Physiological measurements of the effect of cord clamping strategies

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PART FOUR

General discussion and Summary

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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.

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 ^{125}I-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 breath 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).

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.

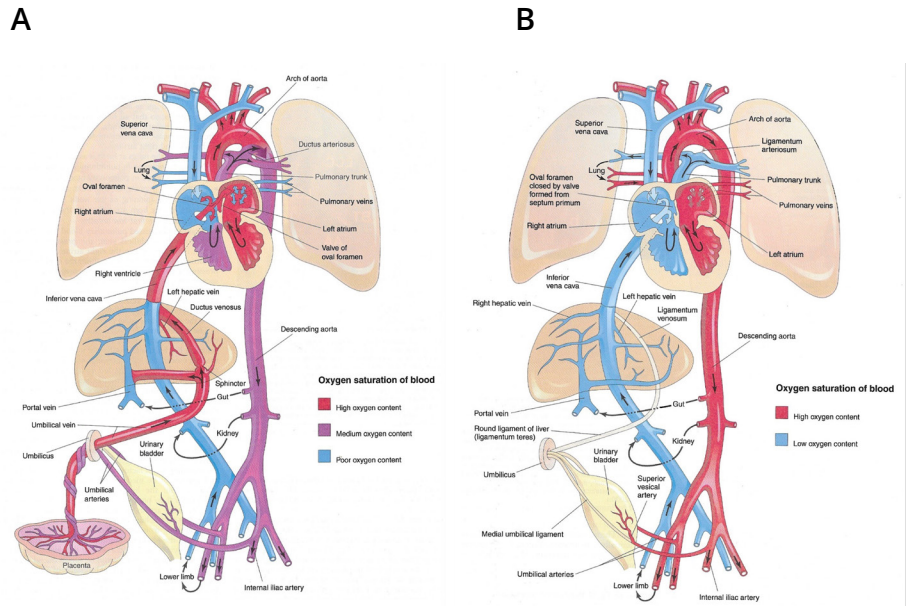

Figure 1 | Circulation before (A) and after (B) birth
"This figure was published in The Developing Human – Clinically Oriented Embryology, 8th edition, KL Moore & TVN Persaud, Page 328-329, Copyright 2008 by Saunders, an imprint of Elsevier Inc."

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 (SpO_2) > 90%, fractional inspired oxygen (FiO_2) < 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 SpO_2 > 90% while the FiO_2 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 SpO_2 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 SpO_2 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 SpO_2/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.

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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