient and maternal demographics, multiple secondary outcomes were collected as described in the study protocol.⁽¹²⁾ These outcomes were related to the procedure itself and to the transition of the infant. Furthermore, short-term neonatal and maternal outcomes were collected. Important safety outcomes were infant temperature at admission at the neonatal intensive care unit (NICU) and maternal peripartum blood loss, postpartum haemorrhage and surgical site wound infection after caesarean section.

Statistical analysis

When designing this study, no data were available on the effectiveness of stabilising infants during PBCC and comparing PBCC with DCC to conduct a power analysis. Few studies have performed initial respiratory support before cord clamping, but all used predefined timing of cord clamping, varying from 30 to 120 s. Data from our feasibility study showed that the median [IQR] time to obtain respiratory stability was 4:23 min [3:00 – 5:11] during PBCC.

In our unit, the mean time (from birth) needed to stabilise an infant using DCC is 5 ± 2 minutes.⁽¹³⁾ If the time to stabilisation was not different between DCC and PBCC, we calculated that 64 infants (32 in each treatment arm) were required. This would give 80% power to assess whether the upper limit of a one-sided 97.5% confidence interval (equivalent to a 95% two-sided confidence interval) around the difference in mean time to stabilisation would be below the non-inferiority limit of 75 s. A non-inferiority margin of 75 s was chosen as current clinical guidelines for targeting of oxygen saturation indicate that this time difference is acceptable during stabilisation.⁽¹⁴⁾

Normally distributed data is presented as means $\pm$ standard deviations, whereas data that is not normally distributed is presented as medians and interquartile ranges. Categorical data were analysed using the Chi-square test or Fisher's exact test. Continuous data were analysed using the Student's t test or Mann-Whitney test as appropriate. Intention-to-treat analysis was performed. For the primary outcome an additional as-treated analysis was performed, analysing results based on the received rather than allocated treatment. The effect of PBCC on the primary outcome was assessed by multivariable linear regression analysis including GA as additional covariate. An additional sensitivity analysis correcting for other possible confounders that were unbalanced between the groups was performed. The primary test of non-inferiority is reported with a one-sided p-value and compared to an alpha of 2.5%; all other (superiority) p-values are reported two sided and compared to an alpha of 5%.

RESULTS

Following a discussion between the investigators and three independent experts (an ethicist, a statistician and an epidemiologist), it was decided to stop this trial before reaching the target sample size due to a slower than anticipated recruitment. The recruitment period for this trial was predetermined and limited by the funding conditions of the next-stage, and larger, ABC3 trial. Ceasing the trial at this time allowed an analysis of trial outcomes in recruited infants and review by the research ethics committee so that ABC3 trial could be approved by the required date, as long as the results of ABC2 trial were satisfactory. The analysis occurred at 50% recruitment and found that the predefined non-inferiority limit was already met.

A total of 191 women were assessed for eligibility (figure 2). Of these women 158 were deemed eligible and 120 approached for study participation. A total of 74 parent couples consented for study participation and 39 infants (35 pregnancies) were randomised, allocating 22 infants to PBCC and 17 infants to DCC. Two

infants were excluded either because of lung hypoplasia or due to an emergency caesarean section. Another two infants in the PBCC group received standard treatment, due to technical failure of the radiant heater of the Concord and birth prior to timely preparation of the Concord. Baseline characteristics of the study patients are presented according to their allocation (table 1).

Table 1 | Baseline characteristics

	PBCC group (n=20)	DCC group (n=17)
Gestational age, weeks	28 ⁺⁴ [27 ⁺⁶ – 30 ⁺³]	30 ⁺² [27 ⁺⁵ – 31 ⁺⁰]
Birthweight, grams	1155 [1043 – 1349]	1200 [895 – 1620]
Female	16 (80.0)	8 (47.1)
Twins	5 (25.0)	3 (17.6)
Antenatal steroids		
Yes	20 (100)	17 (100)
Complete (≥ 48h)	16 (80.0)	9 (52.9)
Caesarean Section	9 (45.0)	9 (52.9)
PE/HELLP	3 (15.0)	2 (11.8)
Premature contractions	15 (75.0)	12 (70.6)
Chorioamnionitis	7 (35.0)	6 (35.3)

Data presented as median [IQR] or n (%). PBCC, physiological-based cord clamping; DCC, delayed cord clamping; PE, preeclampsia; HELLP, Haemolysis Elevated Liver-enzymes Low Platelets syndrome.

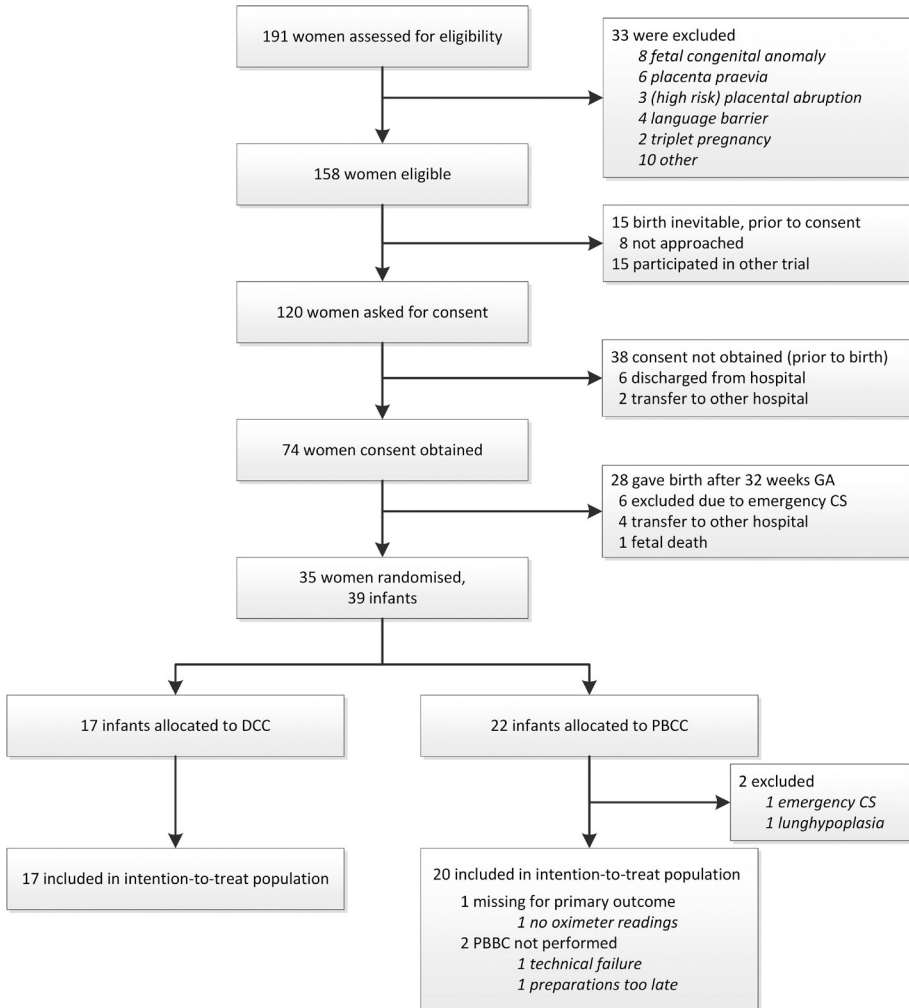


Figure 2 | CONSORT diagram

CONSORT, Consolidated Standards of Reporting Trials; GA, gestational age; CS, caesarean section; DCC, delayed cord clamping; PBCC, physiological-based cord clamping.

Primary outcome

Primary outcome was recorded for 36 infants, as pulse-oximeter measurements were not obtained for one infant and primary outcome could therefore not be determined. Intention-to-treat analysis showed that the mean time to stabilisation was $5:54 \pm 2:27$ min for infants in the PBCC group and $7:07 \pm 2:54$ min for infants in the DCC group. Mean difference, corrected for GA, was $-1:19$ min with a 95% confidence interval of $-3:04$ to $0:27$ min. The pre-defined non-inferiority limit of 75 s fell outside of the confidence interval ($p < 0.01$; one sided non-inferiority test, figure 3). A total of three infants did not achieve the primary outcome within 10 min. Two of these infants were allocated to DCC and one to PBCC.

The baseline characteristics revealed a higher proportion of females in the PBCC group. Additionally, correcting for gender showed a mean difference of $-0:45$ min with a 95% confidence interval of $-2:32$ to $1:02$ min ($p = 0.01$; one-sided non-inferiority test).

As-treated analysis showed the mean time to stabilisation was $5:25 \pm 1:35$ min for infants in the PBCC group and $7:25 \pm 3:10$ min for infants in the DCC group. Mean difference, corrected for GA, was $-2:02$ min with a 95% confidence interval of $-3:42$ to $-0:22$ minutes ($p < 0.01$, one sided non-inferiority test; $p = 0.019$, two-sided superiority test, figure 3).

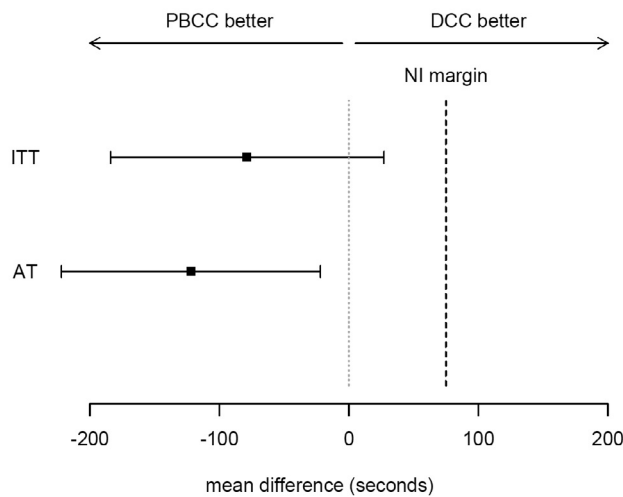


Figure 3 | Forest plot for time to stabilisation

Mean differences and 95% confidence intervals for intention-to-treat (ITT) and as-treated (AT) analysis, and the predefined non-inferiority (NI) margin of 75 seconds.

DCC, delayed cord clamping; PBCC, physiological-based cord clamping.

Secondary outcomes

Infants in the PBCC group were more likely to receive respiratory support earlier, and cord clamping was performed later at a mean time of $5:49 \pm 2:37$ min (table 2). No statistically significant differences were shown between the two groups for labour room or short term neonatal secondary outcomes, except for umbilical cord pH (table 2 and 3).

No postoperative infections were reported after caesarean sections. Median maternal blood loss was 300 [200-700] ml and 450 [263-538] ml for the PBCC and DCC group, respectively ($p=0.53$, table 2).

Table 2 | Secondary outcomes, labour room

	PBCC group (n=20)	DCC group (n=17)	P value
Time until cord clamping, min	$5:49 \pm 2:37$	$1:02 \pm 0:30$	0.00
Time until start support, min	$1:11 \pm 1:18$	$2:00 \pm 0:47$	0.04
Respiratory support			
None	1 (5.0)	0 (0.0)	1.00
CPAP	18 (90.0)	17 (100)	0.18
PPV	14 (70.0)	10 (58.8)	0.48
Intubation	1 (5.0)	0 (0.0)	1.00
Maximum FiO ₂ used, %	70 [50 – 90]	50 [30 – 88]	0.24
Apgar 1 minute	6 [5 – 8]	7 [6 – 8]	0.58
Apgar 5 minutes	8 [7 – 9]	9 [8 – 9]	0.39
Apgar 10 minutes	9 [8 – 10]	9 [9 – 10]	0.49
Umbilical cord pH	7.21 ± 0.09	7.29 ± 0.09	0.01
Temperature at admission, °C	36.5 ± 0.8	36.7 ± 0.6	0.61
Temperature < 36.0 °C	4 (20.0)	1 (5.9)	0.21
Maternal blood loss, ml	300 [200-700]	450 [263-538]	0.53
Postpartum haemorrhage (>1000 ml)	2 (11.1)	2 (12.5)	0.90

Data reported as analysed per intention-to-treat. Data presented as mean $\pm$ SD, n (%) and median [IQR]. PBCC, physiological-based cord clamping; DCC, delayed cord clamping; min, minutes; CPAP, continuous positive airway pressure; PPV, positive pressure ventilation; FiO₂, fraction of inspired oxygen.

Table 3 | Secondary outcomes, short term neonatal

	PBCC group (n=20)	DCC group (n=17)	P value
Haemoglobin first 24 h, g/dL	16.3 ± 2.9	16.8 ± 3.4	0.76
Haematocrit first 24 h, l/l	0.49 ± 0.07	0.50 ± 0.09	0.80
First infant pH after admission	7.27 ± 0.09	7.31 ± 0.10	0.27
Intubated during NICU stay	5 (25.0)	5 (29.4)	0.76
Need for surfactant	8 (40.0)	6 (35.3)	0.77
Treatment for hypotension <72h	3 (15.0)	0	0.23
Patent ductus arteriosus	6 (30.0)	4 (23.5)	0.66
IVH (all grades)	3 (15.0)	2 (11.8)	0.77
IVH ≥ grade 3	0	1 (5.9)	0.46
Maximum bilirubin	169.2 ± 43.2	163.7 ± 43.5	0.70
Need for phototherapy	20 (100)	15 (88.2)	0.12
Need for RBC transfusion	4 (20.0)	6 (35.3)	0.30
Early onset infection <72h	1 (5.0)	2 (11.8)	0.45
Late onset infection >72h	7 (35.0)	6 (35.3)	0.99
NEC ≥ grade 2A	2 (10.0)	1 (5.9)	0.65
Death	0	2 (11.8)	0.20
ROP (all grades)	7 (35.0)	2 (14.3) (n=14)	0.18
BPD (all grades)	4 (20.0)	4 (26.7) (n=15)	0.64

Data reported as analysed per intention-to-treat. Data presented as mean ± SD and n (%). PBCC, physiological-based cord clamping; DCC, delayed cord clamping; NICU, neonatal intensive care unit; IVH, intraventricular haemorrhage; RBC, red blood cells; NEC, necrotizing enterocolitis; ROP, retinopathy of prematurity; BPD, bronchopulmonary dysplasia.

DISCUSSION

This is the first reported trial comparing physiological-based cord clamping to delayed cord clamping treatment in very preterm infants. The timing of cord clamping in the PBCC group was based on the infant's transitional status, which in contrast with the time-based approaches used previously, lead to a considerably longer cord clamping time of 5:49 min. Our results demonstrate that stabilisation of preterm infants when performing PBCC using the Concord, is at least as effective as stabilising infants using the current routine DCC approach and a standard resuscitation table.

Few studies have investigated the provision of respiratory support to very preterm infants before cord clamping. All previous studies have used a time-based approach, with cord clamping varying between 60 s and 3 min after birth.⁽¹⁵⁻¹⁹⁾ These studies either used a bedside trolley or performed resuscitation while the neonate was positioned on the mother's leg or abdomen, reporting feasibility of the approach between 59% and 100%. In the present study, in all but two infants allocated to the PBCC approach this was feasible resulting in a 90% success rate of the PBCC approach using the Concord, which is comparable to our first cohort.⁽¹¹⁾ The two most important predefined safety parameters were maternal blood loss and admission temperature of the infant at the NICU. Although cord clamping is performed considerably later in the PBCC group than in all previous reported studies performing DCC in very preterm infants, maternal blood loss was not increased. This observation is in concordance with earlier studies showing that maternal blood loss is not increased after DCC.^(20,21) Mean infant temperature at NICU admission was not different, although four infants had moderate hypothermia in the PBCC group as compared to one infant in the DCC group. Temperature management from birth to NICU admission during PBCC was performed conform standard care, but remains an important focus during training for the PBCC approach, which is also emphasised by others using slightly different approaches.^(17,18) No important safety concerns were observed for the PBCC approach using the Concord, as all other short term neonatal outcomes were not different between groups. Three infants in the PBCC group were treated for hypotension in the first 72 h after birth by the administration of one bolus of fluid. This occurred twice after the administration of propofol prior to intubation. No infant needed inotropes during the first 72 h after birth.

This study showed stabilisation using PBCC is at least as effective as stabilising infants according to the current DCC approach. Interestingly, the as-treated analysis, based on actual received treatment, showed infants were stabilised faster when the PBCC approach was performed with a statistically significant difference between the two groups ($p=0.019$). This may reflect the positive effect PBCC has on cardiorespiratory

stability and ultimately stabilisation of preterm infants. Umbilical cord pH was lower for the PBCC group, whereas Apgar scores and first neonatal pH values during NICU admission did not differ between the two groups. The delay in obtaining umbilical cord blood after the PBCC approach could have resulted in lower umbilical cord blood pH values, as described previously after delayed cord clamping.^(22,23) Since first neonatal pH values and Apgar scores were not different, clinical relevance of the difference in cord blood pH is most likely minimal.

This study has some important limitations. Infants were only included after having received antenatal consent from both parents. Women giving birth soon after admission, were not approached since this was deemed inappropriate. Using deferred consent for this type of interventional labour room studies may overcome this problem and increase generalisability.⁽²⁴⁾ Likewise, infants born by emergency caesarean section were also not eligible, as there would be insufficient time for equipment set-up. In contrast, these infants probably may benefit most from PBCC and including these infants in future studies may further increase generalisability. Lastly, respiratory support in infants in the PBCC group was initiated earlier compared to the control group, which is simply a hallmark and consequence of the overall PBCC approach. Thus, evaluation of the PBCC approach in this study includes earlier start of respiratory support, later umbilical cord clamping and the utilization of the Concord.

Previous animal models already showed increased respiratory and haemodynamic stability when using the physiological-based cord clamping approach.⁽⁶⁾ Increased stability during PBCC could explain for infants in this group to be stabilised faster. The aim of the PBCC approach in preterm infants is to establish lung aeration, adequate pulmonary blood flow and pulmonary gas exchange prior to cord clamping. No clear criteria are available to define when an infant is respiratory stable. In this study, stabilised was defined as the infant having established regular spontaneous breathing with SpO_2 above 90%, $\text{HR} > 100$ bpm while using $\text{FiO}_2 < 0.40$. Others used different criteria for cord clamping in a physiological approach, for example including exhaled carbon dioxide as a marker for pulmonary gas exchange.⁽²⁵⁾ Our definition of being stable was useful in our study as SpO_2 and HR are monitored for every preterm infant. However, the target of 90% may be set relatively high, as current international guidelines aim for SpO_2 of 85% at 5 min. We previously demonstrated the feasibility and now tested the effectiveness of the PBCC approach using the Concord.⁽¹¹⁾ The next step is to determine whether this approach results in beneficial effects on important clinical outcomes in very preterm infants. In 2019, a large multicentre randomised clinical trial (ABC3 trial) has been started in the Netherlands.

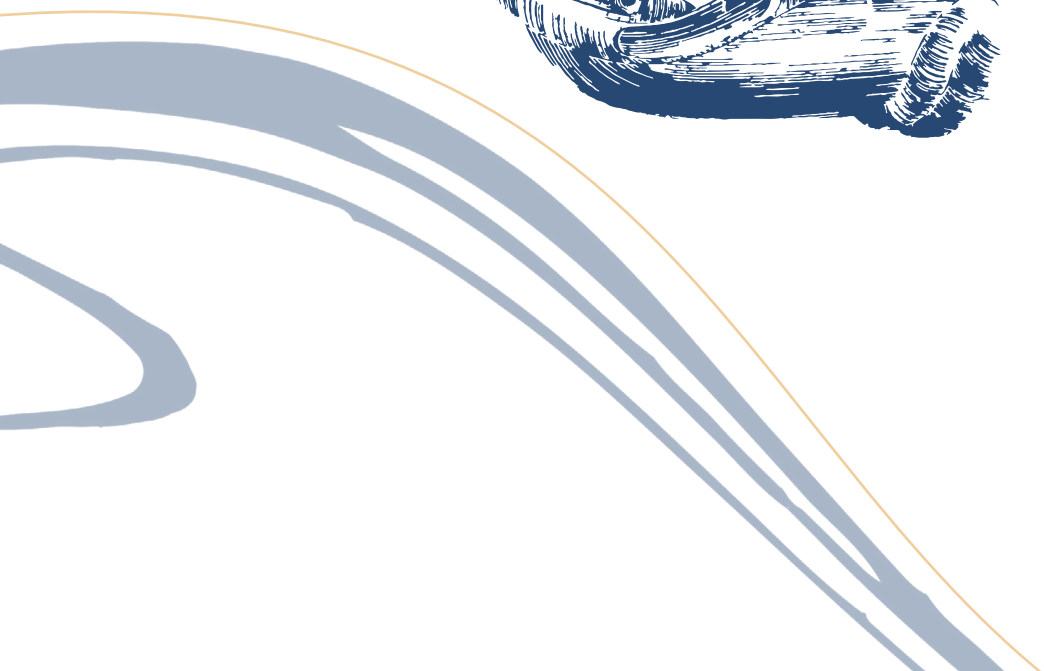
CONCLUSIONS

In conclusion, stabilisation of very preterm infants performing the PBCC approach results in considerably longer cord clamping times and is at least as effective as stabilisation according to the current routine DCC approach. Larger randomised clinical trials are needed to show potential beneficial clinical effects for preterm infants.

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6

CHAPTER 6

Ductal flow ratio as measure of transition in preterm infants after birth: a pilot study

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ABSTRACT

BACKGROUND

Cardiovascular changes during the transition from intra- to extrauterine life, alters the pressure gradient across the ductus arteriosus (DA). DA flow ratio (R-L/L-R) has been suggested to reflect the infant's transitional status, and could potentially predict neonatal outcomes after preterm birth.

AIM

Determine whether DA flow ratio correlates with oxygenation parameters in preterm infants at 1 hour after birth.

METHODS

Echocardiography was performed in preterm infants born <32 weeks gestational age (GA), as part of an ancillary study. DA flow was measured at 1 hour after birth. DA flow ratio was correlated with FiO_2 , SpO_2 and $\text{SpO}_2/\text{FiO}_2$ (SF) ratio. The DA flow ratio of infants receiving physiological-based cord clamping (PBCC) or time-based cord clamping (TBCC) were compared.

RESULTS

Measurements from 16 infants were analysed (median [IQR] GA 29 [27-30] weeks; birthweight 1176 [951-1409] grams). R-L DA shunting was 16 [17-27] ml/kg/min and L-R was 110 [81 – 124] ml/kg/min. The DA flow ratio was 0.18 [0.11-0.28], SpO_2 94 [93-96]%, FiO_2 was 23 [21-28]% and SF ratio 4.1 [3.3-4.5]. There was a moderate correlation between DA flow ratio and SpO_2 (correlation coefficient (CC) -0.415; $p=0.110$), FiO_2 (CC 0.384; $p=0.142$) and SF ratio (CC -0.356; $p=0.175$). There were no differences in DA flow measurements between infants where PBCC or TBCC was performed.

CONCLUSION

In this pilot study we observed a non-significant positive correlation between DA flow ratio at 1 hour after birth and oxygenation parameters in preterm infants.

INTRODUCTION AND RATIONALE

Directly after birth, major cardiovascular changes occur during transition from intra-uterine to extra-uterine life. These cardiovascular changes are triggered by lung aeration and entail a decrease in pulmonary vascular resistance (PVR) and a corresponding increase in pulmonary blood flow.^(1, 2) After umbilical cord clamping, there is an increase in the systemic vascular resistance (SVR) following the loss of the low resistance placental circulation.⁽³⁾ The pressure changes in both the pulmonary and systemic circulation that accompany transition are reflected in the ductus arteriosus (DA) flow.

DA flow has been evaluated in both clinical and preclinical studies to entirely flow from right-to-left (R-L) prior to birth, due a high PVR, with flow continuing throughout diastole due to retrograde flow in the pulmonary arteries.^(4, 5) As PVR decreases with lung aeration after birth, DA flow shifts from R-L flow to predominantly left-to-right (L-R) flow in the first 10 minutes after birth.⁽⁴⁻⁶⁾ It was suggested that echocardiographic measurements of DA flow ratio (R-L flow/ L-R flow) can be used as a measure of neonatal transitional, as duration, direction and the amount of DA flow are taken into account in this measure as well as pressure changes in the systemic circulation.⁽⁴⁾ DA flow ratio accurately reflects differential pressure changes between the pulmonary and systemic circulation. DA flow ratio is likely to be influenced by the increase in SVR after clamping and the degree of lung aeration and decrease in PVR. As such, it can be used to indicate a successful or disturbed neonatal transition, and therefore has the potential to predict (adverse) neonatal outcomes. However, data on these transitional changes in preterm infants is lacking.

Preterm born infants often fail to aerate their immature lungs and need respiratory support in order to transition from a fetus into a neonate. The success of transition is usually reflected by heart rate and pulmonary gas exchange efficiency, which is largely determined by the gas exchange surface area of the lungs and the gas diffusion distance, and is reflected by the need for altering the alveolar oxygen gradient. Currently, there are various versions of an oxygenation index (OI) used to identify hypoxic respiratory failure in neonates and paediatric patients.⁽⁷⁻⁹⁾ We recently used the $\text{SpO}_2/\text{FiO}_2$ (SF) ratio as OI to reflect the gas exchange potential after birth.⁽¹⁰⁾ The oxygenation of preterm infants in the first hour after birth not only reflects lung immaturity, but also the infants transitional status, particularly the degree of lung aeration. In this study, we aimed to investigate whether DA flow ratio is correlated with oxygenation parameters in preterm infants 1 hour after birth as a measure of the success of neonatal transitional.

Experimental and clinical studies showed that infants went through transition more successfully when physiological based cord clamping (PBCC) was performed.⁽¹¹⁻¹³⁾ As this observational study was performed while infants were recruited for an RCT comparing PBCC to time-based cord clamping (TBCC), we were able to compare the DA flow ratio between both groups.⁽¹⁴⁾

METHODS

This pilot study is an ancillary study to the Aeration Breathing and Clamping (ABC) 2 and 3, multicentre randomised controlled trials (NTR7194; NCT03808051) performed in the Leiden University Medical Centre (LUMC). The ABC trials compared PBCC to TBCC on both short and long term outcomes in infants born <32 weeks gestation.⁽¹⁴⁾ This study was approved by the Institutional Review Board of the LUMC.

Measurements

To assess DA flow and DA flow ratio, an echocardiographic examination was performed using a Vivid-S6 or Vivid-S60 (GE Healthcare) ultrasound system with a neonatal/paediatric 12S probe (4.0-11.0MHz) 1 hour after birth. Standard two-dimensional grey-scale images were acquired from the parasternal view and stored in digital format. The DA was visualised in the parasternal short axis view (figure 1). To assess velocity time integral (VTI), pulsed wave Doppler measurements were obtained. Using pulsed wave Doppler measurements, the peak velocity of the R-L and L-R shunt was assessed (figure 2). VTI evaluation of DA flow included analysis of three consecutive flow profiles, providing a mean VTI for each infant. Variation between the three consecutive flow profiles was calculated using standard deviation. DA shunt volume was calculated using the formula $((\pi \times \text{Annulus}^2 / 4) \times \text{HR} \times \text{VTI}) / \text{birthweight}$.⁽⁴⁾ The net difference in DA shunting was calculated (L-R flow – R-L flow) as well as the DA flow ratio (R-L flow VTI/L-R flow VTI) to assess relative differences between R-L versus L-R shunting, for each infant.⁽⁴⁾

Measurements of SpO₂ and FiO₂ were averaged over a period of 5 minutes directly prior to the echocardiographic examination and SF ratio was calculated subsequently. The SF ratio represents the gas exchange efficiency of the lungs and, next to lung immaturity, is largely influenced by the transitional status of the infant. The correlation between DA flow ratio and FiO₂, SpO₂ and SF ratio was calculated (SpO₂/FiO₂; maximum is 4.8; SpO₂ 100%/ FiO₂ 21%) as well as the correlation between L-R DA flow and FiO₂, SpO₂ and SF ratio.

For this pilot study a convenience sample was used. The enrolment was based on the availability of the researcher and whether the echocardiographic ultrasound measurements would not interfere with essential neonatal care.

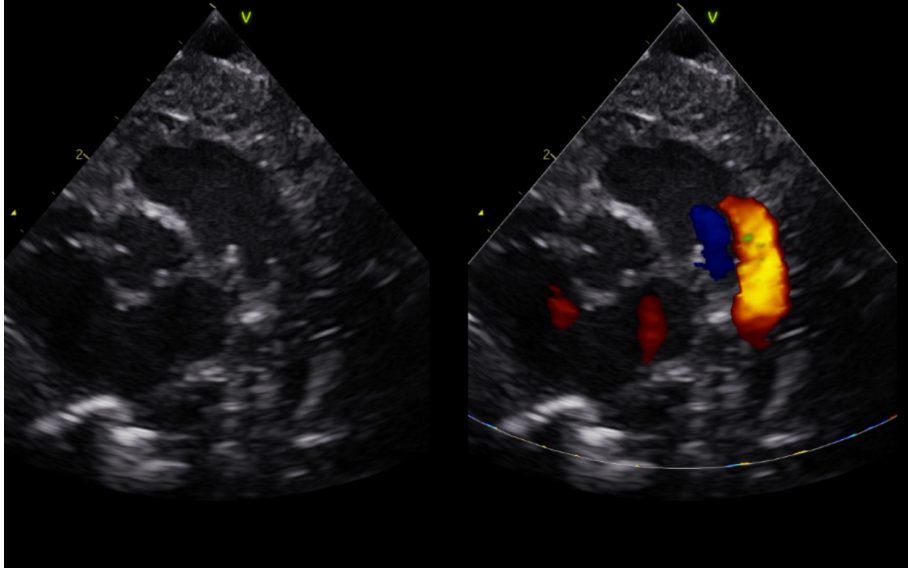


Figure 1 | Short axis parasternal view of ductus arteriosus and pulmonary arteries (3 vessel view)

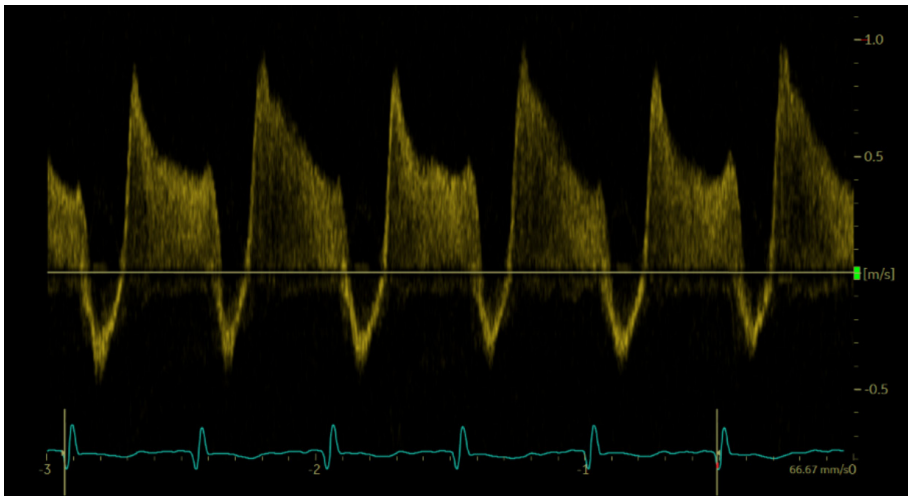


Figure 2 | Doppler pulse wave measurements of ductus arteriosus flow

Three consecutive flows profiles were used to average measurements.

Statistics

Data were analysed using SPSS software version 24.0 (SPSS IBM, Chicago, Illinois, USA). Results are presented as mean (standard deviation; SD), median [interquartile range; IQR] and n(%). Continuous data were analysed using the sample t-test or Mann-Whitney U test, if appropriate. Correlation between DA ratio and oxygenation parameters was determined using the Pearson correlation or Spearman rank correlation test, if appropriate. A two-sided p value of <0.05 was regarded as statistically significant.

RESULTS

Echocardiographic measurements were performed in 21 out of 47 infants in the period from June 2018 until November 2018 and from October 2019 until September 2020. Ultrasound measurements were not made in a total of 26 infants; in 13 infants the researcher was unavailable for measurements and in 13 infants measurements would have interfered with needed neonatal care. In 5 of 21 infants, DA flow could not be successfully analysed over a full cardiac cycle and measurements were therefore excluded, thus measurements from 16 infants (GA 29 [27 – 30] weeks; birthweight 1176 [951 – 1409] grams) were analysed (table 1). Echocardiographic measurements were obtained at a median time of 01:13:00 [01:07:00-01:24:00] hours after birth. One infant did not receive respiratory support during measurements, while all other infants received CPAP 7-8 cmH₂O with automatic FiO₂ control (SpO₂ target ranges 91%-95%). None of the infants received surfactant. SpO₂ and FiO₂ during measurements were 94 [93-96]% and 23 [21-28]% respectively, with an SF ratio of 4.1 [3.3-4.5] (table 2).

Table 1 | Baseline characteristics, N=16

Gestational age, weeks	29 [27-30]
Birthweight, grams	1176 [951-1409]
Female	11 (68.8)
Antenatal corticosteroids	
Yes, complete course	11 (68.8)
Yes, incomplete course	5 (31.3)
No	0 (0)
Caesarean section	7 (43.8)
Apgar 1 min	7 [4-8]
Apgar 5 min	8 [8-9]
Apgar 10 min	9 [9-10]
Respiratory support in delivery room	
None	0 (0)
CPAP	16 (100)
PPV	7 (43.8)
Intubation	0 (0)
FiO ₂ max	85 [50-100]

Values are presented as median [IQR] and n (%).

CPAP, Continuous positive airway pressure; PPV, positive pressure ventilation

Table 2 | Neonatal variables during echocardiographic measurements, n=16.

Respiratory support	
None	1 (6)
CPAP	15 (94)
SpO ₂	94 [93-96]
FiO ₂	23 [21-28]
Heartrate	152 [136-160]
Blood pressure	
Systolic	52 [47-58]
Diastolic	34 [26-41]
MAP	41 [34-49]
First neonatal blood gas	
pH	7.24 [7.16-7.30]
lactate	2.2 [1.6-6.1]

Values are presented as n (%) and median [IQR].

CPAP, Continuous positive airway pressure; MAP, Mean arterial blood pressure

Median R-L DA shunting was 16 [17-27] ml/kg/min, with a peak velocity of 0.52 [0.41-0.61] m/s. L-R shunting was 110 [81 – 124] ml/kg/min, with a peak velocity 0.83 [0.65-1.08] m/s. The net difference in DA shunting (L-R flow – R-L flow) was 89 [56-109] ml/kg/min. The DA flow ratio was 0.18 [0.11-0.28]. There was a moderate positive correlation between DA flow ratio and FiO₂ (correlation coefficient (CC) 0.384; p=0.142). There was a moderate negative correlation between DA flow ratio and SpO₂ (CC -0.415; p=0.110), and SF ratio (CC -0.356; p=0.175), however correlations remained non-significant (figure 3).

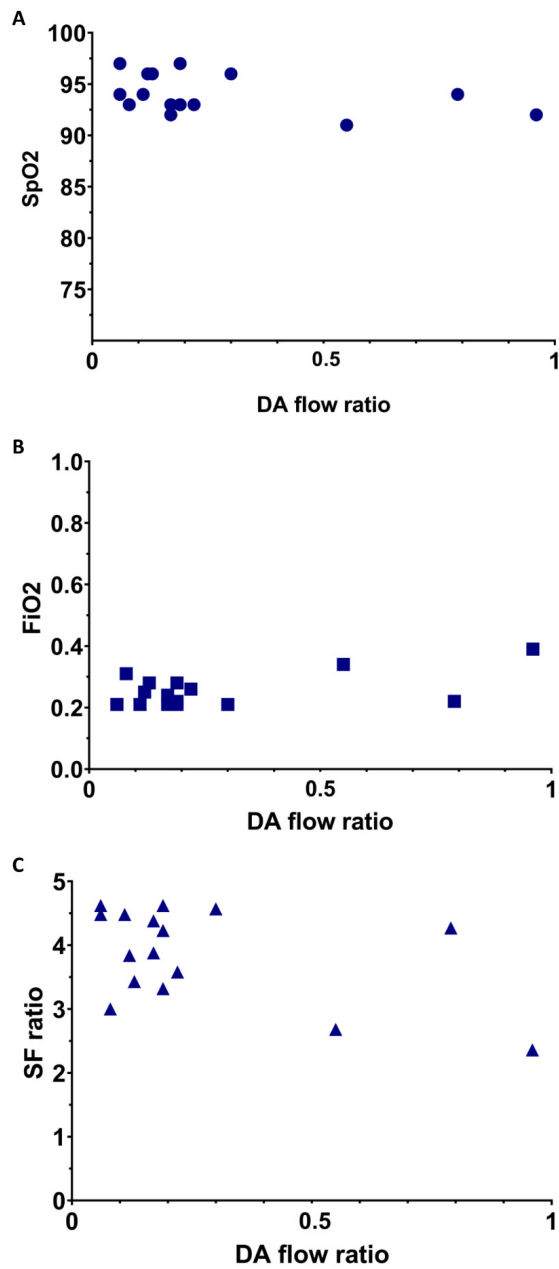


Figure 3 | Simple scatterplot of the relation between DA flow ratio with oxygenation parameters
A: Relation between DA flow ratio and the SF ratio. B: Relation between DA flow ratio and FiO_2 . C: Relation between DA flow ratio and SpO_2 .

DA flow measurements and ratio were compared between infants who received PBCC or TBCC. One infant was excluded from this analysis based on an increased risk for pulmonary hypertension, based on rupture of membranes nearly 2 weeks prior to birth. Thus, 15 infants were compared (8 PBCC vs 7 TBCC). We found no differences between groups for either R-L shunting (13 [8-44] vs 17 [14-23] ml/kg/min, $p = 0.30$), L-R shunting (113 [86-122] vs 97 [77-130] ml/kg/min, $p = 0.82$) or net difference in DA shunting (20 [7-23] vs 14 [10-19], $p = 0.42$). No differences were found in DA flow ratio between groups (0.12 [0.07-0.46] vs 0.19 [0.17-0.22], $p = 0.23$).

DISCUSSION

In this pilot study we evaluated, for the first time, DA flow measurements in preterm infants at 1 hour after birth. In this study we observed a bidirectional flow over the DA with a predominance of L-R shunting. While, the correlation between DA flow ratio and the SpO_2 , FiO_2 and SF ratio were not significant, the tendency towards significance (p values < 0.18) indicates that further investigation with a larger group of infants is required to determine whether the DA flow ratio has utility in indicating a successful or disturbed neonatal transition. Moreover, if DA flow ratio can successfully indicate a disturbed transition, based on the differential pressure changes in the pulmonary and systemic circulation, this measure can potentially predict underlying neonatal disease and (adverse) neonatal outcome.

We were able to successfully measure DA flow in 16 preterm infants at 1 hour after birth and demonstrated DA flow to be predominantly L-R, indicated by the shunting volumes as well as the small DA flow ratio. Our findings are difficult to compare with the current literature as echocardiographic measurements in preterm infants after birth have previously focused on the presence of DA patency and not on DA flow changes after birth.^(15, 16) However, we previously demonstrated that in term born infants the DA flow shifts from R-L to predominantly L-R in the first minutes after birth⁽⁴⁾ and remains bi-directional in the majority of infants on the first day of life. Similarly, we have now shown that preterm infants follow a similar temporal pattern.⁽¹⁷⁾ After birth, spontaneous breathing or assisted ventilation aerates the lung and thereby decreases PVR and increases pulmonary blood flow. This, combined with the increase in SVR that follows umbilical cord clamping, changes the pressure gradient across the DA and causes DA flow to reverse from R-L to predominantly L-R.⁽¹⁸⁻²⁰⁾ The cardiac cycle dependent bidirectional flow pattern within the DA is thought to be due to the time differences between when the pressure waves generated by ventricular contraction reach each end of the DA. Early during systole the pressure wave emanating from the right ventricle reaches the pulmonary artery end of the DA first, resulting in R-L flow. Then as the pressure wave generated by the left ventricle reaches the aortic end of the DA, the pressure

gradient across the DA reverses and the flow becomes L-R. As such, measuring DA flow after birth, particularly during diastole, provides a direct indication of the pressure gradient across the DA and is highly indicative of the PVR relative to SVR.

Based on the correlation coefficient DA flow ratio showed a moderate negative correlation with both SpO_2 and SF ratio and a moderate positive correlation with FiO_2 , however correlations remained non-significant. The negative correlation between SpO_2 and SF ratio and DA ratio were expected, as good oxygenation with little use of additional oxygen would indicate a successful transition, and therefore low DA ratio's. Similarly, the positive correlation with FiO_2 was expected, as high levels of additional oxygen would indicate a less successful transition, and therefore higher DA ratio's. All three oxygenation parameters are influenced by the changes during transition to extra-uterine life. After birth the primary site of gas exchange shifts to the lungs, after which oxygenation is largely determined by the surface area available for gas exchange, the gas diffusion distance and the partial pressure gradient for oxygen between the alveoli and adjacent capillaries. Thus, oxygenation depends on adequate lung aeration (surface area), gas diffusion distance and the amount of additional oxygen given during neonatal support (partial pressure gradient), all of which are largely dependent on the success of neonatal transition especially in preterm infants.^(21, 22)

The association between neonatal oxygenation and PVR has been demonstrated, with a reduced risk for an elevated PVR in preterm born infants with higher oxygenation targets (90-95%).⁽²³⁾ As both PVR and SVR are the main determinants of the DA pressure gradient, changes in PVR based on oxygenation status would subsequently result in changes in DA flow. In addition, studies investigating various oxygenation index's (such as SF ratio) demonstrated that SpO_2 is moderately correlated to PVR in lambs.⁽²⁴⁾ Nevertheless, while these oxygenation parameters can provide an indication of PVR, measuring DA flow using echocardiography would present a more direct and possibly a more realistic evaluation of PVR. If this is correct, this could explain why we observed moderate correlations between the oxygenation parameters and the DA flow ratio. As this was a pilot study with a small sample size and a relatively homogenous cohort, the correlations we observed were not statistically significant.

There are various factors that could influence DA shunting, i.e. changes in pulmonary pressure and haemodynamics, which could have had an effect on the obtained DA flow measurements. Infants in this study however, all received similar respiratory support, decreasing the possible influence of pressure change on our measurements. Changes in pulmonary venous return, heart rate, cardiac output and peripheral vascular resistance are all linked to either PVR or SVR, reflecting the neonatal transition, and will therefore be represented in the DA flow ratio.

Previous studies have already demonstrated that only 30-60 seconds of DCC has a positive effect on blood pressure and the need for inotropics in preterm infants in the first hours after birth.⁽²⁵⁻²⁷⁾ As PBCC improves hemodynamic stability during neonatal transition,^(13, 28) it is very well possible that this will further improves blood pressure for these infants. Therefore, DA flow could potentially demonstrate a more predominant L-R flow when compared to infants who received TBCC. However, the aim of this study was to investigate if DA flow ratio was correlated with oxygenation parameters and not to observe differences between groups who received different cord clamping strategies. Indeed, the convenience sample used for this study was too small to find a significant difference in DA flow ratio, but the results from this study could be used to calculate an appropriate sample size to demonstrate differences between groups. In a considerable number of infants who were eligible for this study echocardiographic measurement could not be obtained as the time point was not convenient and intervened with the clinical care, or the researcher was not available. When DA flow ratio were to be further assessed in a larger trial, echocardiographic measurements at different moments after birth would be recommended as well as having measurements performed by caregivers themselves.

In conclusion, this is the first study to report ultrasound measurements of DA flow at 1 hour after birth in infants born <32 weeks gestational age. No differences were found when comparing measurements of infants who received PBCC and TBCC. We observed a non-significant positive correlation between the DA flow ratio and oxygenation parameters in preterm infants. Whether this parameter reflects the state of neonatal transition remains to be further investigated.

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PART FOUR

General discussion and Summary





GENERAL DISCUSSION

INTRODUCTION

Approximately 1 in 10 babies are born premature, before 37 weeks of completed gestation.⁽¹⁾ Currently, prematurity remains the main cause of neonatal mortality and considerably increases the risk of neonatal morbidities. The risks of mortality and morbidities are directly associated with gestational age (GA) at birth, and infants born <28 weeks GA are at highest risk for adverse outcomes.⁽²⁾ While most preterm infants breathe spontaneously at birth, they often fail to achieve lung aeration and sufficient gas exchange due to the immaturity of their respiratory system. These infants require respiratory support to establish lung aeration and gas exchange in order to successfully transition from intrauterine to extrauterine life.^(3, 4) To provide this necessary support, the umbilical cord of preterm infants is clamped immediately after birth in order to transport the infant to the resuscitation table.⁽⁵⁾ Immediate cord clamping (ICC) was also recommended as part of a postpartum strategy to reduce the risk of post-partum haemorrhage, although evidence to support this was lacking.⁽⁶⁾

There has been a renewed interest in delaying the moment of cord clamping, which has led to an ongoing debate as to the optimal timing of cord clamping. By the late 1960s, studies already advocated delaying cord clamping so the infant could benefit from the transfer of extra placental blood, known as placental transfusion.⁽⁷⁾ More recently, several randomised trials were performed and demonstrated beneficial effects when the moment of cord clamping was delayed.⁽⁸⁾ The beneficial effects entailed an increase in haemoglobin and haematocrit, and a reduced risk of iron deficiency in term infants. Furthermore, several small trials in preterm infants showed that clamping the cord at 30-60 seconds after birth (delayed cord clamping; DCC) led to a higher level of haemoglobin, less need for blood transfusion, less intraventricular haemorrhage (IVH) and less necrotising enterocolitis (NEC) compared to ICC.⁽⁹⁾ A large international multicentre trial could not confirm these beneficial effects for preterm infants, but mortality was significantly lower when DCC was performed compared to ICC.⁽¹⁰⁾ When combining all trials in a meta-analysis, the risk of mortality and the need for blood transfusion were both lower and there was also a trend towards less IVH in preterm infants when DCC was performed.⁽¹¹⁾

Currently, DCC is recommended in infants who are not in need of resuscitation, based on the beneficial effects demonstrated in clinical studies.^(12, 13) Preterm infants in need of stabilisation or resuscitation were excluded from the studies and so ICC is currently still recommended for these infants. Furthermore, it is unclear whether infants that received DCC were in need of stabilisation, as this relied on the caregiver's interpretation. Indeed, the infant's vital parameters were not monitored, and infants did not receive respiratory support before the cord

was clamped.⁽⁹⁾ This is probably also the reason why a large proportion of infants who were allocated to DCC were not treated as intended and received ICC instead. Nevertheless, the demonstrated beneficial effects of DCC were attributed to placental transfusion, as infants were allowed time to receive this net increase in blood volume. Although placental transfusion was described in studies performed in the 1960s, the exact physiological mechanism responsible for the net transfer of blood volume from placenta to neonate remains elusive.

While delayed cord clamping studies have used placental transfusion as a rationale, recent experimental studies in preterm lambs demonstrated an even larger benefit when cord clamping was postponed until **after** ventilation onset and lung aeration had occurred (physiological-based cord clamping; PBCC).^(14, 15) These studies demonstrated that immediate cord clamping **prior** to ventilation reduces preload and cardiac output due to a loss of umbilical venous return.^(14, 16) The large swings in haemodynamic function increase the risk of IVH, NEC and associated increased rates of mortality and morbidity.⁽¹⁷⁾ Deferring cord clamping until after ventilation onset sustains preload and cardiac output, and avoids the large disturbances in systemic and cerebral haemodynamics and oxygenation during transition.^(14, 16) Although the physiological effect of PBCC has been clearly demonstrated in experimental studies, the benefits in human infants requires further investigation.

The studies described in this thesis were designed to improve the current state of knowledge of the physiological mechanisms underpinning umbilical cord clamping strategies. In this section the studies performed are discussed and related to what is currently known about the effects of these strategies. Specifically, we will discuss 1) spontaneous breathing as a possible driving force for placental transfusion, and 2) the implementation of PBCC in a clinical setting as well as physiological changes during fetal to neonatal transition when PBCC is performed.

G

PLACENTAL TRANSFUSION

Placental transfusion as a result of DCC is a widely accepted phenomenon and has been the subject of several studies. One of the first studies that aimed to demonstrate placental transfusion, or the net shift in blood volume from placenta to neonate, measured increasing neonatal blood volumes, using ¹²⁵I-labelled human serum albumin, after increasing delays in cord clamping. They also measured a decrease in residual placental blood volume, with an increasing delay in cord clamping.⁽⁷⁾ However, as ICC after birth can have a severe impact on cardiovascular function,⁽¹⁴⁾ it is possible that the measuring techniques used in that study were not optimised for newborn infants. The reduced cardiovascular

function following ICC could have reduced the mixing efficiency of the labelled albumin throughout the circulation, which is necessary for an accurate volume estimate. The lower blood volumes measured in the ICC group could therefore also have reflected the lower cardiovascular function.

Measuring neonatal weight during DCC is potentially a more robust method for assessing placental transfusion, and indeed this was demonstrated in healthy term infants.⁽¹⁸⁾ Neonatal weight increased during DCC with 24-32 ml/kg over the first 5 minutes of life. However, the authors clearly stated the difficulties of obtaining measurements, as artefacts were easily introduced due to movement or touching of the infant.

Studies demonstrated that, when compared to ICC, DCC led to an increased blood flow in the superior vena cava,^(19, 20) a higher haemoglobin and haematocrit⁽⁸⁾ and a reduced residual placental blood volume.^(7, 21, 22) Although these were all indirect measurements of a net increase in neonatal blood volume, placental transfusion became a broadly accepted feature of DCC and has become synonymous with this procedure. As such, the benefits of DCC, which is currently recommended by international (resuscitation) guidelines, have been entirely attributed to placental transfusion,⁽²³⁾ while the underlying mechanism responsible for placental transfusion remains unclear.

Various theories have been raised to explain the mechanism responsible for placental transfusion.^(24, 25) One of these theories was that gravity acts as a driving force, leading to an increase in placental transfusion if infants were held below the introitus during DCC.^(24, 26) However, in a recent large randomised trial using neonatal weight increase after birth as a measure of placental transfusion, no difference was observed between infants held above or below the introitus.⁽²⁷⁾ In addition, in a preterm lamb model, placental transfusion was measured using umbilical blood flows and a biotin-labelled red blood cell technique.⁽²⁸⁾ There were no detectable changes in placental transfusion if the lamb was held below the mother, confirming the findings of the human trial, but there were also significant adverse effects on umbilical blood flows.^(27, 28) As both experimental and clinical studies demonstrated that gravity does not increase placental transfusion, it is unlikely to be a driving force behind it.

The experimental data examining the effect of gravity on placental transfusion showed that arterial and venous umbilical flow are closely interrelated, as would be expected since they form an integral part of the fetal circulatory system.⁽²⁸⁾ When placing the lamb below the mother, umbilical arterial blood flow decreased simultaneously with a reduction in umbilical venous blood flow. The reduction in umbilical venous flow, which occurred despite the assistance of gravity, was

thought to result from the decrease in umbilical arterial flow. As such umbilical arterial blood flow into the placenta is a major determinant of venous flow out of the placenta. This mechanism was further substantiated when the lamb was placed above the mother. While gravity reduced, it did not halt umbilical venous flow, which indicates venous pressure within the placenta must have increased to exceed the pressure head caused by the vertical height difference.⁽²⁸⁾ Nevertheless, these findings showed that what goes into the placenta largely determines what comes out of it, and positional changes do not enhance placental transfusion.

It has been suggested that uterine contractions during labour may also be a driving force for placental transfusion. After birth, while the placenta is still attached to the uterus, it has been suggested that uterine contractions “squeeze” blood out of the placenta towards the infant with each contraction.⁽²⁵⁾ However, this assumption is not consistent with the observed changes in uterine and placental blood flow. A reduction in uterine and fetal placental blood flow and fetal oxygenation is present during uterine contractions, resulting in intrapartum decelerations of fetal heart rate.^(29, 30) In addition, fetal hypoxia is associated with a reduction in umbilical venous flow, and uterine contractions have been shown to increase umbilical vascular resistance. This suggests that uterine contractions are likely associated with a reduced umbilical blood flow. Therefore, as uterine contractions are likely to markedly reduce (rather than increase) umbilical venous blood flow, the theory that uterine contraction is a driving force for transfusion can be refuted.^(29, 30)

Spontaneous breathing has also been suggested as a driving force for placental transfusion. The sub-atmospheric intrathoracic pressures created during inspirations are thought to increase umbilical venous flow towards the infant during delayed cord clamping. The subsequent increase in umbilical venous return could explain both the maintenance of cardiac output as well as the net increase in neonatal blood volume associated with DCC.^(30, 31) Indeed, studies that measured residual placental blood volumes as an indication of placental transfusion showed that residual placental blood volumes were reduced if the onset of respiration occurred prior to cord clamping.^(21, 22) In addition, prenatal ultrasound studies in human fetuses have shown that fetal breathing movements (FBM) are associated with a distention of the umbilical vein and an increase in umbilical venous blood flow. In contrast, they also showed that inspiratory movements were associated with a concentric reduction in the cross-section of the inferior vena cava (IVC) and a significantly reduced IVC blood flow.^(32, 33) A recent observational study using ultrasonography demonstrated that umbilical blood flow continues for much longer than previously assumed during delayed cord clamping after birth. In addition, the umbilical venous blood flow pattern was intermittent, with umbilical venous blood flow increasing and decreasing at a frequency that most likely reflected respiratory rate, while crying stopped or even reversed flow. These

findings suggested that spontaneous breathing may influence umbilical venous flow, although the exact relationship between inspiration and expiration and umbilical venous flow could not be assessed as breathing was not recorded.⁽³¹⁾

In **chapter 1** we examined the effect of spontaneous breathing on umbilical venous flow in newborn lambs during DCC. In contrast to the findings in human infants, we observed an inverse correlation between inspiration depth and umbilical venous flow, as flow decreased or ceased during deep inspirations.⁽³¹⁻³⁴⁾ This prompted us to investigate the effect of spontaneous breathing on blood flow in the hepatic vein (HV) and ductus venosus (DV) (as a measure of umbilical venous flow) in infants at birth. In this study, described in **chapter 2**, we observed that blood flow in both vessels flows in an antegrade direction (towards the infant) and increases during inspiration. We also observed that in the majority of inspirations the IVC collapsed. This collapse was consistently located directly caudal to the inlet of the HV and DV into the subdiaphragmatic venous vestibulum. These observations confirmed previous prenatal and perinatal ultrasound studies in humans, but still differed from our experimental findings in newborn lambs.⁽³¹⁻³⁵⁾ We now speculate that the different effect of breathing can be explained by the anatomical differences of the DV and IVC in relation to the diaphragm between lambs and humans. In contrast to humans, lambs have a lengthy (3-4 cm) intrathoracic segment of the IVC and the DV joins the IVC before it enters the chest. As such, diaphragmatic contractions therefore reduce both DV flow as well as IVC.⁽³⁶⁾ However, in the human circulation the IVC, DV and HV pass through the diaphragm enter the subdiaphragmatic venous vestibulum via separate inlets, before continuing directly into the right atrium (RA). This is the most likely explanation for the different effects of respiration on DV and IVC flow in humans versus sheep.

Based on the human data we provided, inspiration seems to be associated with an increase in antegrade flow in both the umbilical vein and DV. In addition, there seems to be a correlation between inspiration and the collapse of the IVC, which presumably leads to a simultaneous decrease in blood flow in the IVC. When combined, these two findings indicate that during inspiration placental blood flow is preferentially directed towards the RA. This preferential blood flow could explain the increase in neonatal blood volume observed during DCC in spontaneously breathing infants,⁽¹⁸⁾ and could also explain why infants who breathe prior to umbilical cord clamping have less residual placental blood volume.⁽²²⁾ As such, spontaneous breathing may partially explain the net increase in neonatal blood volume by providing a driving force for placental transfusion.

It is likely that the effects of spontaneous breathing on circulation at birth are not limited to umbilical flow and venous return. Indeed, we observed another effect in our study where we compared preductal (right hand) and umbilical pulse oximetry in preterm infants at birth. Although, plethysmography was of equally good quality at both locations, heart rate (HR) measured at the umbilical cord was consistently lower when compared to measurements at the right hand (**chapter 3**). This finding was in line with previous studies demonstrating that, when compared to ECG, counting HR by umbilical cord palpation led to underestimation of the HR.^(37, 38) This underestimation was attributed to miscalculation by the caregiver,⁽³⁹⁻⁴¹⁾ but based on our recent findings it is possible that the observed HR difference is a true physiological phenomenon. Indeed, a difference in pulse waves between pre and post ductal vessels has been observed in experimental animal models.⁽⁴²⁾ It is possible the negative intra-thoracic pressures created during inspiration leads to an increase in left to right (L-R) shunt through the ductus arteriosus (DA), which then creates a disruption in the systemic blood flow towards the lower body.⁽⁴³⁾ This would subsequently lead to a reduced volume change in the umbilical vessel during a heartbeat, which is then not detected by the plethysmography algorithm. This warrants further investigation but could explain why differences in plethysmography waveforms were observed between the preductal and umbilical measurements. If systemic blood flow is indeed disrupted by inspiration while the DA remains intact, evaluation of an infant's clinical condition based on HR by palpation of the umbilical cord should be reconsidered, as this will lead to underestimation of the true HR.

PHYSIOLOGICAL-BASED CORD CLAMPING

While DCC has been shown to be beneficial for both term and preterm infants,^(8, 9) these benefits have been largely attributed to placental transfusion. However, recent experimental studies demonstrated an even larger benefit when PBCC is performed. Prior to birth, the placenta is the primary site of gas exchange, and normally has a low resistance with a highly compliant vascular bed to ensure that it receives a large proportion (30-50%) of fetal cardiac output.⁽³⁶⁾ Oxygenated blood from the placenta flows through the umbilical vein and is directed through the DV towards the left atrium, via the Eustachian valve and foramen ovale, thereby providing the majority of preload for the left ventricle.⁽³⁵⁾ As such, left ventricular output is largely dependent on this placental venous return, as the majority ~50% of left ventricular preload is derived from the placenta. Deoxygenated blood from the superior and inferior vena cava is directed towards the common pulmonary trunk, via the RA.⁽³⁵⁾ The lungs are liquid-filled and as pulmonary vascular resistance (PVR) is high, most of the blood exiting the right ventricle is directed towards the systemic circulation through the ductus arteriosus (figure 1A).

DISCUSSION AND SUMMARY

After birth, liquid is cleared from the airways, to allow the entry of air due to the sub atmospheric pressures generated during inspiration. Lung aeration triggers a sudden decrease in PVR and a large increase in pulmonary blood flow.^(30, 44) Once pulmonary blood flow has increased, cardiac output is less dependent on placental venous return as the supply of preload for the left ventricle has switched to pulmonary venous return. When the umbilical cord is clamped **after** the lungs have aerated, cardiac output remains stable while the lungs function as the primary source of gas exchange (figure 1B).⁽¹⁴⁾ However, when the umbilical cord is clamped **prior** to lung aeration, which is often the case in preterm infants, cardiac output is compromised as pulmonary blood flow and subsequent pulmonary venous return remain low and placental venous return is now absent, resulting in reduced preload for the left ventricle. The cardiovascular instability resulting from cord clamping prior to lung aeration produces large fluctuations in cardiac output, blood pressure, and blood flow,^(14, 15) which likely increases the risk of adverse outcomes in preterm infants, such as IVH, NEC, and death. PBCC, then, has the potential to decrease the risk for adverse outcomes.

In most clinical studies that compared DCC with ICC, the moment of cord clamping was based on a fixed time.^(8, 9) However, when performing DCC in healthy term infants who spontaneously breathe directly at birth, the lungs are likely to be sufficiently aerated before the defined moment of cord clamping. This means that when time-based DCC was performed the criteria for PBCC were also met, and infants received the haemodynamic benefits of PBCC that have recently been demonstrated in experimental studies.^(14, 16, 45, 46) The haemodynamic stability that accompanies PBCC has the potential to improve neonatal outcomes. It is therefore possible that part of the beneficial effects that were demonstrated for DCC in these studies could be attributed to the haemodynamic effect of PBCC.

As in the clinical studies of healthy term infants, the studies investigating the effect of DCC in preterm infants also delayed clamping of the cord for only 30-60 seconds. However, in contrast to healthy term infants, most preterm born infants have difficulty aerating their lungs within 30-60 secs of birth, and so a proportion of these infants might not have (sufficiently) aerated their lungs at the moment of cord clamping. In this case, they will not have received the haemodynamic benefits that accompany PBCC. As the risks of neonatal mortality and morbidities are directly associated with GA, preterm infants especially could benefit from the improved haemodynamic stability of PBCC. In addition, the vital parameters of the infants receiving DCC were not monitored; the decision on whether the cord should be clamped before 60 seconds depended on the caregiver's interpretation.⁽⁹⁾ This could have led to a delay in stabilisation or unneeded crossover to immediate clamping. While these studies had some limitations, DCC for 30-60 seconds was still demonstrated to have beneficial effects for preterm infants. However, these

beneficial effects might be greater if PBCC were performed.

When translating PBCC to human infants, it is important that lung aeration and the subsequent increase in pulmonary blood flow are established. This indicates that respiratory support should be initiated prior to the moment of cord clamping in infants who have difficulty aerating their lungs. It also indicates that the moment of clamping should not be fixed (time-based) but should depend on when the infant establishes lung aeration, which varies per infant (i.e. physiological-based). Before embarking on large trials to evaluate important clinical outcomes, studies are needed to investigate whether true PBCC can be performed in a clinical setting and whether a direct effect on haemodynamic stability can be observed, similar to the experimental studies.

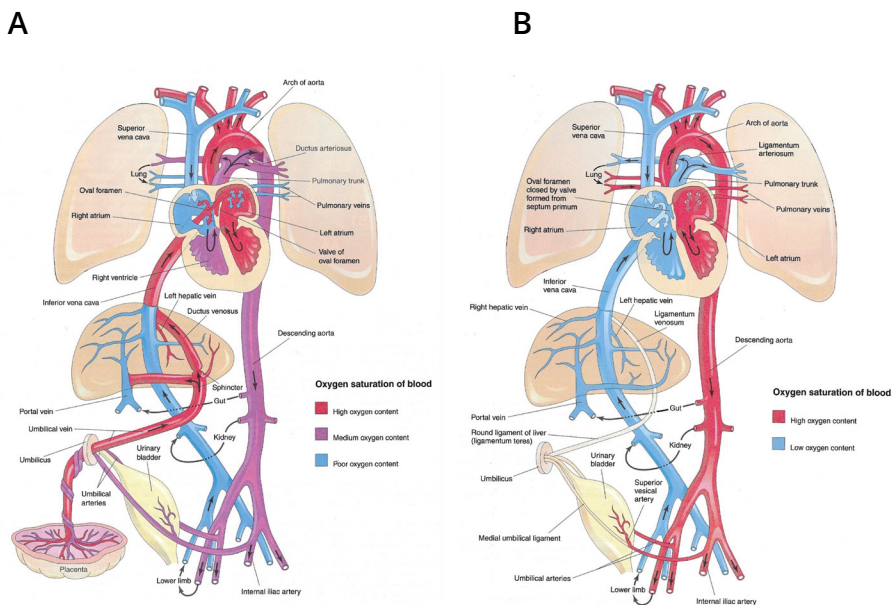


Figure 1 | Circulation before (A) and after (B) birth

"This figure was published in *The Developing Human – Clinically Oriented Embryology*, 8th edition, KL Moore & TVN Persaud, Page 328-329, Copyright 2008 by Saunders, an imprint of Elsevier Inc."

Before being able to translate PBCC to clinical practice, several issues needed to be addressed.⁽¹⁷⁾ For instance, when performing PBCC whilst delivering respiratory support it is necessary to continuously assess the cardiopulmonary condition in order to determine if the infant is adequately stabilised and if the optimal moment of umbilical cord clamping has been reached. We predefined 'stabilised' by the following criteria: HR > 100bpm, adequate oxygen saturation (SpO₂) > 90%, fractional inspired oxygen (FiO₂) < 0.4 whilst spontaneously breathing.^(45, 47) When reaching the moment of stabilisation, these criteria inform the clinician that it is likely that the lungs have been aerated, pulmonary blood flow has increased, and the umbilical cord can be clamped with minimal haemodynamic disturbances.⁽¹⁷⁾ Also, when performing PBCC it is important that the umbilical cord is not stretched or kinked, so umbilical cord length is an important limiting factor for this approach. During PBCC, infants should be stabilised as close as possible to the mother in order to include infants with a short umbilical cord. In addition, normal precautions should be taken to prevent hypothermia during the PBCC procedure.⁽¹⁷⁾ Furthermore, the neonatal team should be able to adequately provide respiratory support without obstructing the obstetric team's maternal care, as they need to assess maternal blood loss and uterine contractions, and monitor the second twin in case of a twin pregnancy. In order to translate PBCC into clinical practice and overcome the issues mentioned, changes are needed in the working logistics for both the neonatal and obstetrical personnel. In addition, new equipment is needed to enable neonatal stabilisation, according to standard care, with an intact umbilical cord. The neonatal and obstetrical team should be trained in this new procedure, and communication is essential to optimise this multidisciplinary team approach.

Providing respiratory support to (very) preterm infants prior to the moment of cord clamping has only been investigated by a few studies.⁽⁴⁸⁻⁵²⁾ In these studies, a bedside trolley was used or resuscitation was performed while the infant was positioned on the mother's legs or abdomen. While these studies reported the approach to be feasible in infants, with success ranging between 59% and 100%, the moment of clamping was still time-based (between 60 seconds and 3 minutes) and no true PBCC was performed.^(49, 52, 53)

We considered that performing true PBCC was not feasible using the currently available resuscitation tables, and therefore a new resuscitation table was constructed: The Concord birth trolley. This mobile resuscitation table is designed to provide the highest standard of care for preterm infants while the umbilical cord remains intact. All equipment needed for stabilisation and/or resuscitation is included to ensure that even infants in need of immediate resuscitation can receive the potential beneficial effects of PBCC. In addition to the potential cardiovascular benefits, the PBCC approach allows for both parents to remain

close to their baby in the first minutes after birth.⁽⁴⁵⁻⁴⁷⁾ To investigate the PBCC approach in clinical care, using the Concord, a clinical project (Aeration, Breathing, then Cord clamping; ABC trials) was started for which three studies were designed: a feasibility and safety study, an effectivity study and a efficiency study.

During these ABC projects, the moment of cord clamping depended on the transitional status of the infant and was not based on a fixed time. We could do this as we aimed to provide all the standard care needed, while waiting with clamping until the criteria were met. Based on previous studies in which physiological parameters of preterm infants were measured during transition, infants were considered to have reached lung aeration and an increase in pulmonary blood flow when they were stable and breathing with a HR > 100 bpm and SpO₂ > 90% while the FiO₂ was less than 0.4.^(45, 47) The ability to truly perform PBCC in a clinical setting was demonstrated in the ABC1 study, which therefore presented the proof of concept (**chapter 4**).⁽⁴⁵⁾ Indeed, cord clamping based on the infant's clinical condition led to variable clamping times (4:23 [3:00-5:11] min:sec) and no bradycardia occurred after the cord was clamped. After having shown the feasibility of the PBCC approach in the ABC 1 study, we also demonstrated the approach to be non-inferior to standard time-based DCC in the time needed to stabilise preterm infants according to the predefined criteria.^(45, 47) Indeed, when performing an 'as treated' analysis, infants who received PBCC were stabilised significantly faster than infants who received the time-based DCC approach, indicating the superiority of the PBCC approach (**chapter 5**).⁽⁴⁷⁾ Moreover, we demonstrated that this true PBCC approach could successfully be performed in > 89% of infants in both the ABC 1 and ABC 2 studies, with unsuccessful events declining over time which suggests a caregiver learning curve.^(45, 47)

In our ABC studies, physiological parameters such as HR and SpO₂ were continuously monitored during neonatal stabilisation while the cord was intact. Previous studies that investigated respiratory support with the cord intact did not report HR and SpO₂ or did not record them continuously, making it difficult to compare our findings with the literature.^(48, 49, 52) However, in our cohort we found that HR was higher and more stable than previously described for other methods of cord clamping.^(54, 55) In addition, we observed no bradycardia and a stable HR at the moment of cord clamping when performing PBCC, suggesting that pulmonary blood flow had successfully increased and that cardiac output therefore was less dependent on placental venous return. These observations correspond with the results of the experimental PBCC studies and imply that the preterm infants were haemodynamically stable, with a gradual transition and without adverse events.^(14, 16) When performing PBCC, infants also experienced less hypoxemia in the first 10 minutes of life while less oxygen was needed compared to infants who received cord clamping within 60 seconds of birth.^(54, 56, 57) The stable HR and the decrease in the duration of hypoxemia is likely to be the effect of haemodynamic stability

created by PBCC. The stable cardiac output, as part of the haemodynamic stability, provides a stable supply of oxygenated blood and explains the higher SpO_2 values and absence of bradycardia after PBCC. Avoiding hypoxemia and bradycardia, especially in the first minutes after birth, is pivotal as this is associated with an increased risk for IVH and death.⁽⁵⁸⁾

Failure to successfully transition to extra-uterine life increases the risks for neonatal morbidities and mortality. Adequate assessment of neonatal respiratory and cardiovascular transition after birth is therefore critical.⁽¹³⁾ If the neonatal transitional status could be measured in the first hours after birth, this could potentially be used as a predictor for (adverse) neonatal outcome. While both HR and SpO_2 are indicative of the infant's clinical condition, DA flow ratio is likely to give a better estimate of PVR and neonatal transition. We therefore obtained echocardiography measurements of DA flow in preterm infants who had received either PBCC or standard DCC in the ABC 2 and ABC 3 trial as part of a feasibility study (**chapter 6**).⁽⁴⁷⁾ DA flow measurements were obtained at 1 hour after birth using a short axis parasternal view. DA flow ratio was calculated for all infants and was correlated with FiO_2 given, SpO_2 and $\text{SpO}_2/\text{FiO}_2$ ratio (SF) and the DA flow ratio of infants receiving PBCC or time-based DCC were compared. Similar to measurements in term born infants, we found DA flow to have shifted to predominantly L-R and to remain bi-directional.^(59, 60) In addition, DA flow ratio showed a moderate negative correlation with both SpO_2 and SF ratio and a moderate positive correlation with FiO_2 . All three oxygenation parameters are largely dependent on the success of neonatal transition, especially in preterm infants.^(61, 62) However, as both systemic and pulmonary vascular resistance are the main determinants of the pressure gradient over the DA, and therefore DA flow, DA flow measurements potentially present a more direct and possibly a more realistic evaluation of PVR and neonatal transition. As PBCC improves haemodynamic stability during transition,^(45, 47) we expected DA flow measurements to demonstrate a more predominant L-R flow compared to that of infants who received the time-based DCC approach. However, due to a small sample size, we did not observe a significant difference in DA flow measurements between infants who received PBCC or DCC. Nevertheless, obtaining DA flow measurements at 1 hour after birth is feasible and our current findings provide a strong incentive for further research in using DA flow ratio as a research or clinical parameter for neonatal transition.

LIMITATIONS

In **chapters 1 and 2** the effects of spontaneous breathing on umbilical venous flow and placental transfusion are described. In both studies, the quality of measurements was often hampered by movements of the infant, either spontaneously or caused by the handling of the caregiver. The recordings were reviewed second by second, and any measurements containing artefacts were excluded from the analysis in order to observe the true effect of breathing on umbilical blood flow. Nevertheless, as the patterns observed were quite consistent, the data was sufficient to demonstrate the effect of breathing on blood flow in human infants, which was quite different from that observed in lambs.

In **chapter 3**, the results of an observational study on measuring umbilical heart rate are described. This study is limited by the sensor and algorithm of the pulse oximeter used, which were not designed to measure heart rate at the umbilical cord. Even though the pulse oximeter algorithm might have difficulties detecting a signal through tissues other than skin and subcutaneous fat of the hand and feet, we were able to detect a reliable heart rate in all infants. The results of this small observational study might not be conclusive; however, it creates a rationale for further investigation.

Chapters 4 and 5 describe the feasibility and efficacy of the PBCC approach using the Concord resuscitation table. Infants were only eligible for inclusion after having received antenatal consent from both parents. Women who gave birth soon after hospital admission were therefore not approached. Infants born by emergency caesarean section were also ineligible due to insufficient time for equipment set-up. However, these infants could potentially benefit most from the PBCC approach and their inclusion would increase generalisability. In addition, the study described in **chapter 4** was not powered for physiological measurements and measurements were only available in 26 out of 37 infants. However, the physiological measurements obtained demonstrated little variability (small IQR) and are therefore likely to represent a good estimation.

In **Chapter 6** the results of an observational study on ductal flow measurements is described. Although echocardiographic measurements were easy to perform, measurements in a considerable number of infants could not be obtained at 1 hour after birth, either because it would have interfered with clinical care or because the researcher was not available during non-daytime hours. Indeed, the sample size was too small to find significant differences between groups. However, results from this study create a rationale for further investigation, and the current data can be used to calculate an appropriate sample size.

GENERAL CONCLUSION

In this thesis we have investigated spontaneous breathing as a possible driving force for placental transfusion and the implementation of PBCC in a clinical setting, as well as the physiological changes during neonatal transition when PBCC is performed. Spontaneous breathing at birth, and more importantly lung aeration, is pivotal to accomplish the increase in pulmonary blood flow and decrease in pulmonary vascular resistance needed for a successful transition to extra-uterine life. However, based on the studies described in this thesis, spontaneous breathing has more haemodynamic effects at birth. Indeed, we demonstrated spontaneous breathing to influence umbilical blood flow as inspiration was associated with an increase in umbilical venous blood flow in humans. When combined with the association between inspiration and collapse of the IVC, this potentially preferentially directs placental venous return through the foramen ovale and into the left atrium. It is possible that the anatomical differences between humans and the animal models used have prevented the experimental studies from demonstrating a similar association. Moreover, we found spontaneous breathing not only to influence umbilical and pulmonary blood flow but also to cause a disruption of the systemic blood flow towards the lower body while the DA remains intact. We therefore conclude that spontaneous breathing is likely to be a driving force in placental transfusion.

Experimental studies have clearly demonstrated the beneficial physiological effects of PBCC and that lung aeration, with the subsequent prompt increase in pulmonary blood flow, is vital for the success of this approach. When successfully translating PBCC to human infants it is pivotal that lung aeration has been established. The moment of cord clamping should therefore not be at a fixed time but rather based on the infant's transitional status. With the studies performed in this thesis we demonstrated the proof of concept for performing PBCC in preterm infants in a clinical setting: that it is both possible and safe, and that a similar effect can be observed as in an experimental setting. We also demonstrated that resuscitation on the cord is at least as effective and potentially even superior to the standard approach, while allowing longer and variable cord clamping times.

FUTURE PERSPECTIVES

The physiological mechanism responsible for the net transfusion of blood volume from the placenta and into the neonate has long remained elusive. While we demonstrated in this thesis that spontaneous breathing is likely to be a driving force for placental transfusion, more detailed data is needed to better understand the placental transfusion and how infants can benefit from this in an optimal

manner. We were not able to quantify the net increase in blood volume and how much breathing would influence this. To measure this in a clinical setting, probes placed around the umbilical vessels measuring blood flow would be ideal but are not yet available. This would also allow us to measure the effect of breathing on umbilical blood flow set out in time.

Preterm infants often receive positive pressure ventilation. While we demonstrated the effect of sub atmospheric pressures generated during breathing, it is likely that positive pressure ventilation might lead to a different effect. While these infants may potentially still benefit from the haemodynamic effects of PBCC, the effect of ventilation on placental transfusion is not clear. The effect of ventilation on umbilical blood flow, creating a positive intrathoracic pressure, should therefore be further evaluated. The anatomical differences between humans and sheep should also be considered when deciding whether to investigate this experimentally or clinically.

Palpation of the umbilical cord to determine HR is often used to obtain an indication of the clinical condition of an infant. However, we demonstrated umbilical HR to be consistently lower when compared to the HR measured at the right hand (preductal). We speculated that this difference could be explained by a disruption in the systemic blood flow towards the lower body, caused by an increased left-to-right DA shunt based on the sub atmospheric pressures created during inspiration. If this is indeed true, determining the clinical condition of an infant based on a HR obtained by palpation of the umbilical cord needs to be reconsidered. Echocardiographic measurements of the effect of spontaneous breathing on systemic blood flow and pulse waves directly at birth is therefore needed to investigate this further.

If the neonatal transitional status could be measured in the first hours after birth, this measurement could be used as a predictor for (adverse) neonatal outcome. We demonstrated DA flow ratio to be correlated to oxygenation parameters that depend on the success of neonatal transition. However, DA flow ratio might provide a more direct and realistic evaluation of PVR and neonatal transition. We demonstrated that it was feasible to obtain DA flow measurements at 1 hour after birth. However, the usefulness of DA flow ratio as a research or clinical parameter of neonatal transition still needs to be determined. This would require a large observational study. Echocardiographic measurements should be obtained at various times to determine the optimal moment of obtaining this measurement.

Before implementing PBCC as standard practice, more knowledge of long-term outcomes of preterm infants having received this approach is needed. Implementing the PBCC approach now, based on the current feasibility and efficacy trials, would nullify the incentive to further investigate long-term effects in a randomised trial.

DISCUSSION AND SUMMARY

If PBCC indeed improves long-term neonatal outcomes and intact survival, this approach could improve and save neonatal lives globally. However, before this approach can be implemented on a large scale, there should be a trial to determine whether this hypothesis is true. A large randomised controlled trial, comparing neonatal outcomes of infants who were stabilised with either the PBCC approach or standard delayed/immediate cord clamping, is therefore needed.

In addition, investigating the parental perspectives of the PBCC approach are likely to be of added value. During delayed or immediate cord clamping the woman and infant are separated quickly after birth, which interferes with mother-child bonding. As both parents can stay close to their infant during PBCC, this can be beneficial for the bonding process.

Furthermore, the improved haemodynamic stability that accompanies the PBCC approach during neonatal stabilisation could also be beneficial for infants who fail to go through transition due to reasons other than prematurity. Experimental studies have already shown that PBCC is beneficial in infants with a congenital diaphragmatic hernia and in asphyxiated infants, although the mechanisms are different. While these experimental studies have shown great potential and clinical studies in infants with CDH are currently being undertaken, it will be a logistical challenge to design a trial to demonstrate the benefits in asphyxiated infants.

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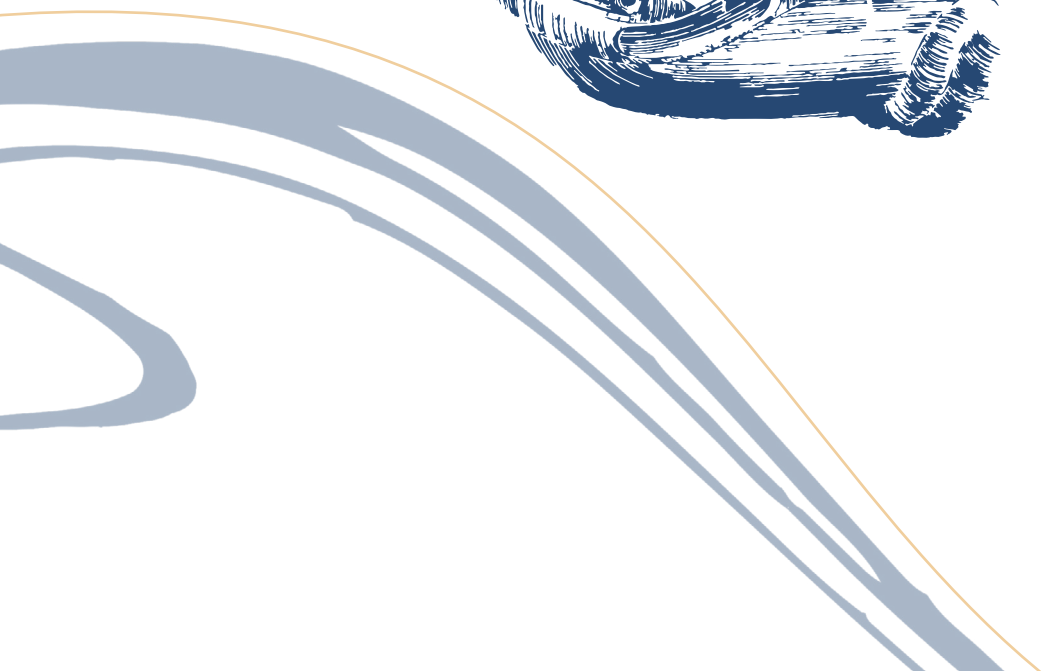
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SUMMARY

INTRODUCTION

Clamping and cutting the umbilical cord after birth is a normal and necessary procedure, but the ideal moment to do this has been the subject of debate since ancient times.⁽¹⁾ Delaying the moment of cord clamping (delayed cord clamping; DCC) was considered advantageous for the newborn and has been advocated for centuries. However, as soon as infants started to be born in hospitals, early cord clamping was implemented as a part of 'active management of the third stage of labour' to reduce the risk of post-partum haemorrhage.⁽²⁾ In the past few decades there has been renewed interest in DCC, and several studies have been performed.^(3, 4) These studies demonstrated beneficial effects if the moment of cord clamping was delayed, which led to changes in both obstetrical and neonatal guidelines recommending DCC for at least three minutes for term born infants and 30-60 seconds for preterm infants who are not compromised at birth.^(5, 6) However, most preterm infants are in need of respiratory support at birth and immediate cord clamping is recommended, denying them the beneficial effects of DCC. To overcome this practice, new resuscitation tables have been developed that enable effective neonatal stabilisation at the maternal bedside, while the umbilical cord remains intact. Implementing this new approach in clinical practice will allow preterm infants to receive DCC and its accompanying beneficial effects.

The beneficial effects seen after DCC have been attributed to the net shift in blood volume from placenta to neonate, or placental transfusion.⁽⁷⁻⁹⁾ Although this is a commonly accepted feature of DCC, the physiology causing this net increase in neonatal blood volume remains unclear. It is possible that spontaneous breathing is the driving force for placental transfusion by generating a sub atmospheric pressure during inspiration.⁽¹⁰⁾ This sub atmospheric pressure is thought to create an increase of umbilical venous blood flow influx towards the neonate, resulting in a net increase in blood volume. In addition to placental transfusion, there has recently been another, perhaps even larger, benefit described.⁽¹¹⁾ Establishing lung aeration at birth, by breathing or positive pressure ventilation, causes a prompt increase in pulmonary blood flow at birth, which will have a major influence on the hemodynamic changes during neonatal transition.⁽¹²⁾ Experimental studies demonstrated that delaying the moment of cord clamping until **after** lung aeration (physiological-based cord clamping; PBCC) leads to a more stable and gentle hemodynamic transition than immediate clamping **before** lung aeration has been established. This PBCC has been shown to lead to less bradycardia and better peripheral and cerebral oxygenation at birth.^(11, 13) In an experimental setting the beneficial effects are clear and look promising for improving neonatal outcomes; this physiological effect and benefit needs to be confirmed in human infants in a clinical setting, however.

The general aim of this thesis was to investigate the physiological changes that occur with physiological and time-based delayed cord clamping strategies. To obtain a better understanding of the optimal moment of cord clamping and factors of influence, this thesis has focused on the effect of spontaneous breathing on placental transfusion as well as the effect of PBCC on preterm neonates during and directly after resuscitation.

PLACENTAL TRANSFUSION

In **chapter 1**, we investigated the effect of spontaneous breathing on umbilical venous flow and body weight in preterm lambs directly after birth. Sterile fetal surgery was performed on six pregnant ewes, which were instrumented at 132-133 days of gestational age to measure fetal pulmonary, cerebral and common umbilical venous (UV) blood flow at delivery. Both doxapram and caffeine were administered after delivery to promote spontaneous breathing, which was assessed using intrapleural pressure changes. Body weight, breathing, and blood flow measurements were continuously measured throughout the experiment. Change in body weight could be analysed in a total of 491 breaths and was found to increase in 46.6% and decrease in 47.5% of breaths with an overall mean increase of 0.02 ± 2.5 g per breath. We did not observe net placental transfusion prior to cord clamping. In addition, we found UV flow to be influenced by breathing, however the relationship was the opposite of our hypothesis. UV flow transiently decreased and, in some cases, even ceased with inspiration before normalising during expiration. This reduction in UV flow was positively correlated with the reduction in intrapleural pressure or increase in inspiratory depth. This finding could be the result of narrowing or closure of the inferior vena cava (IVC) based on diaphragm contraction during inspiration. Although spontaneous breathing had no net effect on body weight it clearly has an effect on UV blood flow in preterm lambs, which warrants further investigation. Unravelling the exact effect of breathing on the mechanisms driving placental transfusion could lead to recommendations for a more optimal moment of cord clamping.

In **chapter 2**, the effect of spontaneous breathing on systemic venous return and flow in the ductus venosus (DV) in healthy term born infants directly after birth was evaluated. Echocardiographic recordings were obtained from infants born between 37 and 42 weeks of gestational age, after an uncomplicated and low-risk pregnancy, if parental consent was obtained prior to birth. A subcostal view was used to obtain an optimal view of the IVC entering the right atrium (RA), including both the DV and hepatic vein (HV). Spontaneous breathing was assessed by diaphragm movements visible in all recordings. Measurements continued until the umbilical cord was clamped at the discretion of the midwife. Flow measurements were observed to be antegrade (towards the infant) in the DV and HV during inspiration in 98% and 82%

respectively, with an increase in flow in 74% of inspirations. Retrograde flow in the DV was observed sporadically and only occurred during expiration. Collapse of the IVC was consistently located caudal of the DV inlet into the subdiaphragmatic venous vestibulum and occurred in the majority of inspirations (58%). These findings clearly demonstrate the association between spontaneous breathing and IVC collapse as well as the increased antegrade flow in the DV and HV during inspiration. Inspiration appears to preferentially direct blood flow from the DV into the RA, indicating that it could be a factor driving placental transfusion.

Chapter 3 describes an observational study investigating the feasibility of measuring heart rate (HR) with the use of umbilical pulse oximetry (PO) and whether reliable HR measurements can be obtained faster when compared to preductal PO in infants who need stabilisation at birth. Both preductal and umbilical HR measurements were obtained in infants >25 weeks of gestational age. During stabilisation, but after cord clamping, a PO sensor was placed around the umbilical cord to obtain measurements in addition to the standard preductal PO measurements. Umbilical PO measurements were shielded and alarms were muted, so caregivers were not informed or distracted by umbilical measurements. HR data of the first ten minutes after birth were reviewed and compared. A HR signal was considered reliable when signal identification and quality >30% and a stable plethysmograph pulse wave was observed. Measurements were obtained from a total of 18 infants, who needed respiratory support at birth. Reliable HRs from umbilical PO were obtained in all infants, but the time between sensor application and obtaining a reliable HR signal was longer than with preductal PO (19 [16-55] seconds vs. 15 [11-17] seconds; $p=0.01$). Umbilical HR was consistently lower than preductal HR (mean($\pm$ SD) difference 36 ($\pm$ 22) bpm; Intraclass Correlation Coefficient (95% CI): 0.1 (0.03-0.22)). Although it is feasible to obtain reliable HRs when using umbilical PO in infants needing stabilisation at birth, obtaining reliable measurements takes longer when compared to preductal PO. The lower HR measured at the umbilical cord compared to preductal HR measurements warrants further studies to confirm this but, if correct, evaluation of the infant's clinical condition using HR by palpating the cord should be reconsidered.

PHYSIOLOGICAL-BASED CORD CLAMPING

In **chapter 4**, we evaluated the feasibility of the PBCC approach in preterm born infants using a new purpose-built resuscitation table (Concord). Infants born <35 weeks of gestational age were stabilised on the Concord resuscitation table, which was supplied with the standard equipment needed for stabilisation. Cord clamping was performed when the infant was considered to be stable according to predefined criteria (HR >100 bpm, spontaneous breathing on continuous positive

airway pressure (CPAP) with tidal volumes >4 mL/kg, oxygen saturation (SpO_2) $\geq 25^{\text{th}}$ percentile and fraction of inspired oxygen (FiO_2) <0.4). The PBCC approach was successfully performed in 33 of 37 infants (89.2%) and resulted in a median cord clamping time of 4:23 [3:00–5:11] min after birth. Measurements on HR and SpO_2 were adequate for analysis in 26 of 37 infants. HR was 113 [81–143] bpm and 144 [129–155] bpm at 1 min and 5 min after birth. SpO_2 levels were 58% [49%–60%] and 91% [80%–96%], while median FiO_2 given was 0.30 [0.30–0.31] and 0.31 [0.25–0.97], respectively. Considering the success percentage in performing PBCC, a more stable HR and faster increase in oxygenation as well as the absence of reported serious adverse events, we consider the PBCC approach in preterm infants using the Concord feasible. Importantly, HR remained stable during and around the moment of cord clamping, which makes it likely that PBCC may result in optimal timing of cord clamping and in optimal pulmonary and cardiovascular transition.

Chapter 5 describes a randomised controlled non-inferiority study to evaluate whether stabilising very preterm infants according to the PBCC approach is at least as effective as the standard approach of time-based DCC. Infants were eligible for inclusion if they were born < 32 weeks of gestational age and if antenatal parental consent was obtained. Infants were either allocated to PBCC and stabilised with an intact cord or allocated to standard DCC. In infants who received PBCC the umbilical cord was clamped when they were deemed stable (regular spontaneous breathing, HR ≥ 100 bpm and $\text{SpO}_2 > 90\%$ while using $\text{FiO}_2 < 0.40$). In infants receiving DCC, the cord was clamped at 30–60 s after birth before they were transferred to the standard resuscitation table for further treatment and stabilisation. The non-inferiority limit was set at 1:15 min. A total of 37 infants (mean gestational age $29+0$ weeks) were included. Mean cord clamping time was $5:49 \pm 2:37$ min in the PBCC ($n=20$) and $1:02 \pm 0:30$ min in the DCC group ($n=17$). Infants receiving PBCC needed less time to reach respiratory stability (PBCC $5:54 \pm 2:27$ min; DCC $7:07 \pm 2:54$ min). The mean difference, corrected for gestational age, in time needed to reach respiratory stability was $-1:19$ min, 95% CI [$-3:04 - 0:27$], demonstrating the non-inferiority of the PBCC approach as the pre-defined limit of 1:15 min falls outside of this confidence interval. Stabilisation of very preterm infants with the PBCC approach is therefore at least as effective as with standard DCC.

In **chapter 6**, the correlation between ductus arteriosus (DA) flow ratio and oxygenation parameters (as a measure of the infant's transitional status) were evaluated. Echocardiography was performed in preterm infants at 1 hour after birth, as part of an ancillary study to the previously described cord clamping studies of ABC 2 and 3. The DA flow ratio was calculated and correlated with FiO_2 given, SpO_2 and $\text{SpO}_2/\text{FiO}_2$ ratio (SF) in a total of 16 infants. The DA flow ratio of infants receiving PBCC and standard DCC were compared. All infants received CPAP of 7–8 cmH₂O at the time of measurements, except for one infant who did not receive any respiratory support.

Right-to-left DA shunting was 16 [17-27] ml/kg/min and left-to-right shunting was 110 [81 – 124] ml/kg/min. The DA flow ratio was 0.18 [0.11-0.28], SpO₂ was 94 [93-96] %, FiO₂ was 23 [21-28] % and SF ratio 4.1 [3.3-4.5]. There was a moderate correlation between DA flow ratio and SpO₂ (correlation coefficient (CC) -0.415; p=0.110), FiO₂ (CC 0.384; p=0.142) and SF ratio (CC -0.356; p=0.175). There were no differences in any of the DA flow measurements between infants who received PBBC or time-based DCC. In this pilot study DA flow ratio at 1 hour after birth seems to be correlated with oxygenation parameters in preterm infants at birth and could reflect transition in preterm infants at birth. DA flow ratio as a measure of transitional status at different moments after birth could be of use to predict adverse outcome.

CONCLUSION

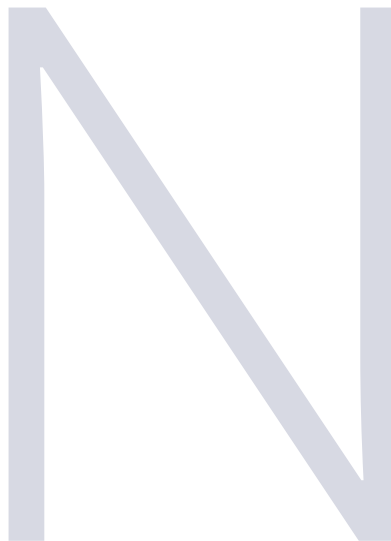
In the **general discussion** we reviewed spontaneous breathing as a possible driving force for placental transfusion, the implementation of PBCC in a clinical setting, and the physiological changes during neonatal transition when PBCC is performed. Placental transfusion is a widely accepted feature of DCC, however the physiological mechanisms driving this net increase in neonatal blood volume remain unclear. The negative sub atmospheric pressures generated during spontaneous inspiration have been suggested as the driving force for placental transfusion. Indeed, we demonstrated that spontaneous breathing greatly influences umbilical blood flow, as inspiration was associated with an increase in umbilical venous blood flow in humans. When combined with the association between inspiration and collapse of the IVC, this potentially creates a preferential placental blood flow towards the RA. Anatomical differences between humans and animal models used have prevented experimental studies from demonstrating a similar association. In addition, we found spontaneous breathing to not only influence umbilical and pulmonary blood flow, but also a disruption of the systemic blood flow to the lower body while the DA remains intact. We concluded that spontaneous breathing is likely to be a force influencing placental transfusion.

Both the physiological effect and the beneficial effects of PBCC are clear in an experimental setting. Lung aeration and the subsequent increase in pulmonary blood flow are vital for the success of this approach. When successfully translating PBCC to human infants it is pivotal that the lungs have been aerated. Hence, the moment of cord clamping should not be at a fixed time but rather based on the infant's transitional status. With the studies performed in this thesis we demonstrated that it is safe and feasible to perform PBCC, and that it has a similar effect to that shown in the experimental setting. We also demonstrated that resuscitation on the cord is at least as effective as the standard approach, while allowing much longer and variable cord clamping times.

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NEDERLANDSE SAMENVATTING

INTRODUCTIE

Het afklemmen en doornemen van de navelstreng, ook wel afnavelen genoemd, is een normale en onontkoombare procedure die plaatsvindt na de geboorte.

Sinds jaar en dag is echter discussie gaande over het juiste moment om deze procedure uit te voeren. Al sinds de tijd van Aristoteles (350 voor Christus) wordt - vanwege de mogelijke voordelen voor de pasgeborenen - gepleit om enkele minuten te wachten met het moment van afnavelen. Alhoewel verlaat afnavelen in theorie beter is voor het pasgeboren kind, werd in de ziekenhuizen al snel gestart met versneld afnavelen om het risico op extra maternaal bloedverlies te verlagen. De laatste decennia is opnieuw interesse ontstaan in verlaat afnavelen en zijn verschillende studies uitgevoerd om de effecten hiervan te onderzoeken. Deze studies lieten zien dat het beter is om verlaat af te navelen, waarna zowel de obstetrische als neonatale richtlijnen zijn aangepast. Volgens de huidige richtlijnen wordt geadviseerd om ten minste 3 minuten te wachten met afnavelen bij gezonde atermen neonaten. Bij premature neonaten, als deze niet direct respiratoire ondersteuning nodig hebben, wordt geadviseerd om 30-60 seconden te wachten. De praktijk leert dat de meeste prematuren wel respiratoire ondersteuning nodig hebben na de geboorte, waarbij de huidige richtlijn dus adviseert direct af te navelen. Inmiddels zijn nieuwe opvangtafels ontwikkeld om zowel de voordelen van verlaat afnavelen als respiratoire ondersteuning aan te kunnen bieden bij premature neonaten. Deze opvangtafels maken het mogelijk om neonaten direct op te vangen en te stabiliseren naast het bed van de moeder, terwijl de navelstreng intact blijft.

De voordelige effecten van het verlaat afnavelen werden voorheen toegeschreven aan het netto bloedvolume, dat zich verplaatst van de placenta naar de neonaat. Dit wordt ook wel placentale transfusie genoemd. Ondanks het feit dat placentale transfusie een algemeen geaccepteerd concept is, blijft het fysiologische mechanisme ervan onduidelijk. Het is mogelijk dat spontane ademhaling van de neonaat de drijvende kracht is achter de placentale transfusie, doordat tijdens de inspiratie intra-thoracaal negatieve druk wordt gecreëerd. Deze negatieve druk heeft mogelijk een aanzuigende werking. Hierdoor ontstaat een toename van de bloedstroom door de navelstreng, hetgeen resulteert in een netto toename van het neonataal bloedvolume. Recent is naast placentale transfusie nog een ander, en wellicht groter, voordeel beschreven van verlaat afnavelen. Wanneer de longen geaereerd worden na de geboorte (door spontane ademhaling of positieve drukbeademing), zorgt dit voor een directe toename van pulmonale bloedstroom. Dit heeft grote impact op de hemodynamische veranderingen, die plaatsvinden tijdens de neonatale transitie direct na de geboorte. Uit experimentele studies is gebleken dat wachten met afnavelen tot nadat de longen geaereerd zijn (fysiologisch afnavelen), leidt tot een meer stabiele hemodynamische transitie

(minder bradycardieën). Daarnaast leidt fysiologisch afnavelen tot een verbeterde perifere en cerebrale oxygenatie na de geboorte. Hoewel deze resultaten en de toegeschreven voordelen in de experimentele setting veelbelovend zijn, zal dit in een klinische setting nog bevestigd moeten worden.

Dit proefschrift beschrijft mijn onderzoek naar de fysiologische veranderingen, die optreden tijdens fysiologisch en verlaagd afnavelen. Het onderzoek is met name gericht op de invloed van spontane ademhaling op placentale transfusie en daarnaast op het effect van fysiologisch afnavelen bij prematuren tijdens en direct na de opvang. Het doel is om het optimale moment van afnavelen en de factoren die hierop van invloed zijn te (kunnen) bepalen.

PLACENTALE TRANSFUSIE

Hoofdstuk 1 van dit proefschrift beschrijft het onderzoek naar het effect dat spontane respiratie heeft op de flow in de vena umbilicalis in prematuur geboren lammeren. Als maatstaf voor placentale transfusie werd tijdens dit onderzoek gekeken naar het verschil in gewicht vóór en na een spontane ademteug. Bij zes zwangere oaien met een zwangerschapsduur van 132-133 dagen werden steriele, foetale operaties uitgevoerd waarbij meetinstrumentaria werden geplaatst. Met deze meetinstrumentaria was het mogelijk om direct na de geboorte, die drie dagen later plaatsvond, zowel de foetaal pulmonale en de cerebrale flow te meten, alsook de vena umbilicalis communis flow. Om spontane ademhaling na de geboorte te stimuleren werd doxapram en coffeïne toegediend. De spontane respiratie werd beoordeeld op basis van intra-pleurale drukverschillen. Tijdens het experiment werden dus gewicht, intra-pleurale druk en pulmonale, cerebrale en umbilicale flow continu gemeten. Veranderingen in lichaamsgewicht werden vervolgens gemeten bij 6 lammeren met in totaal 491 ademteugen, waarbij het gewicht toenam in 46.6% van de teugen en afnam in 47.5% van de teugen. De gemiddelde toename in gewicht per ademteug was 0.02 ± 2.5 g. Daarnaast werd duidelijk dat de respiratie van invloed was op de vena umbilicalis flow. Tijdens de inspiratie nam de flow af - en was soms zelfs volledig afwezig - voordat deze weer normaliseerde tijdens de expiratie.

Dit resultaat staat lijnrecht tegenover de bestaande theorie dat de negatieve intra-thoracale druk tijdens inspiratie een aanzuigende werking heeft op de umbilicale flow. De resultaten toonden een positieve correlatie tussen de afname in flow over de vena umbilicalis en de afname in intra-pleurale druk (ofwel diepte van de inspiratie). Het is mogelijk dat dit effect wordt veroorzaakt door contractie van het diafragma tijdens inspiratie, waarbij de vena cava inferior gedeeltelijk of volledig wordt geobstrueerd.

In het diermodel dat is gebruikt werd geen significant effect gevonden van spontane ademhaling op gewicht en dus geen effect op de placentale transfusie. Wel werd een duidelijk effect geconstateerd van de ademhaling op de vena umbilicalis flow. Aanvullend onderzoek is echter nodig om het exacte effect van spontane respiratie op placentale transfusie te bepalen. Deze kennis zou ertoe kunnen leiden dat nieuwe aanbevelingen moeten worden geformuleerd aangaande het meest optimale moment om af te navelen.

In **hoofdstuk 2** wordt het onderzochte effect van de spontane ademhaling op zowel de veneuze return als op de bloedstroom door de ductus venosus beschreven, bij gezonde atermen neonaten direct na de geboorte. Bij neonaten van 37-42 weken, waarbij sprake was geweest van een ongecompliceerde zwangerschap en *informed consent* van de ouders, werden vóór het afnavelen echografiemetingen verricht. Tijdens deze metingen werd door middel van een subcostale probe positie de overgang van de vena cava inferior naar het rechter atrium in beeld gebracht, samen met de ductus venosus en vena hepatica. Spontane respiratie werd beoordeeld op basis van diafragmabewegingen, welke eveneens zichtbaar waren in de opnames. De metingen werden voortgezet tot het moment van afnavelen; waarbij het moment van afnavelen werd bepaald door de verloskundige. In zowel de ductus venosus als de vena hepatica werd antegrade flow geconstateerd bij respectievelijk 98% en 82% van de metingen. Hierbij was sprake van een toename van flow in 74% van de inspiraties. Retrograde flow in de ductus venosus werd sporadisch geconstateerd en dan enkel tijdens de expiratie. De vena cava inferior collabeerde in de meerderheid van de inspiraties (58%). Deze collaps bevond zich consistent caudaal van de overgang van ductus venosus naar het subdiafragmatisch vestibulum (direct voor het rechter atrium). Deze bevindingen tonen duidelijk een associatie tussen zowel spontane respiratie en collaps van de vena cava inferior, alsook de toename van flow in de ductus venosus en vena hepatica tijdens inspiratie. Deze resultaten wijken af van de resultaten zoals beschreven in **hoofdstuk 1**. Dit kan mogelijk verklaard worden door anatomische verschillen tussen schaap en mens, met name het verschil in systemisch veneuze return. Op basis van de observaties in **hoofdstuk 2** is *waarschijnlijk* sprake van een preferentiële flow van de ductus venosus naar het rechter atrium tijdens inspiratie. Spontane respiratie is derhalve potentieel de drijvende kracht voor placentale transfusie.

Hoofdstuk 3 beschrijft een observationele studie, waarin de mogelijkheid om umbilicale hartslag metingen te verkrijgen middels pulsoximetrie werd onderzocht. Daarnaast werd de betrouwbaarheid van deze metingen geanalyseerd, evenals de snelheid waarmee ze verkregen kunnen worden ten opzichte van preductale pulsoximetrie metingen van de rechterhand. De preductale meting wordt standaard verricht bij pasgeborenen, die ondersteuning nodig hebben na de

geboorte. Tijdens dit onderzoek werd een pulsoximetrie sensor om de navelstreng en rechterhand van de baby geplaatst. De verkregen hartslagmetingen van de eerste tien minuten na de geboorte, op zowel de navelstreng als rechterhand gemeten, werden geanalyseerd en met elkaar vergeleken. Metingen werden beoordeeld als “betrouwbaar” als de ‘signal identification and quality’ hoger was dan 30% en indien een stabiele ‘pulse wave’ werd geobserveerd op het plethysmogram. In totaal werden metingen verzameld van 18 pasgeborenen, die na de geboorte respiratoire ondersteuning nodig hadden. Van alle 18 neonaten werden betrouwbare umbilicale hartslagmetingen verkregen. De tijd tussen het plaatsen van de sensor en het verkrijgen van een betrouwbare meting aan de navelstreng was echter langer ten opzichte van de preductale metingen aan de rechterhand, namelijk 19 [16-55] seconden versus 15 [11-17] seconden; $p=0.01$). Daarnaast werd consistent een lagere hartslag gemeten aan de navelstreng dan aan de rechterhand (gemiddeld verschil (mean ($\pm$ SD)) 36 ($\pm$ 22) slagen/min; Intraclass Correlation Coefficient (95% CI): 0.1 (0.03-0.22)). Op basis van deze resultaten werd geconcludeerd dat het mogelijk is om betrouwbare hartslagmetingen te verkrijgen van de navelstreng, maar dat het verkrijgen van deze meting langer duurt in vergelijking met de standaardmeting aan de rechterhand. Verder onderzoek is essentieel om te bepalen of de te meten umbilicale hartslag daadwerkelijk lager is. Het beoordelen van de klinische conditie van een pasgeborene op basis van de umbilicale hartslag, die voelbaar is in de navelstreng, moet op basis van de resultaten mogelijk worden heroverwogen.

FYSIOLOGISCH AFNAVELEN

Hoofdstuk 4 beschrijft een observationele studie waarin wordt geëvalueerd of het toepassen van fysiologisch afnavelen met gebruik van een nieuwe opvangtafel (Concord) veilig is. Pasgeboren neonaten met een zwangerschapsduur van <35 weken, waarvoor antenataal *informed consent* van de ouders was verkregen, werden opgevangen en gestabiliseerd op de Concord opvangtafel. Deze opvangtafel is uitgerust met alle standaardapparatuur die nodig is voor het stabiliseren van een pasgeborene. Pas op het moment dat de pasgeborene stabiel werd geacht, werd de navelstreng doorgenomen. Dit moment werd bepaald op basis van de vooraf vastgestelde criteria (te weten: hartslag > 100 slagen/minuut, spontane ademhaling met continu positieve luchtdruk en een teugvolume van > 4ml/kg, zuurstofsaturatie (SpO_2) $\geq$ 25^{ste} percentiel van de standaard curve en een fractionele zuurstofconcentratie van ingeademde lucht (FiO_2) van < 0.4). Fysiologisch afnavelen werd succesvol uitgevoerd bij 33 van de 37 pasgeborenen (derhalve 89.2%). De gemiddelde tijd tot afnavelen was 4:23 [3:00–5:11] minuten na de geboorte. Zowel de hartslag- en SpO_2 metingen tijdens de opvang waren beschikbaar voor 26 van de 37 pasgeborenen. De hartslag was 113 [81-143]

slagen/minuut en 144 [129-155] slagen/minuut bij respectievelijk 1 en 5 minuten na de geboorte. Vervolgens was de SpO_2 bij 1 en 5 minuten na de geboorte 58% [49%-60%] en 91% [80%-96%], terwijl de FiO_2 respectievelijk 0.30 [0.30-0.31] en 0.31 [0.25-0.97] was.

Op basis van deze resultaten werd fysiologisch afnavelen bij premature neonaten met gebruik van de Concord opvangtafel beschouwd als een succes, ook gezien de hartslag op het moment van afnavelen stabiel bleef. Fysiologisch afnavelen lijkt op basis van het voorgaande te resulteren in optimale transitie, zowel pulmonaal als cardiovasculair, waardoor fysiologisch afnavelen mogelijk de optimale manier om af te navelen is.

In **hoofdstuk 5** wordt een gerandomiseerde, maar gecontroleerde, non-inferiority studie beschreven. Middels deze studie werd onderzocht of fysiologisch afnavelen bij prematuren minstens zo effectief is als de standaardbenadering, waarbij wordt afgenveld op een vast tijdstip na de geboorte. Neonaten waren geschikt voor deelname aan de studie indien ze werden geboren na een zwangerschapsduur van minder dan 32 weken en waarvoor uiteraard antenataal toestemming was verkregen van ouders om deel te nemen aan de studie. Bij deelname werden pasgeborenen gerandomiseerd voor fysiologisch afnavelen óf voor standaard afnavelen op basis van een vastgestelde tijd. Wanneer een neonaat was geselecteerd voor fysiologisch afnavelen, werd de neonaat gestabiliseerd met een intacte navelstreng. Deze werd doorgeknipt als de pasgeborene stabiel werd geacht (dat wil zeggen: spontane en reguliere ademhaling, hartslag > 100 slagen/minuut, $\text{SpO}_2 > 90\%$ met $\text{FiO}_2 < 0.40$). In het geval dat de selectie was gemaakt voor afnavelen op basis van een vast tijdstip, werd afgenveld tussen de 30 en 60 seconden na de geboorte. Hierna werd de pasgeborene verplaatst naar een standaard opvangtafel voor verdere behandeling en stabilisatie.

Bij deze studie werd de non-inferiority limiet gesteld op 1:15 minuten. In totaal werden 37 neonaten geïncludeerd met een gemiddelde zwangerschapsduur van 29+0 weken. Bij de groep pasgeborenen geselecteerd voor fysiologisch afnavelen gold een gemiddelde tijd van $5:49 \pm 2:37$ minuten voor afnavelen na de geboorte ($n=20$) en bij de groep pasgeborenen geselecteerd voor afnavelen op basis van tijd gold een gemiddelde tijd van $1:02 \pm 0:30$ min voor afnavelen na de geboorte ($n=17$). Het (gemiddelde) verschil in de tijd die nodig was om een pasgeborene te stabiliseren, was $-1:19$ min, 95% CI $(-3:04-0:27)$ waarbij werd gecorrigeerd op basis van de zwangerschapsduur. Deze resultaten laten zien dat de vooraf vastgestelde non-inferiority limiet van 1:15 minuten buiten het betrouwbaarheidsinterval valt. Concluderend is fysiologisch afnavelen tijdens het stabiliseren van premature neonaten minstens zo effectief als het standaard afnavelen op basis van tijd.

In **hoofdstuk 6** wordt de correlatie tussen de flowratio van de ductus arteriosus en oxygenatie parameters (als maat voor de neonatale transitie) onderzocht. Dit was aanvullend onderzoek op de eerder beschreven onderzoeken naar fysiologisch afnavelen (hoofdstuk 4 en 5; ABC2 en 3 studie). Hiertoe werden een uur na de geboorte echocardiografische metingen verricht bij premature neonaten, die al waren geïncludeerd voor de ABC2 of 3 studie. De flowratio van de ductus arteriosus werd berekend en gecorreleerd aan de FiO_2 , SpO_2 en de ratio tussen $\text{SpO}_2/\text{FiO}_2$ (SF ratio) van in totaal 16 neonaten. De ductus arteriosus flowratio werd vergeleken van de groepen waarbij fysiologisch afnavelen was toegepast en de groepen waarbij afnavelen op basis van tijd was toegepast. Alle neonaten kregen daarbij continu positieve luchtdruk met 7-8 cmH_2O ten tijde van de metingen, met uitzondering van één neonaat die geen respiratoire ondersteuning kreeg.

De gemeten shunting over de ductus arteriosus van rechts-naar-links was 16 [17-27] ml/kg/min en shunting van links naar rechts was 110 [81 – 124] ml/kg/min. De ductus arteriosus flowratio was 0.18 [0.11-0.28], de SpO_2 was 94 [93-96] %, de FiO_2 was 23 [21-28] % en de SF ratio was 4.1 [3.3-4.5].

Een matige correlatie werd aangetoond tussen de ductus arteriosus flowratio en SpO_2 (correlatiecoëfficiënt (CC) -0.415; $p=0.110$), FiO_2 (CC 0.384; $p=0.142$) en SF ratio (CC -0.356; $p=0.175$). De metingen waren niet verschillend voor de groepen neonaten, die fysiologisch afnavelen danwel afnavelen op basis van een vaste tijd hadden ondergaan. De resultaten van deze pilotstudie tonen aan dat *mogelijk* sprake is van een correlatie tussen ductus arteriosus flowratio en de bijbehorende oxygenatie parameters bij premature neonaten 1 uur na de geboorte. Mogelijk kan de ductus arteriosus flowratio gebruikt worden als maatstaf van de neonatale transitie na de geboorte en kan deze ook dienen als voorspeller voor negatieve gevolgen van premature geboorte.

CONCLUSIE

In de **algemene discussie** evalueren we spontane ademhaling als mogelijk drijvende kracht voor placentale transfusie, het implementeren van fysiologisch afnavelen in een klinische setting en de fysiologische veranderingen tijdens de neonatale transitie bij fysiologisch afnavelen. Hoewel placentale transfusie een algemeen geaccepteerd aspect is bij het wachten met afnavelen, blijft de drijvende kracht, die verantwoordelijk is voor de toename in neonataal bloedvolume, nog onduidelijk. In de literatuur wordt gesuggereerd dat de intra-thoracaal negatieve druk tijdens spontane inspiratie de drijvende kracht is voor placentale transfusie. De resultaten van de studies beschreven in dit proefschrift tonen inderdaad de impact van spontane ademhaling op de umbilicale bloedstroom aan. In de studies

werd met name de inspiratie geassocieerd met een toename van de umbilicale flow. Daarnaast werd een verband tussen de inspiratie en het collaberen van de vena cava inferior waargenomen. Wanneer deze resultaten worden gecombineerd, toont dit mogelijk een preferentiële bloedstroom van de placenta naar het rechter atrium tijdens inspiratie aan. Naast de eerder beschreven effecten op umbilicale en pulmonale flow werd ook een effect op de systemische flow geconstateerd, vooropgesteld dat de ductus arteriosus intact is. De anatomische verschillen tussen mensen en de eerder gebruikte diermodellen van experimentele studies hebben waarschijnlijk als gevolg gehad dat deze verbanden niet eerder zijn aangetoond. Op basis van deze resultaten werd geconcludeerd dat spontane respiratie zeer waarschijnlijk een factor van invloed is op placentale transfusie.

De voordelige effecten van fysiologisch afnavelen zijn duidelijk aangetoond in experimentele onderzoeken. Voor deze benadering zijn het lucht houdend worden van de longen en de bijbehorende toename van pulmonale flow van essentieel belang. Het aereren van de longen is uitermate belangrijk voor het succesvol vertalen van fysiologisch afnavelen naar de klinische setting. Het moment van afnavelen zou daarom niet afhankelijk moeten zijn van een vooraf vastgesteld tijdstip, maar zou gebaseerd moeten worden op de kliniek van de pasgeborene in kwestie. De resultaten van het onderzoek in dit proefschrift tonen aan dat fysiologisch afnavelen niet alleen veilig en haalbaar is, maar ook dat de effecten ervan vergelijkbaar zijn met hetgeen werd geconstateerd in de experimentele setting. Daarnaast is aangetoond dat het opvangen en stabiliseren van een pasgeborene met een intacte navelstreng minstens zo effectief is, als toepassing van de standaardbenadering. Indien fysiologisch afnavelen wordt toegepast, is het moment van afnavelen meer variabel en vindt het afnavelen met name later plaats in vergelijking met de standaardbenadering.



PART FIVE

Appendices

LIST OF ABBREVIATIONS

ABC	Aeration, Breathing, Clamping
BPD	Bronchopulmonary dysplasia
CA	Carotid arterial
CC	Correlation coefficient
CPAP	Continuous positive airway pressure
DA	Ductus arteriosus
DA flow ratio	R-L DA flow/L-R DA flow
DCC	Delayed cord clamping
DV	Ductus venosus
ECG	Electrocardiogram
FBM	Fetal breathing movements
FiO ₂	Fraction of inspired oxygen
FRC	Functional residual capacity
GA	Gestational age
HELLP	Haemolysis Elevated Liver enzymes Low Platelet syndrome
HR	Heart rate
HV	Hepatic vein
ICC	Immediate cord clamping
IP	Intra-pleural
IQR	Interquartile range
IRB	Institutional Review Board
IRDS	Infant respiratory distress syndrome
IVC	Inferior vena cava
IVC-Dia	Inferior vena cava diameter
IVH	Intraventricular haemorrhage
LUMC	Leiden University Medical Center
L-R	Left-to-right
NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit
NIRS	Near-infrared spectroscopy
OI	Oxygenation index
PA	Pulmonary artery
PBCC	Physiological-based cord clamping
PBF	Pulmonary blood flow
PDA	Persistent ductus arteriosus
PE	Preeclampsia
PEEP	Positive end-expiratory pressure
PIP	Positive inspiratory pressure
PO	Pulse oximetry

PPH	Post-partum haemorrhage
PPV	Positive pressure ventilation
PVR	Pulmonary vascular resistance
RA	Right atrium
RBC	Red blood cell
RCT	Randomized controlled trial
RFM	Respiratory function monitor
R-L	Right-to-left
ROP	Retinopathy of prematurity
SF ratio	SpO ₂ /FiO ₂ ratio
SpO ₂	Oxygen saturation
SVR	Systemic vascular resistance
TBCC	Time-based cord clamping
SD	Standard deviation
SI	Sustained inflation
SIQ	Signal identification and quality
UA	Umbilical arterial/umbilical artery
UV	Umbilical venous/umbilical vein
UVBF	Umbilical venous blood flow
VTI	Velocity time integral

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CURRICULUM VITAE

Emma Brouwer was born on the 30th of March 1991 in Mariekerke, the Netherlands. She grew up in Zeeland together with her brother and sister. After completing secondary school at CSW van de Perre in Middelburg, she started to study Biomedical Sciences at Leiden University in 2009. After having successfully finished the first year, she changed to studying Medicine at the Leiden University in 2010. During her research internship in 2014 she studied the effect of doxapram, used for apnea of prematurity, on long-term neurodevelopmental outcome under the guidance of prof. dr. A.B. te Pas and dr. M. Rijken. This internship influenced her interest in neonatology. After obtaining her medical degree in 2016, Emma started as a PhD candidate under the supervision of prof. dr. A.B. te Pas, prof. dr. S.B. Hooper and dr. A.A.W. Roest. During her PhD training she performed experimental and clinical studies, performing physiological measurements during umbilical cord clamping strategies. She visited the Ritchie Center (Monash University, Melbourne, Australia) for six months, to conduct the experimental arm of her thesis under supervision of prof. dr. S.B. Hooper.

In January 2021, she started working clinically as a registrar (ANIOS) at the department of Pediatrics at Alrijne Hospital in Leiderdorp.

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