Aus dem Zentrum für Kinder- und Jugendmedizin der Universität zu Köln
Klinik und Poliklinik für Kinder- und Jugendmedizin
Direktor: Universitätsprofessor Dr. med. J. Dötsch

The Effect of Extrauterine Placental Transfusion on the Outcome of Very Low Birth Weight Infants

Inaugural-Dissertation zur Erlangung der Doktorwürde
der Medizinischen Fakultät
der Universität zu Köln

vorgelegt von
Michelle Celine Harvey
(geb. Kuckelkorn)
aus Köln

promoviert am 09.01.2024

Gedruckt mit Genehmigung der Medizinischen Fakultät der Universität zu Köln

2024

Dekan: Universitätsprofessor Dr. med. G. R. Fink

1. Gutachterin: Professorin Dr. med. S. Habbig
2. Gutachter: Professor Dr. med. M. C. Liebau

Erklärung

Ich erkläre hiermit, dass ich die vorliegende Dissertationsschrift ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe; die aus fremden Quellen direkt oder indirekt übernommenen Gedanken sind als solche kenntlich gemacht.

Bei der Auswahl und Auswertung des Materials sowie bei der Herstellung des Manuskriptes habe ich Unterstützungsleistungen von folgenden Personen erhalten:

PD Dr. med. André Oberthür, Dr. med. Benjamin Kühne, Frau Dipl.-Math. Ingrid Becker und PD Dr. med. Angela Kribs

Weitere Personen waren an der Erstellung der vorliegenden Arbeit nicht beteiligt. Insbesondere habe ich nicht die Hilfe einer Promotionsberaterin/eines Promotionsberaters in Anspruch genommen. Dritte haben von mir weder unmittelbar noch mittelbar geldwerte Leistungen für Arbeiten erhalten, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertationsschrift stehen.

Die Dissertationsschrift wurde von mir bisher weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde vorgelegt.

Die dieser Arbeit zugrunde liegenden Daten wurden nach Einweisung von PD Dr. med. André Oberthür und Dr. med. Benjamin Kühne von mir selbst erhoben.
Der entwickelte Abnabelungsbogen, welcher zusätzlich die zugrunde liegenden Messergebnisse für diese Arbeit liefert, wurde in Absprache mit PD Dr. med. André Oberthür und PD Dr. med. Kribs erstellt.
Die Krankenakten und Patientenkurven wurden von mir selbst ausgewertet.
Die statistische Auswertung der Daten erfolgte mittels der Software SPSS Statistics, mit Unterstützung von Frau Dipl.-Math. Ingrid Becker, durch mich selbst.

Erklärung zur guten wissenschaftlichen Praxis:
Ich erkläre hiermit, dass ich die Ordnung zur Sicherung guter wissenschaftlicher Praxis und zum Umgang mit wissenschaftlichem Fehlverhalten (Amtliche Mitteilung der Universität zu Köln AM 132/2020) der Universität zu Köln gelesen habe und verpflichte mich hiermit, die dort genannten Vorgaben bei allen wissenschaftlichen Tätigkeiten zu beachten und umzusetzen.

Köln, den 18.10.2023

Unterschrift: .....

Für meine Schwester Nadine, das erste Frühchen, das ich kennenlernte

Für meine Eltern Gabriele und André für ihre unermüdliche Unterstützung

Für meinen britischen Ehemann Samuel Harvey, der mir stets eine Inspiration ist die richtigen Worte zu finden

Table of Contents

List of abbreviations

ACS	antenatal corticosteroids
BPD	bronchopulmonary dysplasia
BW	birth weight
BW7	blood withdrawal over 7 days
CM	cord milking
CPAP	continuous positive airway pressure
CRIB	clinical risk index for babies
DCC	delayed cord clamping
EPT	extrauterine placental transfusion
ELBW	extremely low birth weight
FiO2	fraction of inspired oxygen
GA	gestational age
ICC	immediate cord clamping
IMV	invasive mechanical ventilation
Hct	haematocrit
IVH	intraventricular haemorrhage
IQR	interquartil range
NEC	necrotising enterocolitis
PDA	patent ductus arteriosus
PT	phototherapy
PTx	pneumothorax
PVL	periventricular leukomalacia
RBC	red blood cell
RBC7	red blood cell transfusion after 7 days
RBC28	red blood cell transfusion after 28 days

RDS	respiratory distress syndrome
ROP	retinopathy of prematurity
SBR	peak serum bilirubin
SGA	small for gestational age
SIP	spontaneous intestinal perforation
SRT	surfactant replacement therapy
VLBW	very low birth weight
VLGA	very low gestational age
WHO	World Health Organization

1. Zusammenfassung

Hintergrund: Die Transition vom Fetus zum Neugeborenen ist gekennzeichnet durch das Einsetzen der Spontanatmung, welche zu der Belüftung der Lunge und darauf folgender Zunahme des pulmonalen Blutflusses führt. Studien haben gezeigt, dass ein verzögertes Abnabeln (delayed cord clamping (DCC)) oder auch Ausstreichen der Nabelschnur (cord milking (CM)) zu einem erhöhten neonatalen Blutvolumen führt. Weiterhin gibt es Hinweise, dass ein verzögertes Abnabeln zu einer Verbesserung der zerebralen Oxygenierung und beispielsweise zu einer Reduktion der Inzidenz von intraventrikulären Blutungen (IVH) bei unreifen Frühgeborenen führt.

Die jeweils gewählte Abnabelungsstrategie hat eine direkte Auswirkung auf das neonatale Outcome. Dennoch haben die genannten Verfahren Limitation, zum Beispiel, dass parallel zum verzögerten Abnabeln nur eingeschränkt supportive Beatmungs- oder andere Behandlungsmaßnahmen möglich sind.
Daher wurde im Level 1 Perinatalzentrum der Uniklinik zu Köln das Verfahren der extrauterinen plazentaren Transfusion (EPT) entwickelt, welches in den letzten Jahren insbesondere bei sehr unreifen Frühgeborenen zunehmend Anwendung fand. Für dieses Verfahren wird das Kind per Sectio caesarea gemeinsam mit der Plazenta, häufig in noch intakter Fruchthöhle, entwickelt. Auf der Erstversorgungseinheit wird die Plazenta im Folgenden über mehrere Minuten hochgehalten, so dass über die noch intakte Nabelschnur eine Transfusion von plazentarem Blut zum Kind hin erfolgen kann, während zeitgleich eine Atemunterstützung mittels CPAP durchgeführt wird.

Ziel: Da das EPT-Verfahren bislang nur wenig evaluiert ist, untersuchte die vorliegende Studie das Outcome von Frühgeborenen mit sehr niedrigem Geburtsgewicht (<1500g, very low birth weight (VLBW) Kindern), die im Rahmen der Erstversorgung eine EPT erhielten und verglich deren neonatale Outcome-Parameter mit VLBW-Kindern, die ein alternatives Abnabelungsverfahren erhalten hatten.

Methoden: Die vorliegende Beobachtungsstudie erfasste über den Zeitraum eines Jahres insgesamt 102 Frühgeborene mit einem Geburtsgewicht von <1500g , die

per Sectio caesarea in der Uniklinik Köln entbunden wurden. Die Kinder wurden in zwei Gruppen unterteilt: 67 Frühgeborene, die eine EPT erhalten hatten (EPT-Kohorte) und 35 Frühgeborene, die entweder sofort abgenabelt wurden (immediate cord clamping (ICC)) oder mittels DCC oder CM versorgt wurden (nonEPT-Kohorte).

Kriterien, die zum Ausschluss von der Studie führten, waren vaginale Entbindung (n=2), notfallmäßige Sectio caesarea (n=13), schwerwiegende kongenitale Anomalien (n=2) und ein Versterben des Kindes innerhalb der ersten 24 Lebensstunden (n=0).

Im Rahmen der Studie wurden neben basalen klinischen Kovariaten (z.B. Geburtsgewicht und Gestationsalter), zahlreiche Outcome-Parameter erhoben und zwischen den Kohorten verglichen. Ein wichtiges Zielkriterium hierbei war der durchschnittliche Hämatokrit in den ersten 24 Lebenstunden. Weitere Parameter umfassten die Notwendigkeit bzw. die Menge von Erythrozytenkonzentrat-Transfusionen nach 7 und 28 Tagen, die Höhe der Bilirubinkonzentration im Blut, das Auftreten einer intraventrikulären Blutung (IVH), einer spontanen intestinalen Perforation (SIP), einer Frühgeborenen-Retinopathie (ROP) oder einer Bronchopulmonalen Dysplasie (BPD).

Ergebnisse: Frühgeborene der EPT Gruppe waren sowohl signifikant kleiner (916 ± 43 g versus 1192 ± 43 g; $p<0.001$) als auch unreifer (27+6 Wochen ± 3 Tage versus 30+1 Wochen ± 3 Tage; $p=0.002$), als VLBW-Frühgeborene der nonEPT Gruppe.

Die mittlere Dauer einer durchgeführten EPT lag in der entsprechenden Kohorte bei circa 6 [5:14] Minuten.

Die Anzahl der Frühgeborenen in der EPT Gruppe, die eine Transfusion von Erythrozytenkonzentraten erhielten, war signifikant größer als in der nonEPT Kohorte ($p=0.025$). Allerdings unterschied sich die innerhalb von 28 Tagen transfundierte Menge von Erythrozytenkonzentraten nicht signifikant zwischen den beiden Kohorten

(*p=0.070*). Auch bezüglich des Hämatokrits (*p=0.655*), der Bilirubinkonzentration im Serum (*p=0.060*), der Rate an IVH (*p=0.142*), SIP (*p=0.054*) und BPD (*p=0.116*), zeigte sich kein signifikanter Unterschied im Vergleich der beiden Kohorten.

Eine Analyse bezüglich des Überlebens ohne schwerwiegende Komplikationen, definiert als Vorliegen entweder einer schweren IVH (Grad III/IV), einer periventrikulären Leukomalazie, einer BPD, einer ROP >Grad III+ oder die Notwendigkeit einer operativen Versorgung aufgrund einer SIP oder Nekrotisierender Enterokolitis, zeigte einen signifikanten Vorteil der nonEPT Kohorte (*p=0.023*).

Schlussfolgerung: Obwohl bei den Frühgeborenen der EPT Gruppe aufgrund ihres höheren Grades der Unreife eine erhöhte Inzidenz typischer neonataler Komplikationen zu erwarten gewesen wäre, als bei den reiferen Kindern der nonEPT-Gruppe, zeigte sich ein vergleichbares Outcome in Hinblick auf die Mehrheit der untersuchten Parameter.

Bezüglich des Parameters “Überleben ohne schwerwiegende Komplikationen” zeigte sich zwar eine niedrigere Rate gegenüber der nonEPT-Vergleichskohorte, jedoch finden sich dem Gestationsalter und Geburtsgewicht entsprechend vergleichbare Angaben bezüglich der Inzidenz der jeweiligen neonatalen Komplikation in der Literatur. Dies lässt darauf schließen, dass dieses Ergebnis eher im höheren Unreifegrad der EPT-Kohorte als im Verfahren der EPT selbst begründet ist.

Insgesamt unterstützt die vorliegende Arbeit die Hypothese, dass das EPT-Verfahren das Outcome von sehr kleinen Frühgeborenen nicht negativ beeinflusst. In der Zukunft braucht es jedoch weiterführende randomisiert-kontrollierte prospektive Untersuchungen, um die Einflüsse des EPT-Verfahrens auf die Entwicklung sehr kleiner Frühgeborener eindeutig zu erfassen.

1. Summary

Objective: To investigate the outcome of very low birth weight (VLBW) infants who received an extrauterine placental transfusion (EPT) and to compare outcome data of these patients with VLBW infants who received either immediate cord clamping (ICC), delayed cord clamping (DCC) or cord milking (CM).

The EPT technique comprises the simultaneous delivery of the infant and the placenta en caul with an intact umbilical cord. Then, the placenta is held 30-40 cm above the infant's chest while respiratory support by mask continuous-positive-airway-pressure (CPAP) is initiated. The cord is then clamped after several minutes when spontaneous breathing is established and the lungs are aerated. In comparison to DCC or CM, EPT allows for a simultaneous combination of transfusing placental blood and providing respiratory support. However, the effect of EPT on VLBW infants' outcomes has not been evaluated.

Aim: The presented study investigated the neonatal outcome parameters of VLBW infants who received an EPT at neonatal resuscitation compared to VLBW infants who received alternative cord clamping procedures (ICC, DCC, CM).

Method: This observational study was conducted over a one year period and included preterm infants who were delivered via caesarean section with a birth weight of ≤1500g at the tertiary care center at the University Hospital of Cologne. The study comprised a total of 102 infants who were divided into two cohorts: 67 infants who received EPT (EPT cohort) and a nonEPT cohort of 35 infants who received either ICC, DCC or CM.

Criteria of exclusion included: vaginal delivery (n=2), emergency cesarean section (n=13), major congenital abnormalities (n=2) and death within the first 24 hours of life (n=0).

In order to compare the EPT and nonEPT cohort comprehensive clinical characteristics and outcome parameters of the two cohorts were monitored. Important

outcome parameters included the mean haematocrit (Hct) over the first 24 hours of life, the incidence and volume of red blood cell (RBC) transfusions during the first 7 and 28 days of life, peak bilirubin serum levels, incidence of IVH, BPD, SIP and ROP were registered.

Results: Compared to the nonEPT-group, infants of the EPT-group weighed significantly less (916 ± 43 g compared to 1192 ± 43 g; *p<0.001*) and were delivered at a lower gestational age ($27^{6/7}$ weeks ± 3 days compared to $30^{1/7}$ weeks ± 3 days; *p=0.002*).

The median duration of performed EPT was 6 [5:14] minutes.

The need (*p=0.025*) for RBC transfusion but not the amount after 28 days (*p=0.070*) was lower within the nonEPT-group. Concerning Hct (*p=0.655*), peak bilirubin serum levels (*p=0.060*), the incidence of IVH (*p=0.142*), SIP (*p=0.054*) and BPD (*p=0.116*) there was no significant difference between the two groups.

The analysis of the composite outcome criteria "survival without major complications" defined as survival without the incidence of either IVH grade III/IV, periventricular leucomalacia, BPD, intervention at ROP and the need for surgical intervention at SIP or necrotising enterocolitis, showed a significantly better outcome for patients of the nonEPT-group (*p=0.023*).

Conclusion: Although children of the EPT-group were more immature and therefore had a higher risk for an adverse outcome, the majority of investigated parameters showed no difference between the two groups.

Even though "survival without major complications" was significantly lower within the EPT-group, the incidence of the investigated morbidities corresponds with those that can be found in published data concerning infants with an equivalent birth weight and gestational age, respectively. This led to the conclusion that higher rates of certain neonatal complications of infants in the EPT-group are attributed to their higher degree of prematurity rather than the procedure of EPT they had received.

Therefore, the present study supports the hypothesis that EPT provides an alternative cord clamping approach for VLBW infants. However, future randomised controlled trials on EPT are necessary to further investigate the impact of this procedure on extremely immature infants' outcomes.

2. Introduction

The transition from fetal to neonatal life has become the focus of increased research in recent years. Procedures that had been established decades ago and have ever since dominated the course of actions in obstetrics and neonatal resuscitation, are now being questioned and new approaches of treatment are being investigated.

The process from intra- to extrauterine life is a vulnerable phase for newborns and is especially challenging for very preterm infants. Younger gestational age and lower birth weight are directly linked to an increased risk of neonatal complications during the postnatal period, when adjustments dictated by the onset of respiration and the conversion from fetal to neonatal circulation take place. 1

The German Neonatal Network published observational data in the year 2017 which showed that the vast majority of preterm infants below 37 weeks of gestational age in Germany are delivered via caesarean section. The rationale standing behind this circumstance is that VLBW infants especially those with a gestational age below 30 weeks, benefited from an elective caesarean section by reduced rates of IVH compared to vaginal delivery. 2

Overall, higher rates of caesarean sections, antenatal and also postnatal corticosteroid application and non-invasive ventilation such as CPAP have led to lower rates of IVH and pneumothoraces. The progress made in neonatal resuscitation and ventilatory support resulted in increasing survival rates and decreasing numbers of complications with VLBW and VLGA infants. 3, 4

As the surfactant production slowly starts to increase at 22 weeks of gestational age and approximately reaches a sufficient amount after 32 weeks of gestational age, very premature infants frequently require exogenous surfactant replacement therapy. 5

Instead of using an endotracheal tube during mechanical ventilation in the University Hospital of Cologne, LISA, meaning less invasive surfactant application, has become the standard procedure. When performing LISA, surfactant is administered via

a thin tube while the infant is breathing spontaneously with CPAP support which reduces the need for mechanical ventilation and increases survival without major complications compared to the conventional method. 6, 7, 8

2.1 The „First Golden Minutes of Life" – Fetal to Neonatal Transition

The aeration of the lungs after birth leads to a sudden reduction of pulmonary vascular resistance causing an increased pulmonary blood flow. In this phase of transition a raised venous back flow to the heart is necessary, otherwise a deficit of the cardiac preload could develop. Only the blood that is provided through the placenta which makes up for 30-50% of the cardiac output, is able to compensate the increased pulmonary blood flow. Thus, this compensating mechanism cannot apply if the infant is separated from the placenta and the umbilical cord is clamped before the lungs are aerated. Hypoxia and a decreased cardiac output are observable complications. 9

The effects of clamping the umbilical cord before the initiation of respiration on the physiological processes in the body, have been investigated in previous studies on lambs. Results revealed that clamping the cord after the aeration of the lungs led to an improved cardiovascular function and a more stable haemodynamic transition after birth. 10, 11

2.2 Cord Clamping Strategies

The right timing for the clamping of the umbilical cord is considered to be of vital importance to the outcome of preterm infants. 12

Current cord clamping procedures include immediate cord clamping (ICC), cord milking (CM) and delayed cord clamping (DCC). 13

With the application of ICC the cord is clamped and cut immediately, meaning that the intact connection from placenta to infant existed for less than 30 seconds after delivery. 12

With both CM and DCC, the connection of the umbilical cord between infant and placenta stays intact for longer. CM refers to the repeated compression and milking (2-4 times) of the cord from the placental side towards the infant before clamping the cord, usually carried out either in a one-handed or two-handed technique. DCC, refers to a technique where the cord is clamped and cut at least 30 seconds or more after delivery. 14 The newborn is held below the level of the birth canal approximately at the height of the mother's legs. The spontaneous contractions of the third stage of labour after vaginal birth add to an additional extrusion of the blood into the newborn. 15

At present, there is no standardised period of time over which the DCC procedure should be performed. 16
Therefore cord clamping is delayed between 30 and 120 seconds after delivery. When the intact placenta-cord connection is preserved, the procedure can be performed after spontaneous vaginal birth as well as caesarean section. 17, 18

Additionally, it is recommended to wrap infants in plastic foil in order to keep the body temperature of the infant at an optimal level which can also be complemented by exercising tactile stimulation on the newborn. 14, 19

Both CM and DCC aim to maintain placental blood transfusion towards the child after delivery. 14

2.2.1 Effects of Different Cord Clamping Strategies

A systematic review and meta-analysis by Backes et al. (2014) investigating placental transfusion strategies in preterm infants of less than 32 weeks of gestation, stands in accordance with the recommendation for term newborns which suggests DCC as the procedure of primary choice. These very preterm newborns especially benefit from this technique because of a reduced risk of IVH, overall mortality and blood transfusion incidence. 20

In addition, another observed benefit of DCC is a decreased incidence of late-onset sepsis in VLBW infants. 21

When DCC is performed, the blood being held by the placenta can shift into the circulatory system of the newborn and increase their volume significantly, this is especially notable in preterm and very preterm infants for who it can represent an increased blood volume of 15%. 22

The blood which is transferred when clamping the cord is delayed, can enhance the preterm infants' cardio-circulatory stability. 23

By increasing postnatal blood volumes, DCC also leads to higher levels of ferritin and possibly prevents iron deficiency before the age of six months and improving neonatal development. 24,25

Iron deficiency is considered one of the possible reasons to affect the neurodevelopmental outcome negatively in term and preterm infants. 26,27

Despite presenting increased peak bilirubin concentrations, preterm infants who have received DCC, particularly profit by requiring less red blood cell transfusions and a reduced risk for NEC. 23

In addition, DCC, even when performed for a minimum time of 30-45 seconds, has been shown to have a positive impact on motor function of very preterm infants at 18-22 months corrected age. 28

In summary, research supports the idea that the procedure of DCC provides a positive influence on the outcome of preterm infants and holds no disadvantages for their neurocognitive development, leading to a decrease in mortality and disability. 29, 30

DCC does not allow for simultaneous respiratory support in the majority of deliveries, despite it being of vital importance that the respiration of the preterm infant is established when DCC is performed. In a study by Nevill and Meyer which compared the outcome of preterm infants who had started to breathe spontaneously during DCC to those who had not, it transpired that non-breathing infants had a significantly higher

incidence of chronic lung disease, severe IVH and a higher probability of requiring intubation and invasive mechanical ventilation. 31
Such data stresses the importance of initiating respiratory support simultaneously to DCC.

The benefits of an increased blood volume due to the remaining postnatal blood supply through the placenta, is also utilised in the procedure of Cord Milking (CM).

Two different studies focusing on CM by Katheria et al. (2015 and 2018) also suggested an improved cardiovascular stability which is accomplished through higher systemic blood flow and a similar neurodevelopmental outcome. 32,33

Another trial indicates that CM, just as DCC, has a positive effect on the haematocrit and therefore decreases the need for RBC transfusions. Additionally, the authors reported a lower incidence of NEC and IVH in CM infants. 34

A meta-analysis by March et al. (2013) who observed the effect of CM in extremely preterm infants (24-28 weeks of gestation), corresponded with the finding of decreased incidence of IVH and less need for RBC transfusion presumably originating from higher initial haematocrit concentrations. 35

Similar positive effects on the neonatal neurocognitive outcome comparable to DCC, in preterm infants were highlighted in a meta-analysis of Al-Wassia et al. (2015). 36

However, a more recent, multi-centered randomised controlled trial by Katheria et al. (2019) comparing the rates of death and severe IVH after CM and DCC, indicated that higher numbers of IVH were noted in the preterm infants below 28 weeks of gestation who had received CM, raising questions about the safety of this procedure for preterm infants. 37

Considering all potential benefits and limitations of DCC or CM the question of the best cord clamping management in resuscitation of very preterm infants still remains unsolved. 38, 39

2.3 Delivering the Preterm Infant en caul with the Placenta

Another perspective opens up from manuscripts from Dunn and Várdi who had already recognised in the last century that placental transfusion plays a key role in providing a physiological transition after birth that is especially beneficial for preterm infants.

Dunn described a technique associated with an improved outcome for preterm infants which comprised the simultaneous delivery of the preterm infant and placenta by cesarean section. 40

The procedure which was developed in 1961 consisted of positioning the infant in an upward position connected to the placenta which is put beside on the same level whilst spontaneous breathing of the newborn was established. Studies that were conducted in the following years revealed a decrease in morbidity and mortality concerning RDS in infants below 35 weeks of gestation. 41

In an article from 1965 Várdi also described a technique in which newborn and placenta were delivered together. The latter was then placed on a board one foot above the level of the newborn and the cord was clamped approximately after two minutes or preferably when the pulsations of the cord had terminated. 42

Due to the fact that in the meantime ICC was propagated and became the new standard in obstetrics next to the development of CPAP in the 70s, the method and its effect on very preterm infants had not been investigated any further.

2.4 The Extrauterine Placental Transfusion

The approach of delivering the infant and placenta en caul has been further developed at the University Hospital of Cologne. In this approach, respiratory support

by mask CPAP is started while the infant who is placed on the resuscitation unit remains still attached to the placenta by an intact umbilical cord. This procedure allows for a physiological transition after birth, meaning that the cord is clamped after aeration of the lung has been established. This approach was named the Extrauterine Placental Transfusion (EPT) and has been performed routinely.

In the year 2015 a retrospective analysis revealed that a cohort of preterm infants born <32 weeks of gestational age (n=60 included) who had received EPT, had a similar outcome concerning neonatal morbidities as compared to a cohort of infants <32 weeks who received standard cord clamping procedures (mostly DCC).
Despite the fact that infants of the EPT-group were significantly smaller and more premature, negative effects of the procedure on the neonatal outcome could not be observed. Additionally, recordings of vital parameters during resuscitations made with a respiratory function monitor were able to show that there was no difference in SpO2 levels and heart rates between the infants of both groups. 43

2.5 Aims of this Trial

The primary aim of the present trial is to further evaluate the impact of the EPT approach on the outcome of VLBW infants in a prospective manner. To this end, both data on the cord clamping approach and clinical data were collected of all VLBW infants born between 29/08/2017 and 01/09/2018 at the University of Cologne.

In order to precisely document which cord clamping strategy was performed a specific Cord Clamping Sheet was developed and the specific parameters concerning the cord clamping strategy were collected. In particular the time until the cord was clamped was documented. Finally, data on different cord clamping procedures (EPT vs. DCC vs. CM vs. ICC) were correlated with data on important neonatal outcome parameters, including the need for RBC transfusion and the incidence of IVH, NEC, FIP, BPD and ROP. Thereby, the study aimed to evaluate the potential impact of EPT on both the mean haematocrit of preterm born infants and important neonatal outcome parameters.

3. Material and Methods

The presented trial was approved by the Ethical Review Board of the University Hospital of Cologne. (ID 18-352)

3.1 Patient Cohort

Infants who were born via cesarean section at the University Hospital of Cologne between 29/08/2017 and 01/09/2018 and who had a birth weight of <1500g were included in the present study.
Infants who were born by vaginal delivery or via emergency cesarean section as well as infants with major congenital malformations or who died within the first 24 hours of life were excluded from the analysis.
Following this criteria, 102 of 119 VLBW infants at our centre were eligible for the trial.

The first step in analysing the remaining 102 preterm infants consisted of building two cohorts. *(view **Figure 1**)*

Our analysis compared infants who had received the EPT procedure (EPT-group) to those who had either received CM, DCC or ICC (nonEPT-group). Summarising the different approaches of DCC, CM and ICC into one cohort allowed us to compare the EPT procedure to a broad spectrum of standard procedures.

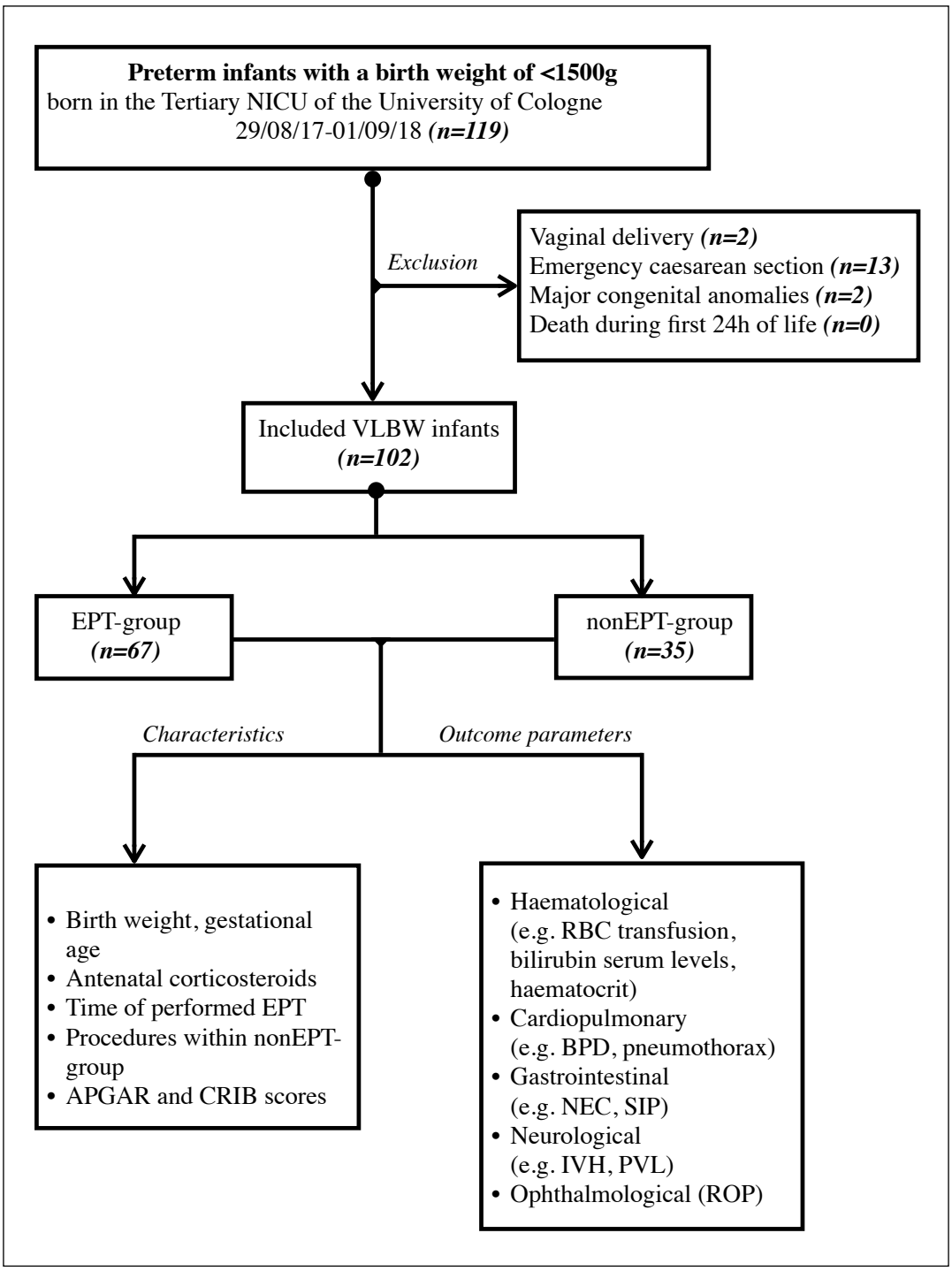


Figure 1: Flowchart of the presented trial

3.2 Procedure of Extrauterine Placental Transfusion in the EPT-group

At the tertiary care department of neonatology at University Hospital of Cologne the procedure of extrauterine placental transfusion (EPT) has been implemented into routine practice for preterm newborns, especially very low birth weight infants.

In order to deliver the preterm neonates in the gentlest way, an experienced obstetrician separates the placenta from the uterine wall whilst the amniotic sac remains intact and the infant remains attached to the placenta via the umbilical cord. It was dependent on the prae- and perioperative judgement of the obstetrician if such an EPT delivery could be safely carried out or not.

The procedure provided the opportunity to transfer the neonate to the resuscitation unit whilst being safeguarded from bruisings inside an intact amniotic cavity. 44

If however, the amniotic sac had been opened during the delivery, it was also possible to only deliver the child with an intact cord connection to the placenta and proceed as described previously.

If the infants were placed on the resuscitation unit in the intact amniotic sac, the sac was incised and the amniotic fluid released. Whilst the CPAP support along with the EPT was initiated, the infant was wrapped in warm sheets and plastic foil, ECG electrodes were fitted and a pulse oximetry electrode was attached to the right hand. The infant's vital parameters were constantly monitored and the placenta was placed into a bowl-shaped vessel that was held ~40 cm above the infants heart level.
After several minutes when the umbilical veins had visibly collapsed, the cord was clamped.

The exact time after birth at which cord clamping occurred, was recorded in the present investigation and described the time during which EPT was performed.
During the administration of EPT every necessary procedure of resuscitation and respiratory support could be performed, without restriction of any kind.

3.3 Procedures in the nonEPT-group

Applied procedures were ICC, DCC and CM.

The most important difference in comparison to the EPT, was that all the procedures within the nonEPT-group were performed by the obstretician in the operating room. Therefore, all these infants received cord clamping before any respiratory support was initiated.

3.4 Data Acquisition, Diagnostics and Scores

Data about the following parameters were collected:

- sex
- birth weight
- gestational age
- antenatal corticosteroid therapy
- nonEPT procedures
- time of performed EPT
- temperature at admission
- APGAR score
- CRIB score
- average haematocrit during the first 24 hours of life
- frequency and amount of red blood cell transfusions
- blood withdrawal
- peak bilirubin serum levels
- days of phototherapy
- patent ductus arteriosus and therapy
- surfactant replacement therapy
- hours of CPAP support
- peak FiO_2
- hours of invasive mechanical ventilation
- pneumothorax
- bronchopulmonary dysplasia
- nectrotising enterocolitis

- spontaneous intestinal perforation
- intraventricular haemorrhage
- periventricular leukomalacia
- retinopathy of prematurity
- survival without major complications

Co-morbidities and specific diagnoses were recorded until discharge and documented digitally in the ORBIS and NEODAT programs.
All further clinical co-variates that were considered in the present trial were taken from patients' medical charts. No extra tests or diagnostic procedures were performed exclusively for the trial. Only parameters that were collected by routine clinical care were included in the dataset for the study.

The APGAR score which was assigned after one, five and ten minutes postnatal, evaluates the status of the neonate concerning respiratory effort, heart rate, muscle tone, colour and reflex irritability. The maximum score per category is two points and higher scores indicate better infant viability. 45

The CRIB score is a tool that helps to determine the mortality risk of very low birth weight infants. Birth weight, gestational age, base excess and sex of the infant are taken into consideration for establishing a score between the numbers 0 and 27. The lower the number the better the prognosis of the infant. 46, 47

Furthermore, it was recorded if antenatal corticosteroids (ACS) had been administered before delivery. The treatment was considered complete if two doses of Bethamethason had been administered. A single dose was registered as an incomplete ACS treatment.

The calculation of the average Hct considered all values that were obtained within the first 24 hours of life (one to three values) from capillary or venous blood withdrawals.

Also we meticulously noted the volume of iatrogenic blood loss caused by taking blood samples and blood gas analysis. The following amount for typically taken

controls were defined: 95 µl for a capillary blood gas analysis, 150 µl for an EDTA blood test tube (for blood count and CRP values), 200 µl for a heparin plasma monovette (for clinical chemistry) and 200 µl for a serum test tube (e.g. for determining vancomycin or aminoglycoside peak or trough levels). Any blood withdrawal was documented in the patients' charts and utilised in the present data set.

Cases of NEC were considered if patients had stage II and III NEC according to the category published by Bell et al. 48

IVH, diagnosed by ultrasound, were graded 1 to 4 and based on criteria according to Papile et al. 49

Diagnosis of BPD was made at day 28 of life and/or 36 weeks postmenstrual age if there was any oxygen or respiratory support by CPAP required, based upon the criteria of Walsh et al. 50

The diagnosis of persistent ductus arteriosus (PDA) was determined by ultrasound as was the assessment of haemodynamic significance. If deemed necessary, medical closure of PDA was conducted by the administering of indomethacin or ibuprofen.

Grading of ROP was distinguished from stages 1-5 complying with the international classification and therapy by either intravitreous antibody injection or by laser therapy was documented. 51, 52

Finally, several co-morbidities were summarised into a composite secondary outcome parameter termed "survival without major complications". This composite outcome parameter was defined as survival until discharge without BPD, severe IVH, PVL, surgery for NEC or SIP, ROP requiring or laser therapy or anti-VEGF treatment. 7

3.5 Statistics

Data analysis was conducted with IBM SPSS Software, Version 25 for Mac (SPSS Inc). Variables are described as mean (± standard deviation), median [interquartile range] or absolute and relative values. Range was indicated from minimum to maximum. Differences between groups were compared using *t*-test for normally distributed data, Mann-Whitney *U* test for other metric data and Fisher Exact Test for categorical data.

Binary logistic regression was applied to test the effects of neonatal parameters in a prediction model.

A two-sided ***p*** value < 0.05 was defined as statistically significant.

When boxplots are displayed, the box itself represents the interquartile range [IQR] and the line in the box represents the median. The end of the whiskers represent the most extreme values inside the 1.5 IQR difference from the box.

Values marked as stars (“ * “) which are 3 IQRs away from the box were labelled as extremes and those which are more than 1.5 IQRs from the box were marked with a small circle (“ ◦ “) and were labelled as outliers.

4.1 One form fits all: The Cord Clamping Sheet

In order to accurately and reliably record the mode and timing of cord clamping, a cord clamping sheet (***Figure 2***) was developed and implemented into the documentation process of both obstetricians and the neonatologists. Only one of the sheets had to be filled in per delivered preterm infant. The obstretrician wrote down the initials of the infant, the date of birth, if it was a single or multiple pregnancy and if so what number of multiples the infant was. In addition to it would be noted if it was a spontaneous vaginal birth, a c-section or emergency c-section and if they had performed ICC, DCC, CM or handed the infant along with the placenta to the paediatrics to perform EPT.

After that the sheet along with the other relevant documentation letters were handed to the paediatricians. They confirmed if they had received the child with or without the placenta, if they had or had not performed an EPT.

The time during which placental transfusion was performed, was monitored for every infant who had received EPT, starting from the moment the infant was placed on the resuscitation unit until the cord was clamped.

In addition, they had the opportunity to note down the first measured Hct and if the neonatal resuscitation procedure was recorded using a NewLifeBox respiratory function monitor.

Although this study has an almost prospective approach, the focus lies on the detailed observations of treatment procedures during and after delivery especially of preterm infants with very low birthweight.

It is the first time the mode of the administered cord clamping procedure (ICC, DCC, CM and EPT) and time of possibly performed EPT were monitored meticulously in our centre.

PLEASE FILL IN FOR ALL INFANTS WITH A BIRTH WEIGHT <1500g

Information provided by attending **obstetrician**:

Initials of born infant: ____.____ Day of delivery: __ __/__ __/__ __

Case-ID: _____

1.) Pregnancy

☐ Single pregnancy

☐ Multiple pregnancy

number of delivered infant: ___

2.) Birth

☐ Vaginal delivery ☐ Caesarean section

☐ Emergency caesarean section

3.) Method of Cord Clamping

☐ Immediate/early cord clamping

☐ Delayed cord clamping

☐ Cord milking

☐ Simultaneous delivery of infant and placenta (attached)

Information provided by attending **paediatrician**:

The infant arrives at the resuscitation unit…

1.) ☐ attached to placenta ☐ without placenta

2.) ☐ and EPT is performed ☐ and EPT is not performed

(Recommendation: Raise placenta approximately 40cm above the infant and hold until umbilical veins have visibly collapsed)

⇩

Umbilical cord clamping after _____ seconds

3.) First measured haematocrit: _____%

4.) Employment of NewLifeBox respiratory function monitor: ☐ yes ☐ no

Figure 2: Cord Clamping Sheet for preterm infants <1500g

4.2 Description of the Patient Cohort

4.2.1 Number of Patients and Excluded Patients

In a one-year period from 29/08/2017 to 01/09/2018, 119 children with a birth weight of less than 1500g were delivered via cesarean section at the at the University Hospital of Cologne, of whom 102 were eligible to be included into the trial.

A total of 17 patients had to be excluded due to vaginal delivery (n=2), emergency cesarean section (n=13) and major congenital abnormalities (n=2; such as one case of tetralogy of Fallot and one case of hypoplastic left heart syndrome).

One hundred of the 102 VLBW infants who were included in the trial survived until discharge. Two newborns died: One of them was born at $22^{6/7}$ weeks of gestation with a birth weight of 290g and developed treatment-refractory circulatory failure due to sepsis with an abdominal focus. The other infant was also born at $22^{6/7}$ weeks and was switched to palliative care because of high grade IVH and SIP.

4.2.2 Patient characteristics

Having included a number of 102 patients into the study, infants were divided into two groups depending on whether they had received an EPT or not. In total, 67 patients received EPT, while the remaining 35 were summarised in the nonEPT-group. Patient clinical co-variates are highlighted in table ***Table 1***.

Characteristics (unit)	**nonEPT-group**	**EPT-group**	***significance***
Sex (male/female)	16/19	34/33	*0.680*
Birth weight (g)	1250 [202]	980 [640]	*<0.001*
Gestational age (days) (weeks)	218 [32] $31^{1/7}$	192 [43] $27^{3/7}$	*0.002*
ACS (none/started/completed)	5 (16)/4(13)/23(72)	12(19)/8(12)/42(68)	*0.943*
Temperature at admisson (°C)	36.9±0.7	36.5±0.5	*0.002*

Characteristics (unit)	nonEPT-group	EPT-group	*significance*
EPT time (s) (min)	— — —	360 [314] 6 [5:23]	— — —
nonEPT procedures (ICC/DC/CM)	20(57)/2(37)/13(6)	— — —	— — —
1-minute APGAR	7 [2]	6 [3]	*<0.0011*
5-minute APGAR	8 [0]	8 [1]	*0.014*
10-minute APGAR	9 [1]	8 [1]	*0.002*
CRIB	1 [1]	3 [8]	*<0.001*

Table 1: Table of characteristics of included infants

4.2.2.1 Sex

There was a total of 50 male and 52 female infants included which were distributed homogeneously within the two groups (***Figure 3***): 16 male and 19 female belonged to the nonEPT-group whilst another 34 male and 33 female infants formed the EPT-group (*p=0.680*).

Within the two cohorts male and female infants were distributed equally.

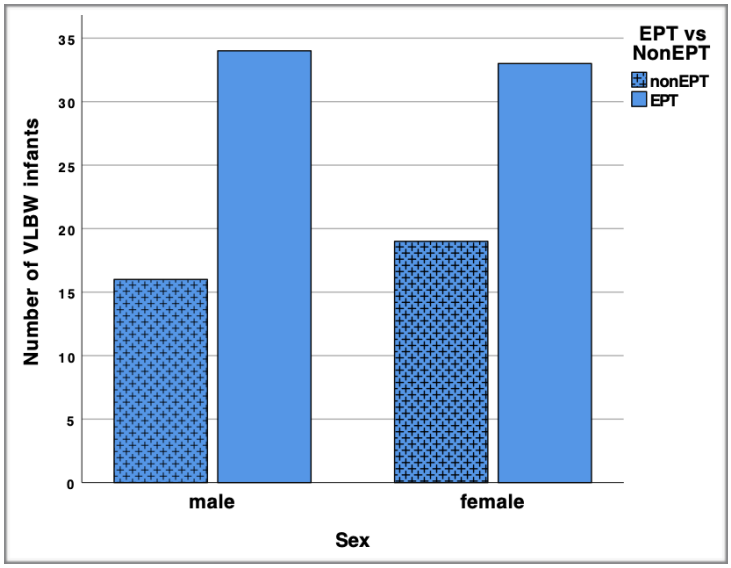
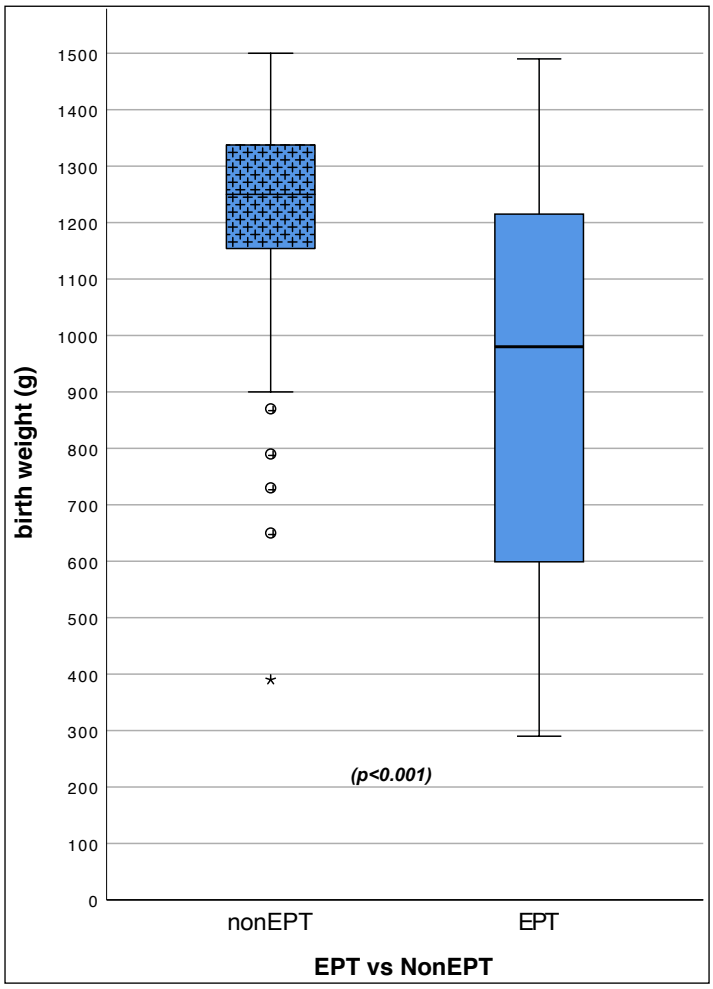


Figure 3: Bar Chart of the distribution of female and male

4.2.2.2 Birth weight

In the non-EPT-group the median birth weight was 1250g [202] and therefore significantly higher than in the EPT-group (980g [640g]; *p<0.001*; ***Figure 4***).



4.2.2.3 Gestational Age

Similarly, gestational age differed significantly between groups. While the median gestational age in the nonEPT-group was 218 days [32 days] (equivalent to $31^{1/7}$ weeks of gestational age), patients in the EPT-group were significantly younger with 192 days [43 days] (equivalent to $27^{3/7}$ weeks of gestational age; *p=0.002*).

Also the range of gestational age was wider in the EPT-group and stretched from 156 to 253 days (equivalent to $22^{2/7}$ to $36^{1/7}$ weeks) of gestation, compared to 157 to 236 days (equivalent to $22^{3/7}$ to $33^{5/7}$ weeks) in the nonEPT-group. (***Figure 5***)

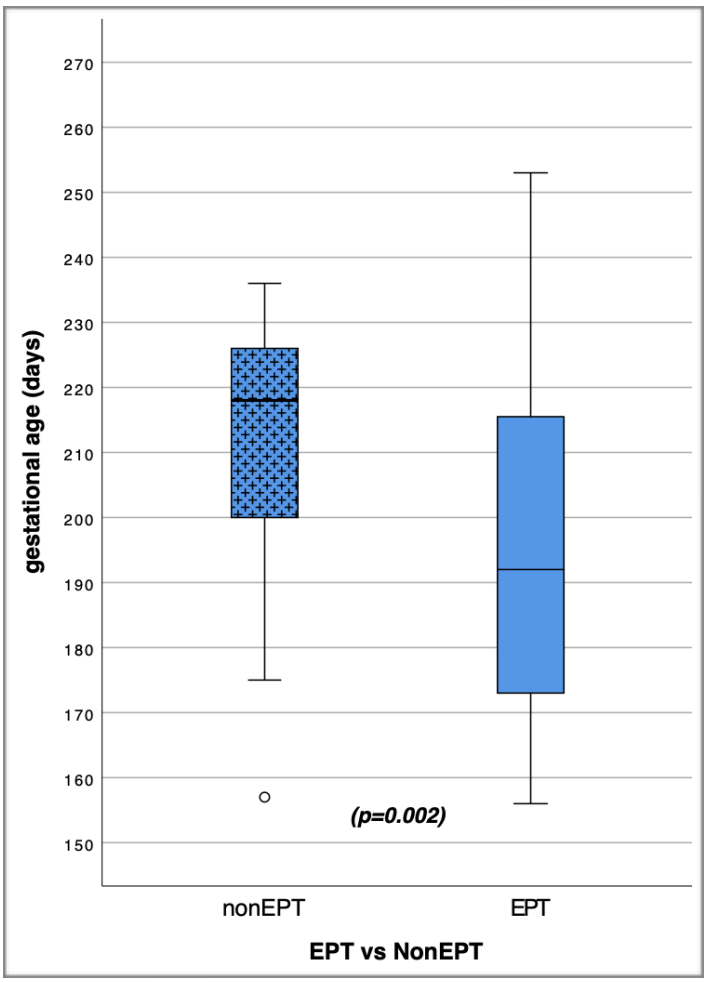


Figure 5: Boxplot showing the gestational age of included infants

4.2.2.4 ACS during Pregnancy

ACS-therapy application did not differ between the groups (*p=0.943)*. In the nonEPT-group ACS was not started in n=5 (16%), started in n=4 (13%) and completed in n=23 (72%). In the EPT-group ACS was not started in n=12 (19%), started in n=8 (12%) and completed in n=42 (68%) of the EPT-group (***Figure 6***).

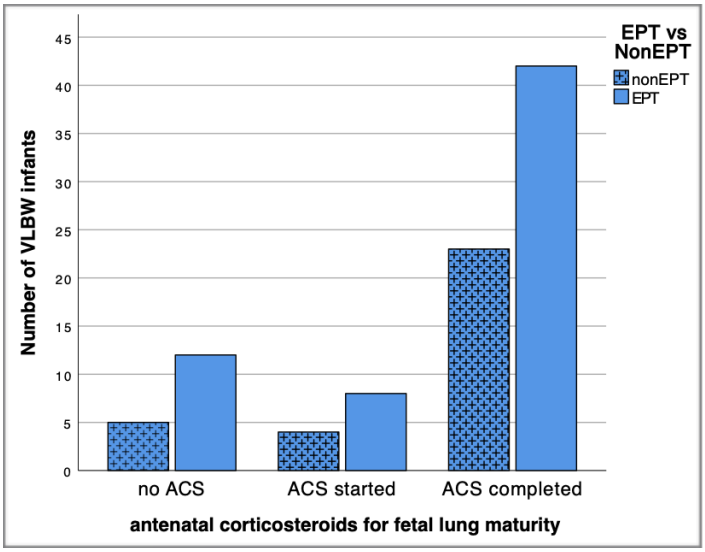


Figure 6: Bar Chart showing the number of not started, started or completed antenatal corticosteroids

4.2.2.5 nonEPT Procedures

Alternatively to the EPT, ICC was performed in n=20 infants (57%), CM in n=13 times (37%) and DCC in n=2 (6%). (nonEPT-group total n=35, ***Figure 7***)

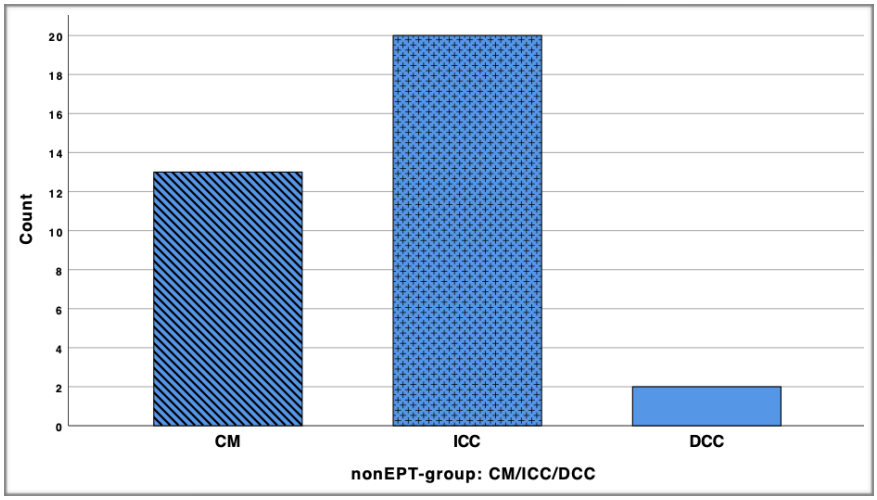


Figure 7: Bar Chart showing the procedures in the nonEPT-group

4.2.2.6 Temperature at Admission

The average temperature at admission within the nonEPT-group was significantly higher with 36.9 ± 0.7 °C compared to 36.5 ± 0.5 °C in the EPT group. (*t*-test: *p=0.002*, ***Figure 8***)

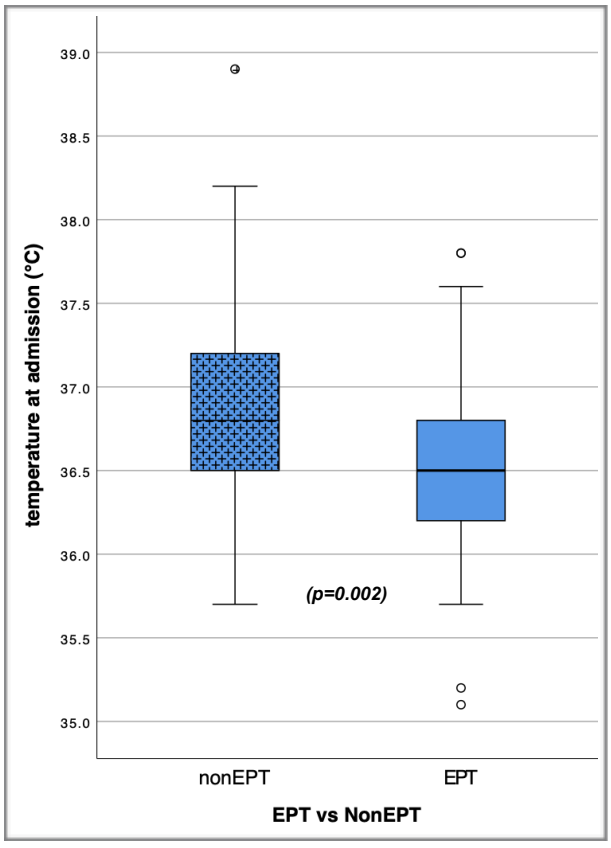


Figure 8: Boxplot showing the temperature at admission of included infants

4.2.3 Duration of EPT

The investigation revealed that EPT was performed for a median of 360 [314] seconds which equals to 6 [5:14] min. The shortest detected time was 100s (1:40 min) and 1260s (21 min) was the longest time. (***Figure 9***)

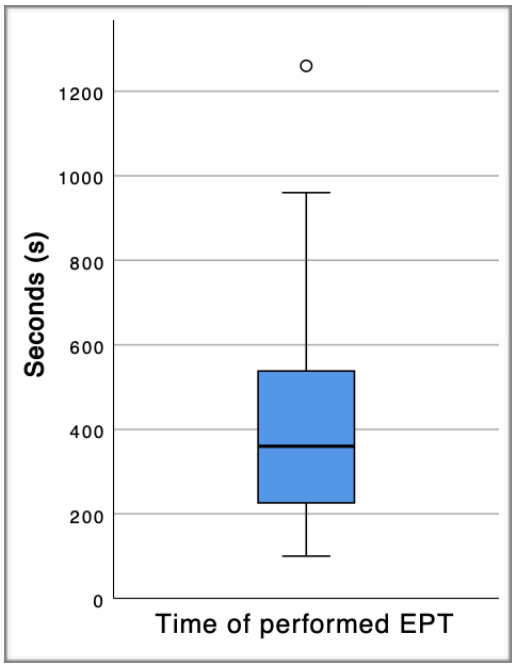


Figure 9: Boxplot showing the time of performance of EPT

4.2.4. Scores: APGAR and CRIB

APGAR-scores were collected for all 102 patients. Overall, it could be noticed that the APGAR scores after 1, 5 and 10 minutes differed significantly between the two groups.

APGAR at one minute of age: The median APGAR score was 7 [2] in the nonEPT-group (ranging from 4 to 8) compared to 6 [3] (ranging from 1 to 8) in the EPT-group ($p<0.001$).

APGAR at five minutes of age: The median APGAR score was 8 [0] in the nonEPT-group, at a (ranging from 6 to 9) compared to 8 [1] (ranging from 3 to 9) in the EPT-group ($p=0.014$).

APGAR at ten minutes of age: The median APGAR score was 9 [1] in the nonEPT-group, (ranging from 6 to 9) compared to 8 [1] (ranging from 4 to 9) in the EPT-group. ($p=0.002$; ***Figure 10***)

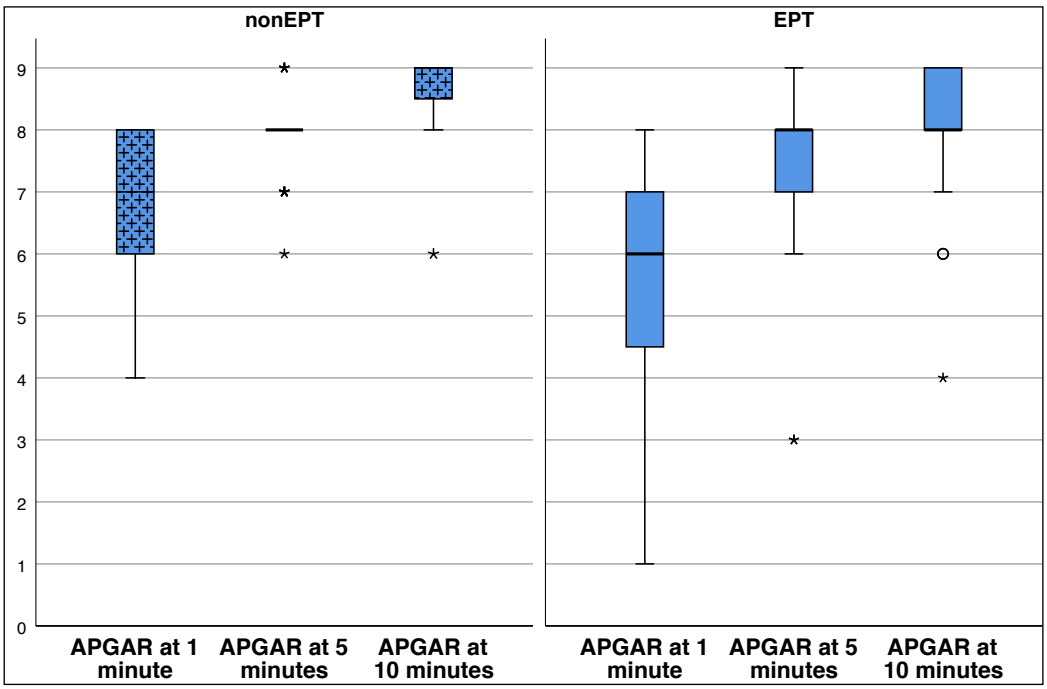


Figure 10: Boxplot showing the APGAR scores of included infants

The median CRIB score in the nonEPT-group was 1 [1] which was significantly lower, compared to the median score of 3 [8] in the EPT-group (Mann-Whitney *U* test: *p<0.001)*. (***Figure 11***)

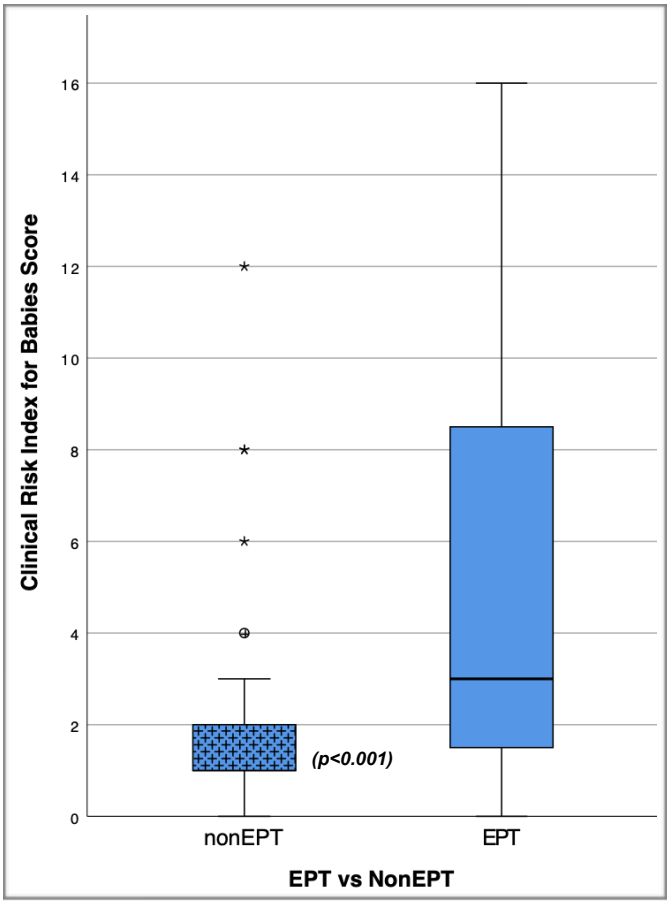


Figure 11: Boxplot showing the CRIB score of included infants

4.3 Outcome Parameters: Complications and Therapies

Outcome parameters are presented in subcategories of haemodynamic, cardiopulmonal, gastrointestinal, neurological, ophthalmological and other outcome parameters. Results are summarised in ***Table 2.***

Outcome parameters (unit)	**nonEPT-group**	**EPT-group**	*significance*
average Hct over 24h (%)	57.0±8.8	56.2±7.9	*0.655*
Need RBC transfusion	6 (17%)	27 (40%)	*0.025*
amount RBC transfusion after 7 days (ml/kg)	15 [0]	20 [35]	*0.817*
amount RBC transfusion after 28 days (ml/kg)	18 [24]	43.5 [33]	*0.070*
blood withdrawal over 7 days (µl)	1945 [1080]	2720 [1290]	*0.005*
peak bilirubin (mg/dl)	10.7 [3.7]	9.1 [4.4]	*0.06*
phototherapy (days)	3 [4]	4 [4]	*0.044*
PDA below 28 weeks gestational age	8 (100%)	29 (81%)	*0.318*
therapy for PDA (number of drug administration)	3 [3]	2 [1]	*0.046*
postnatal surfactant therapy	24 (69%)	53 (79%)	*0.332*
CPAP (hours)	312 [663]	784.5 [1374]	*0.007*
maximum FiO2 level	0.3 [0.19]	0.35 [0.39]	*0.036*
IMV (hours)	398 ± 225	402 ± 71	*0.983*
pneumothorax	1 (3%)	5 (7%)	*0.661*
BPD (mild/moderate/ severe)	4 (11%)/0 (0%)/ 1 (3%)	14 (21%)/4 (6%)/ 0 (0%)	*0.116*
NEC	0 (0%)	1 (1%)	*1.00*

Outcome parameters (unit)	nonEPT-group	EPT-group	*significance*
SIP (diagnosed/ treated)	1 (3%) 1 (3%)	11 (16%) 8 (12%)	*0.054* *0.159*
IVH (Grade I/II/III/IV)	6 (17%)/0 (0)/ 1 (3%)/ 0 (0%)	12 (18%)/ 4 (6%)/ 9 (13%)/ 0 (0%)	*0.142*
PVL	0 (0%)	2 (3%)	*0.545*
ROP (Stage 1/2/3/4/5)	13 (37%)/ 5 (14%)/ 1 (3%)/ 0 (0%)/ 0 (0%)	30 (45%)/11 (17%)/5 (8%)/ 0 (0%)/ 0 (0%)	*0.470*
ROP therapy	0 (0%)	4 (6%)	*0.295*
survival with major complications	5 (17%)	24 (35%)	*0.023*

Table 2: Table displaying all investigated outcome parameters

4.3.1 Haemodynamic Situation

4.3.1.1 Average Haematocrit and Need for Red Blood Cell Transfusions

Compared to the nonEPT-group with a mean of 56.2 ± 7.9 %, the EPT-group had an average Hct of 57.0 ± 8.8 %. The mean Hct of the two cohorts was statistically not significant (*t*-test: *p=0.655*). (***Figure 12***)

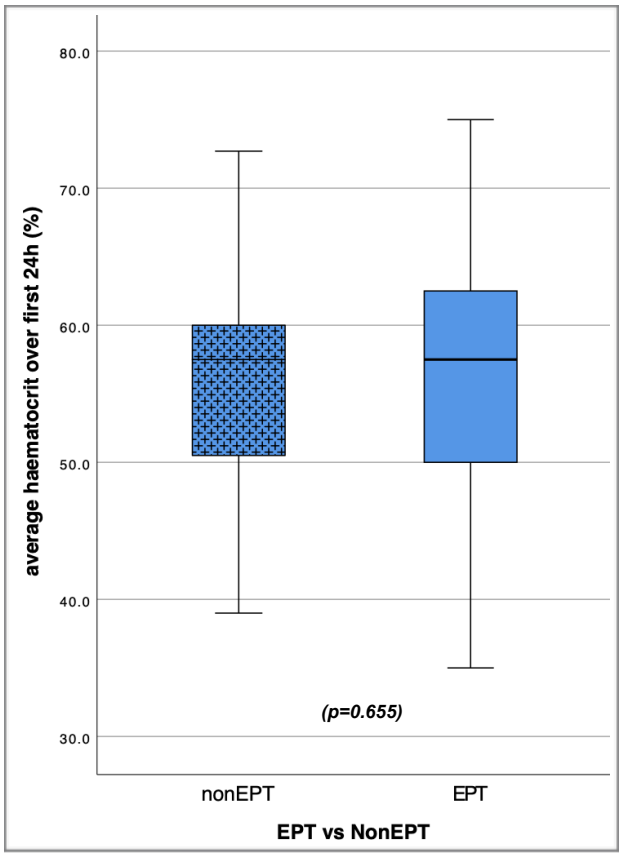


Figure 12: Boxplot of the average haematocrit over the first 24 hours of life

Six of the 35 children in the nonEPT-group required a transfusion of RBC after 28 days (17%) as compared to 27 of the 67 in the EPT-group did (40%; Fisher's Exact Test: *p=0.025*). (***Table 3***)

		EPT vs NonEPT		
		nonEPT	EPT	Total
Red blood cell transfusion	none	29	40	69
	RBC	6	27	33
Total		35	67	102

Table 3: Crosstabulation of the incidence of RBC-transfusion

A logistic regression analysis taking in account the parameters birth weight, gestational age, the performance of EPT and the amount of blood withdrawn within the first 7 days of life shows that only the birth weight (*p=0.010*) had a significant effect on

the occurrence of RBC transfusions. The quality of this regression model settled at 0.741 (Nagelkerke R square) an Odds Ratio at 0.478. The circumstance that the administration of EPT had no influence on the occurrence of RBC transfusions is supported by the univariate logistic regression of every single parameter in which gestational age, birth weight, blood withdrawal within the first 7 days of life were statistically significant factors, but EPT had no statistical significance.

The median amount of RBC transfusion after 7 days was 15 [0] ml/kg in the nonEPT-group and 20 [35] ml/kg in the EPT-group. After 28 days the average added up to 18 [24] ml/kg in the nonEPT-group and to 43.5 [33] ml/kg in the EPT-group. (***Figure 13***)

Comparing the relative volume (in ml/kg) of blood which was transfused after 7 and after 28 days, respectively, the difference between the two groups was not statistically significant (Mann-Whitney *U* test: RBC 7days: *p=0.817*; RBC 28days: *p= 0.070*).

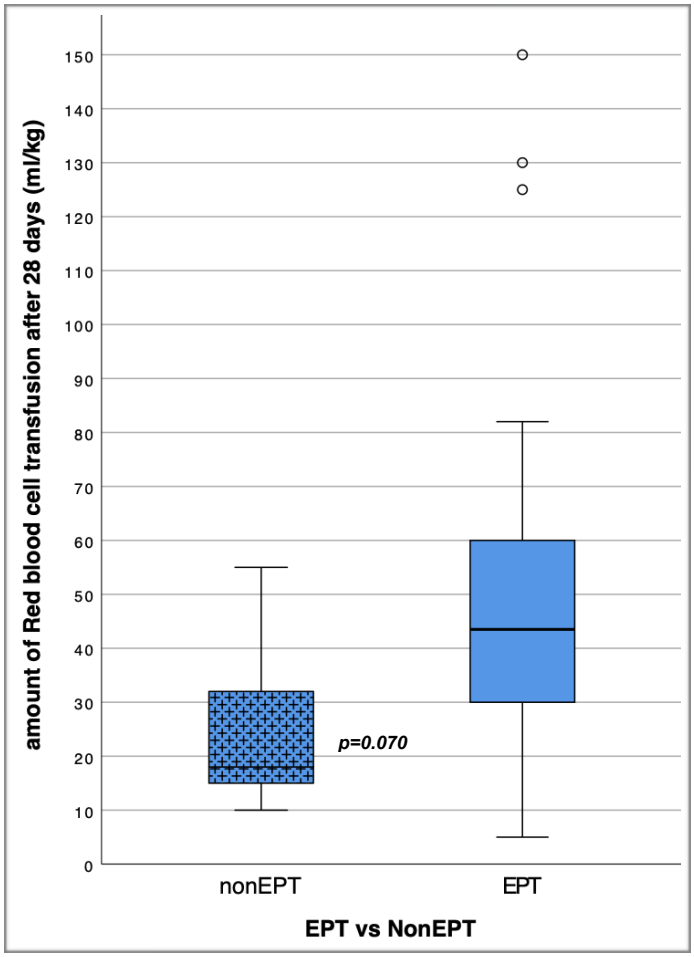


Figure 13: Boxplot of the amount of RBC transfusion after 28 days

4.3.1.2 Blood Withdrawal

In the EPT-group a median amount of 2720 [1290] μl was withdrawn as compared to a median volume of 1945 [1080] μl in the nonEPT-group. (Mann-Whitney *U* test: *p=0.005;* ***Figure 14***).

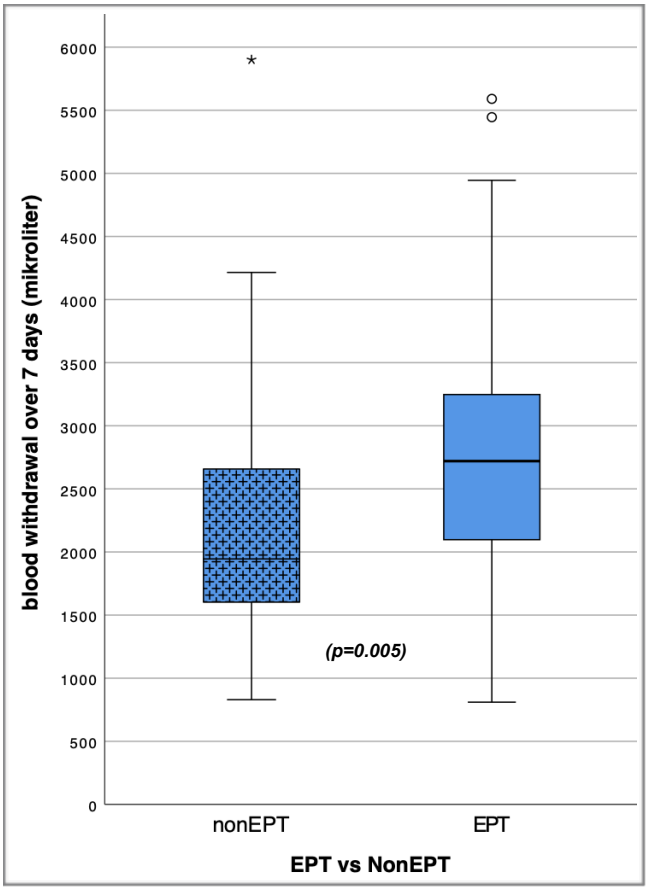


Figure 14: Boxplot showing the amount of blood withdrawal during the first 7 days of life

4.3.1.3 Peak Bilirubin and Phototherapy

The data of all 102 included patients were considered for this analysis. The median peak bilirubin in the nonEPT-group was 10.7 [3.7] mg/dl compared to 9.1 [4.4] mg/dl in infants who had received EPT. (***Figure 15;*** Mann-Whitney *U* test: *p=0.06*).

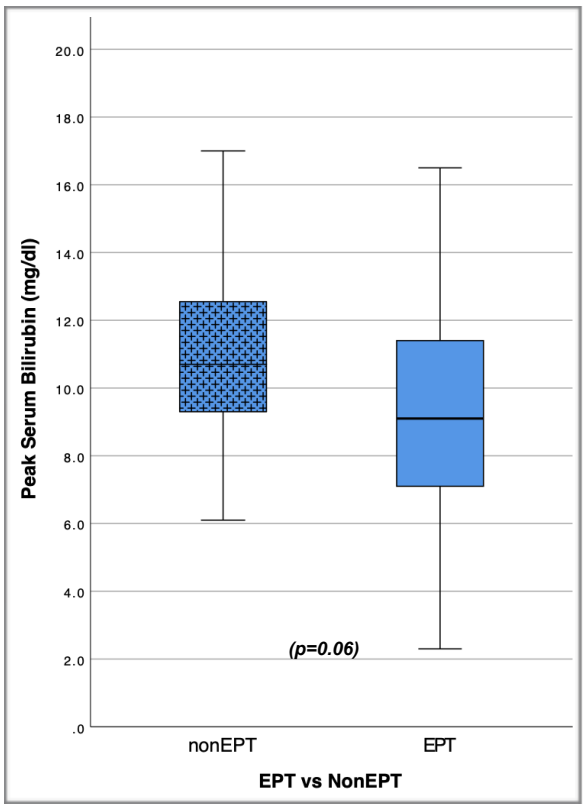


Figure 15: Boxplot showing the peak serum bilirubin levels

The average number of days of phototherapy in the nonEPT-group is 3 [4] days compared to 4 [4] days in the EPT-group. (***Figure 16***) However, a significant difference could be noted (Mann-Whitney *U* test: *p=0.044*) between the cohorts concerning the number of days of phototherapy.

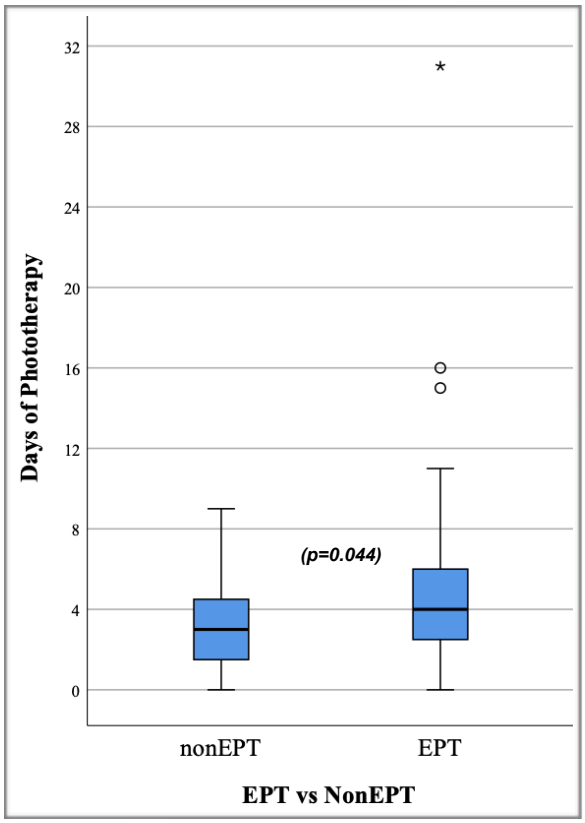


Figure 16: Boxplot showing the number of days of phototherapy

4.3.2 Cardiopulmonal Outcome

4.3.2.1 Persistent Ductus Arteriosus and Indomethacin Therapy

All the infants below 28 weeks of gestational age belonging to the nonEPT-group (n=8), had a manifesting PDA (100%). Compared to that, from the 36 infants below 28 weeks of gestational age within the EPT-group, a number of 29 (81%) was diagnosed with a PDA. (***Figure 17***)
There was no significant difference in between the two groups concerning the number of diagnosed PDAs (Fisher's Exact Test: *p=0.318*).

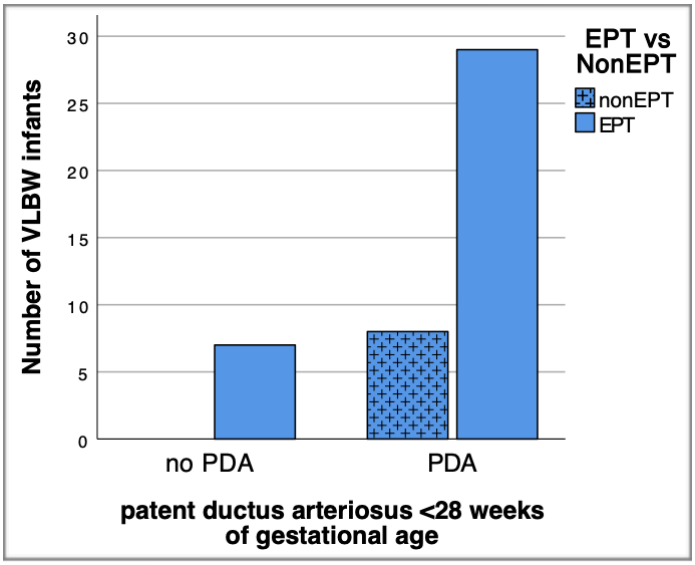


Figure 17: Bar Chart showing the incidence of patent ductus arteriosus

Of those 36 infants diagnosed with PDA, 28 (100%) infants of the EPT (information of Indomethacin treatment of one infant missing) and 7 (88%) of the nonEPT had received a medical therapy which was usually accomplished with a NSAID such as Indomethacin. (*p=0.222*)

The median number of drug administrations in order to close the PDA was higher with infants of the nonEPT-group with 3 [3] times compared to the EPT-group 2 [1] times. (Mann-Whitney *U* test: *p=0.046*). (view ***Figure 18***)

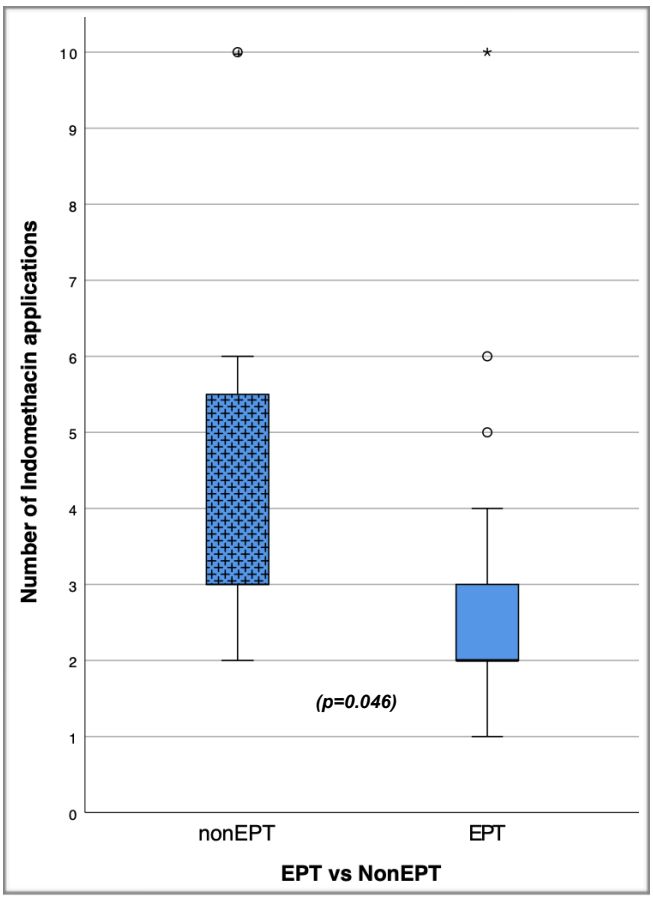


Figure 18: Boxplot showing the number of Indomethacin applications

4.3.2.2 Surfactant, CPAP, FiO_2, Invasive Mechanical Ventilation

A total of 69% of the nonEPT-group (n=24) and 79% of the EPT-group (n=53) were given surfactant therapy postnatal. (***Figure 19***)

There is no significant difference concerning the application of SRT between the two cohorts (Fisher's Exact Test: *p=0.332*)

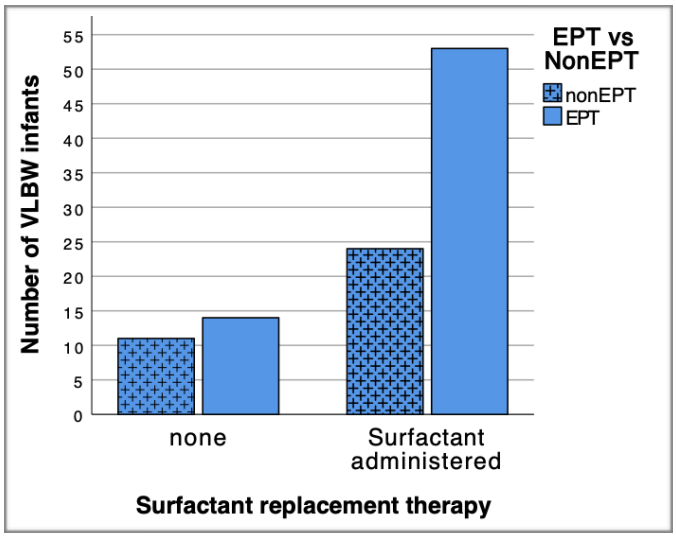


Figure 19: Bar Chart showing the number of surfactant applications

The nonEPT-group recorded a median of 312 [663] hours, compared to the EPT-group's average of 784.5 [1374] hours during their stay. (***Figure 20***)

A statistically significant difference in the amount of hours of CPAP in the two cohorts was observed (Mann-Whitney *U* test: *p=0.007*).

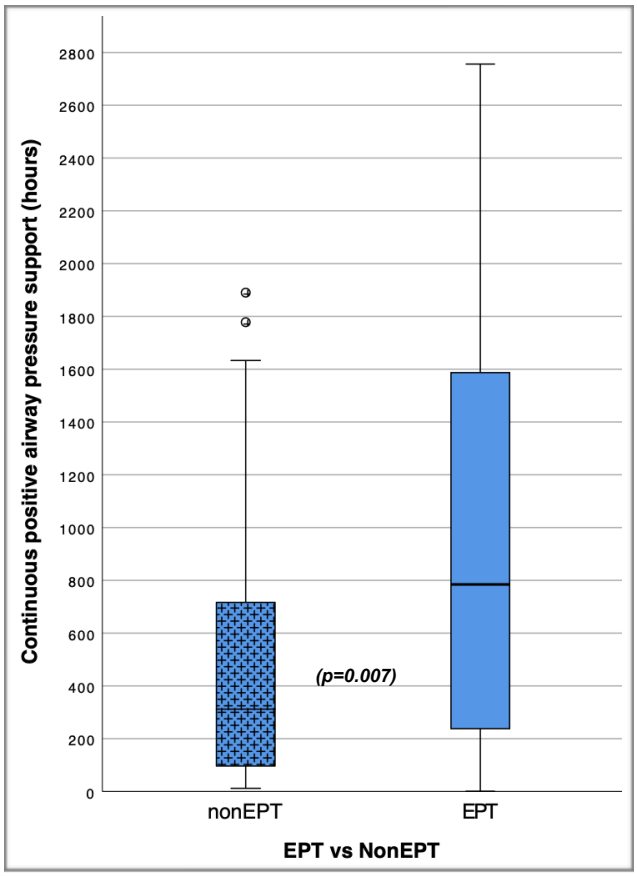


Figure 20: Boxplot showing the hours of CPAP

The maximum fraction of inspired oxygen during the course of respiratory support therapy at CPAP or IMV had a median of 0.3 [0.19] in the nonEPT-group and 0.35 [0.39] in the EPT-group. (***Figure 21***)

The mean FiO2 differs statistically between groups (Mann-Whitney *U* test: *p= 0.036*).

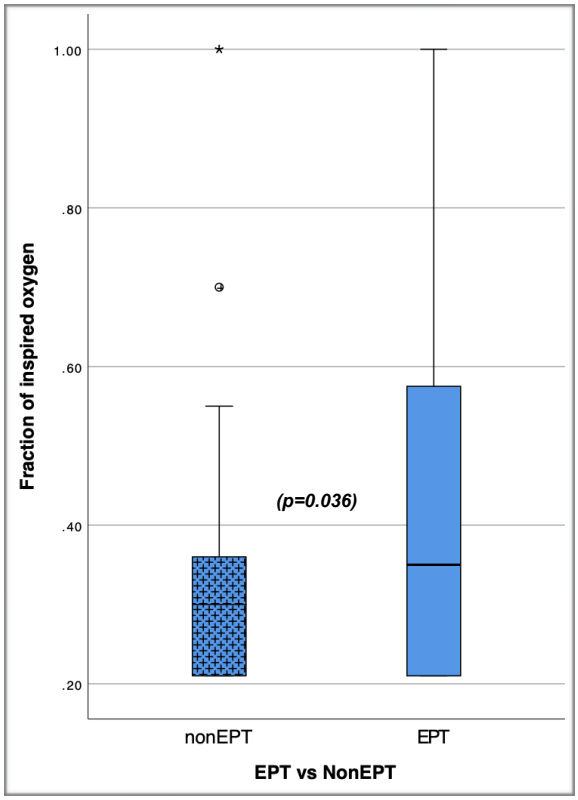


Figure 21: Boxplot showing maximum FiO2 levels

The mean time of invasive mechanical ventilation averages at 398 ± 225 hours for the nonEPT-group compared to 402 ± 71 hours for the EPT-group. (***Figure 22***) A statistic difference was not observed (*t*-test: *p=0.983*).

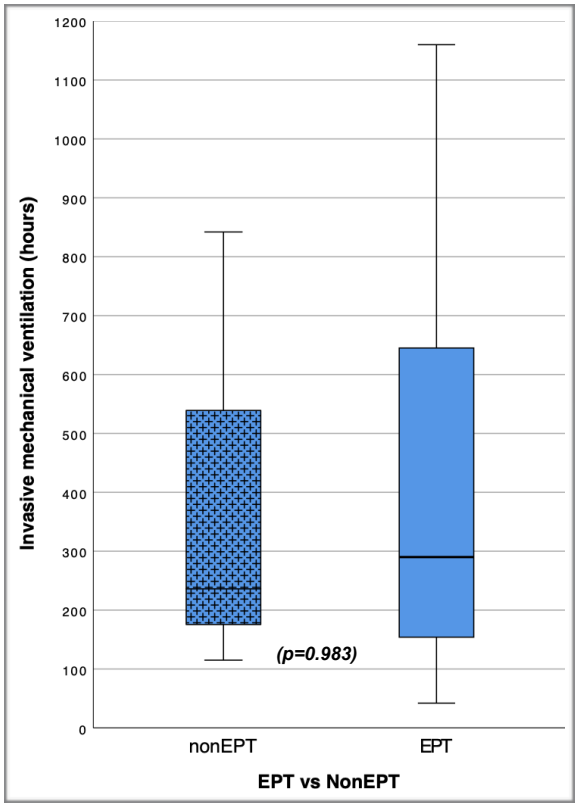


Figure 22: Boxplot showing the amount of hours of IMV

4.3.2.3 Pneumothorax and Bronchopulmonary Dysplasia

Overall, one preterm infant of the nonEPT-group (3%) and five infants of the EPT-group (7%) developed a pneumothorax. (***Figure 23***) The Fisher's Exact Test showed no significant difference (*p=0.661*).

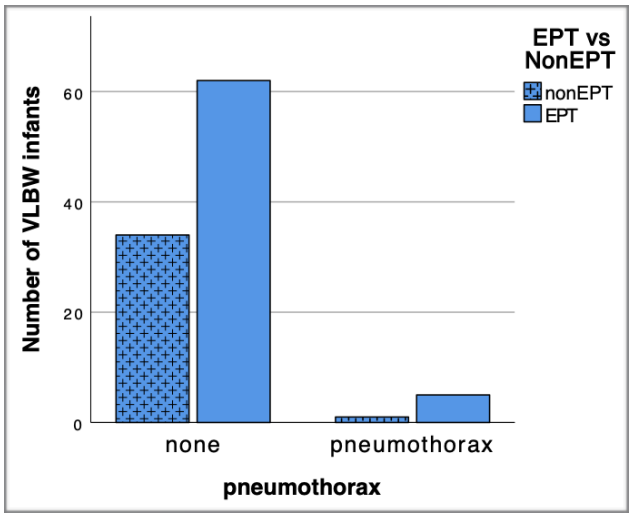


Figure 23: Bar Chart of the incidence of pneumothoraces

Within the group of nonEPT four infants developed a mild form (11%) and one of them a severe form of BPD (3%). In the EPT-group 14 newborn were diagnosed with a mild form (21%) and four with a moderate form (6%) of BPD. (***Table 4*** and ***Figure 24***) The Chi-Square Test reveals no significant differences in the amount of BPD occurrence (Fisher's Exact Test: *p=0.116*).

		EPT vs NonEPT		
		nonEPT	EPT	Total
Bronchopulmonary dysplasia	none	30	48	78
	mild	4	14	18
	moderate	0	4	4
	severe	1	0	1
Total		35	66	101

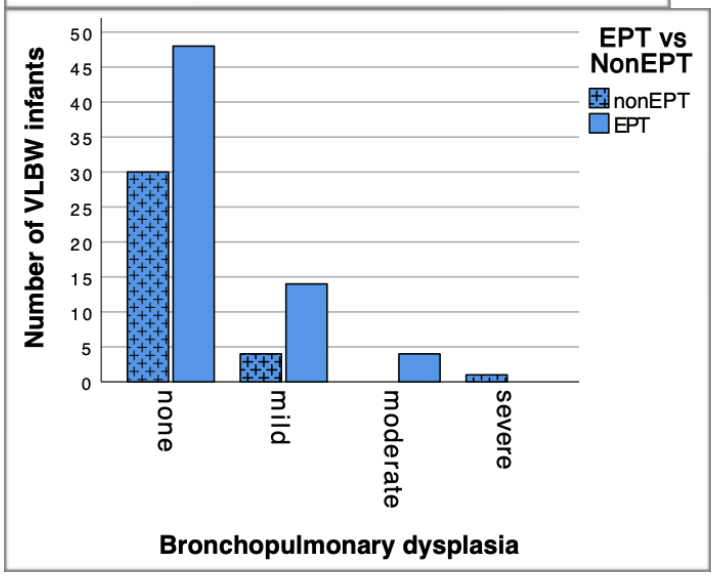


Table 4 and 24: Crosstabulation (above) and Bar Chart (below) of the incidence of different stages of BPD

4.3.3 Gastrointestinal Complications

4.3.3.1 Necrotising Enterocolitis

Out of all the 67 preterm newborn one of them belonging to the EPT-group (2%), developed a NEC which had to be treated by an operation (Fisher's Exact Test: *p=1.00*). (***Table 5***)

		EPT vs NonEPT		
		nonEPT	EPT	Total
Necrotizing enterocolitis	none	35	66	101
	NEC	0	1	1
Total		35	67	102

Table 5: Crosstabulation showing the incidence of NEC

4.3.3.2 Spontaneous Intestinal Perforation

The data of all 102 included infants were evaluated concerning the diagnosis of SIP and its treatment through operation. In total one of the 35 preterm infants in the nonEPT-group developed a SIP (3%) and also needed surgery.

Compared to that in the EPT-group, eleven of the 67 infants were diagnosed with SIP (16%) and eight of them had to be operated on (12%). (***Table 6*** and ***7***)

There was no statistically significant difference looking at the need of therapeutical intervention via operation (Fisher's Exact Test: *p=0.159)*, as there was no statistical difference concerning the number of diagnosed SIP (Fisher's Exact Test: *p=0.054*) .

		EPT vs NonEPT		
		nonEPT	EPT	Total
Focal Intestinal Perforation	none	34	56	90
	FIP	1	11	12
Total		35	67	102

		EPT vs NonEPT		
		nonEPT	EPT	Total
Focal Intenstinal Perforation therapy: Operation	noFipOP	34	59	93
	FipOP	1	8	9
Total		35	67	102

Table 6 and ***7***: Crosstabulation showing the incidence of SIP and the need for surgical treatment

4.3.4 Neurological complications

4.3.4.1 Intraventricular Haemorrhage

In the nonEPT-group six developed a Grade I (17%) and one of them (3%) a Grade III bleeding, whilst in the EPT-group 12 were diagnosed with a Grade I (18%), four with a Grade II (6%) and nine of them with a Grade III (13%) intraventicular haemorrhage. In none of the preterm infants an IVH Grade IV was detected. (***Figure 25***)

The Exact Fisher's Test confirms the difference is not significant ($p=0.142$).

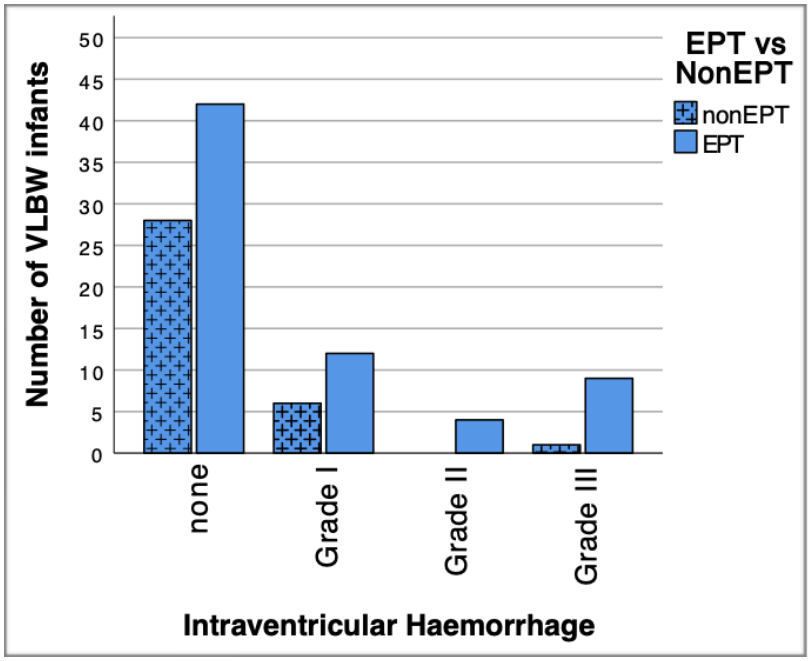


Figure 25: Bar Chart showing the distribution of the different grades of IVH

4.3.4.2 Periventricular leukomalacia

Overall, two of the 67 newborn of the EPT-group developed PVL (3%). One of them born at $29^{3/7}$ weeks of gestational age delivered at a silent cardiotocography and diagnosed intruterine growth restriction, developed severe intraventricular haemorrhage within the first day. The other one delivered at $22^{6/7}$ weeks of gestational age, showed signs of PVL as consequence of severe IVH which had developed. (***Table 8***)

The Exact Fisher's Test shows no statistically significant difference ($p=0.545$).

		EPT vs NonEPT		
		nonEPT	EPT	Total
Periventricular leukomalacia	none	35	65	100
	PVL	0	2	2
Total		35	67	102

Table 8: Crosstabulation showing the incidence of PVL

4.3.5 Ophthalmological Complications

4.3.5.1 Retinopathy of Prematurity

For this analyses the data of 35 newborn in the nonEPT-group and 66 in the EPT-group were taken into account.

Stage 1 ROP developed in 13 of the nonEPT-group (37%) and in 30 of the EPT-group (45%), Stage 2 ROP in 5 of the nonEPT-group (14%) and in 11 of the EPT-group (17%) and Stage 3 in 1 of the nonEPT-group (3%) and in 5 of the EPT-group (8%). No cases was a Stage 4 or 5 diagnosed. (***Figure 26***) The amount and distribution of diagnosed ROPs does not differ between the two cohorts (*p=0.470*).

Four of the newborns diagnosed with ROP within the EPT-group required a therapeutical intervention such as cryotherapy, laser therapy or anti-VEGF treatment (6%), however, statistically there is no difference between the two cohorts (Fisher's Exact Test: *p=0.295*).

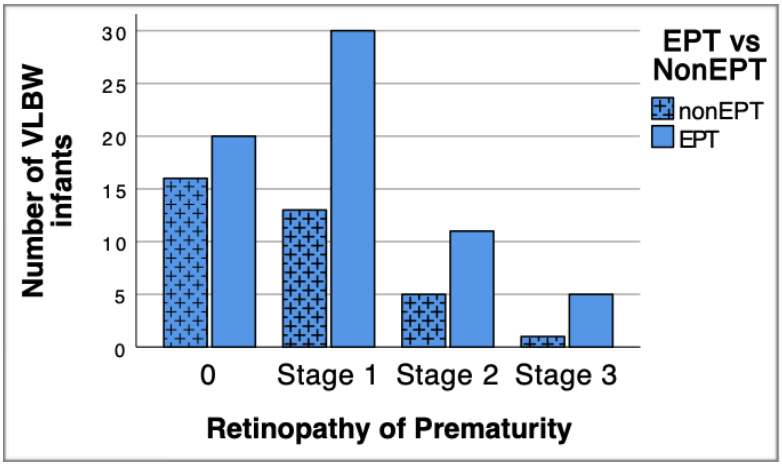


Figure 26: Bar Chart of the diagnosed stages of ROP

4.3.6 Composite outcome: Survival without major complications

The survival without major complications is employed as an indicator for the later quality of life of the included preterm infants. This composite outcome includes the incidence of the following complications: severe IVH (Grade III/IV), PVL, BPD, cryotherapy, laser therapy or anti-VEGF treatment for ROP or required surgery for NEC or FIP. Within the nonEPT-group five infants (17%) and within the EPT-group 24 infants (35%) developed at least one of the severe complications. There was a significantly higher number of cases of survival without major complications within the nonEPT-group (Fisher's Exact Test: *p=0.023)*.

5. Discussion

The concept of performing an extrauterine placental transfusion was first described in the 1960s by Dunn and Várdi but has since not been investigated much further. At the University of Cologne a modified EPT approach has been further developed and has become an important part in neonatal resuscitation of VLBW preterm children for many years. Preterm infants are delivered with the placenta still attached and CPAP support is initiated for several minutes before the cord is clamped.

The present study focused on exploring the impact of different cord clamping procedures on the outcome of preterm infants in a cohort of VLBW infants born in a one-year period (2017-2018) at the University of Cologne. In total, 102 infants were included and important outcome parameters were recorded until discharge.

It was hypothesised that by performing EPT, a significant amount of blood volume which in case of ICC would remain in the placenta, is transfused and added to the infants' total blood volume. Consequently, this would provide for a more stable transition during the postnatal changes of the circulatory system. However, the effects of this approach have not been evaluated sufficiently in scientific studies.

It can be estimated that during a placental transfusion of 2-5 minutes, 24-32 ml/kg of blood are added to a newborns' blood volume. This amount accounts for around 25-30% of the potential blood volume at birth. 53

This increase in blood volume during the transitional period from fetal to neonatal circulation is needed as this period is characterised by the decrease in pulmonary vascular resistance and a consecutively higher pulmonary blood flow which could induce an overall decreased systemic blood flow. Without the "additional" blood volume from the placenta other organs could suffer in deficit perfusion, being especially damaging for the sensitive neural structures and causing higher risks of IVH. 54

Maintaining placental transfusion during the initiation of breathing and the adaption of the new circulatory system, has been shown to reduce the incidence of IVH and also the need for RBC transfusions in preterm infants. 23

Looking at the investigated effects of EPT on the incidence of IVH and PVL in this study, collected data suggests no negative influence on the neurological outcome. This is particularly reassuring as infants of the EPT-cohort were significantly more immature and had lower birth weights, both of which are factors associated with a higher incidence of IVH and PVL, respectively. 55

During the research study average haematocrit levels observed during the first 24 hours of life did not differ between groups. However, blood withdrawal during the first 7 days of life was comparatively higher in the EPT group. A greater incidence of red blood cell transfusions within the EPT group were observed, though the amount of transfused blood volume after 7 or 28 days remained statistically homogenous between groups. This data emphasises the direct link of blood withdrawal and the necessity of RBC transfusions in preterm infants. 56

The fact that the University Hospital of Cologne practices a liberal management of RBC transfusion similar to how it is described in the ETTNO study and the knowledge of the availability of specially prepared extra small packages at the hospital, could further explain the higher rates of RCB transfusion with infants of the EPT-group. 57 Emphasis must be put on the fact that there were no pre-defined transfusion thresholds in the present observational study. Therefore, the finding of a higher RBC transfusion in the EPT group should be tentatively interpreted.
In addition, the logistic regression analysis supports the hypothesis that the need of RBC transfusions is not primarily influenced by the factor EPT but rather dependent on lower birth weight.

Both lower BW and younger GA within the EPT-group also led to a significantly lower CRIB score, a predictor for mortality of the VLBW infants. 58
Similarly, as a potential consequence of higher prematurity, infants of the EPT-group also had lower APGAR scores which are related with an increased mortality. 59

However, while APGAR scores at 1, 5 and 10 minutes were significantly lower in the EPT-group, there has been criticism by the American Academy of Pediatrics about the validity and informational value of APGAR with very premature infants, as they might receive lower scores simply because of their prematurity. 60

A population-based cohort study in the United Kingdom revealed that low APGAR scores (0-3) at 5 minutes are associated with a higher mortality of term and also preterm infants. 61

Only two infants in the EPT-group received a 5 minute APGAR score of 3, one of them being delivered via cesarean section with general anaesthesia which is known to affect postnatal adaptation negatively. 62 Both of those infants were released home without severe morbidities after their hospital stay.

Also, as the APGAR score is assigned by the attending doctor, the score is dependent on individual judgement. 63

In the care of preterm infants, the temperature at admission is another important parameter, as lower temperatures have been associated with both higher incidences of late-onset-sepsis and mortality. 64

Also, temperature is a parameter of the CRIB II score which represents an extension of the CRIB score and is important for predicting the outcome of very small preterm infants. 65,66

Due to the vulnerable nature of immature skin, very small and preterm infants, especially those with a birth weight of below 1500g, may suffer with rapid temperature loss. 67

The mean temperature on admission was significantly lower in infants of the EPT-group. Therefore, it should be discussed if influenceable factors such as higher temperatures in the delivery room and continuous monitoring of body temperature should be implemented when performing EPT.

A limitation of this study is that although the temperature was monitored throughout the procedures at the resuscitation unit, some of the parameters may vary because temperatures had been noted at different timings during resuscitation.

One of the major problems in treating very preterm infants is the immaturity of their lungs and in spite of the progress in treatment, around 25% are still affected by BPD. 68

It is a complication in preterm infants, often characterised by a malfunctioning formation of the alveoli and blood vessels causing pulmonary hypertension. The

administration of surfactant as performed at the University Clinic of Cologne via LISA, is known to support the prevention of developing BPD. 69,70

Also, the occurrence of BPD and mortality due to RDS, is decreased through the administration of corticosteroids before delivery. ACS can reduce preterm infant mortality and morbidity related to the development of pulmonary complications. 71
The same initial situation preexisted in both cohorts concerning the number and way of administration of ACS and surfactant and there was no significant difference in the occurrence of BPD and the incidence of pneumothoraces, a common complication under ventilation, between the preterm infants which had received EPT and those who did not. 72

Another limitation of this study is that EPT constitutes one of many aspects of resuscitation potentially influencing the outcome of preterm infants and is performed with other procedures such high peep respiratory support, LISA or avoidance of invasive mechanical ventilation.

Lower incidences of reported BPD in EBLW infants in this study compared to recently published data, are a promising indicator. 73 Investigating the effects of EPT on the pulmonal development and its possible reduction in rates of BPD is part of current studies and will need further investigation. 74

Neonatal hyperbilirubinaemia is a frequent neonatal complication which is especially dangerous for preterm infants due to its neurotoxicity causing neurological dysfunction. 75
A randomised controlled trial from 2018 suggests that procedures including placental transfusion such as DCC does not lead to polycytaemia and hyperbilirubinaemia and has no negative effects below 34 weeks gestational age. 76

The main intervention in preterm infants of VLBW to prevent brain injuries caused by bilirubin, is phototherapy. 77
Criteria for the phototherapy treatment are often based on gestational age and birth

weight and are based upon an established consensus within the treating NICU. 78
This could explain why the number of days of phototherapy was significantly higher in the EPT-group but no raised bilirubin levels compared the nonEPT-group were detected.

Further severe neonatal complications in VLBW infants are SIP and NEC. Lower gestational age, extreme prematurity and RDS are associated with an increased vulnerability towards SIP compared to NEC. 79 Another risk factor for SIP associated risk factor is the indomethacin therapy for PDA in preterm infants. 80
Rates of SIP were high in both cohorts as compared to rates of SIP in the published literature. A finding that needs further investigation. 81

There was only one reported case of NEC of a preterm infant with extreme low birth weight (470g) and of extreme prematurity ($22^{4/7}$ days of GA) within the EPT-group, which is a remarkably low incidence for this typical neonatal complication. 82,83

Interestingly, there was no increased incidence of ROP and its treatment within the EPT-group, even though low birth weight, small gestational age and the transfusion of blood at EPT leading to an increase of postnatal blood volume, could represent a combination of possible factors to increase rates of ROP. 84,85

Infants of the EPT cohort presented with a lower GA and lower BW, both being risk factors for adverse outcomes with VLBW infants. 86
Therefore, it could be assumed that infants of the EPT-group develop more neonatal complications and show a reduced overall outcome.
With the majority of investigated outcome parameters no increased incidence of complications was detected. However, an investigation of survival without major complications of VLBW infants as a composite outcome criterium, suggested a negative influence regarding the morbidity of infants who received EPT.

Within the EPT-group a higher number of complications occurred. A critical judgement must be taken to determine whether this is directly related to the procedure of EPT itself or rather to other neonatal risk factors such as the lower BW.

Also, with a GA of $27^{6/7}$ weeks, infants of the EPT-group are over two weeks younger than infants of the nonEPT-group. Naturally, this extremely low gestational age is linked to an increased number of neonatal complications within the EPT-group. 87

The hypothesis that higher morbidity within the EPT-group can be explained through lower BW and GA rather than the EPT-procedure, is supported by the comparison of the incidence of neonatal complications in the present cohorts with published data of other VLBW infants. Reviewing recently published literature concerning the incidence of neonatal complications of infants of the same gestational age, shows that those of the EPT-group settle at similar and sometimes even lower incidences. 88,89

However, due to the slight bias of cohorts, the generalisation of our findings remain questionable and further randomised studies are required.

It is now widely accepted that the practice of delayed cord clamping (DCC) in healthy term infants presents the natural gentle transition from fetus to neonate. 90

The practice of immediate or early cord clamping (ICC/ECC) should be viewed critically as it might be cause potential damage to the brain catalysed by intermittent hypoxia. 91

Due to its clear evidence the guidelines for neonatal resuscitation for newborns have been revised. In the year 2012 the World Health Organisation (WHO) declared a recommendation to perform DCC for a minimum of 60 seconds in term and preterm newborn if ventilation is not required. 92

There are also numerous other guidelines and recommendations towards the practice of DCC in preterm infants. The recommendation from the American College of Obstetricians and Gynaecologists considered a delay of 30-60 seconds beneficial. 93

The latest guidelines from the European Resuscitation Council recommend the following: “Clamping after at least 60s is recommended, ideally after the lungs are aerated. Where delayed cord clamping is not possible cord milking should be considered in infants >28 weeks gestation.” 94

However, for very preterm infants it is of vital importance that CPAP therapy can be administered without delay after birth if needed. 95

As an attempt to enable ventilatory support whilst performing DCC, bedside resuscitation trolleys have been developed which are being implemented in controlled studies 96

These trials suggest that it is possible to perform basic resuscitation/respiratory support in the proximity to the mother in order to preserve an intact placenta-child circulation. 97

First trials report that it feasible to perform DCC with the bedside trolley but it remains a challenge, as operating space is limited by the length of the cord which might not be sufficiently long, it has to be ensured that the cord is not stretched at any time. 98,99

For that reason the trolley has to be placed as close as possible to the pelvis of the mother, this may cause restrictions within the setting of the operating room during a cesarean section, which is the frequently chosen mode of delivery for preterm infants.

Also, staff is aware that resuscitation performed in close proximity to the infants parents can cause a certain level of unease from the parents due to the nature of the procedure and from the staff for having to perform the procedure in front of the parents themselves. 100

The majority of included infants (66%) received EPT and within the comparative cohort both DCC, ICC and CM was performed. This imbalance is most likely explained by the long period over which EPT was performed at our institution and has evolved as the standard treatment in the past decade. A limitation of this study is that when certain procedures were performed by the obstetricians, exact data about how long DCC had been performed or how many times the cord was milked was not available.

Within the nonEPT-cohort it was observed that even though DCC is currently recognised as the gold standard in neonatal resuscitation of VLBW infants, DCC was performed in the minority of cases. There is recent data from 2022 by Amendolia et al. which confirm that a high percentage of VLBW infants do not receive DCC despite the general consensus of recommendation for it. 101

Possible explanations are the commonly feared delay of potentially life-saving resuscitation which ultimately prevents the application of DCC. 102

EPT intends to offer a new way for overcoming the previously mentioned concerns and finding the procedure which guarantees the best possible start to life for preterm infants.
Previous studies have indicated that EPT does not influence the outcome of VLBW infants negatively 43,103 Consequently, as it has been performed for many years in the University Hospital of Cologne and proven to be feasible, it has to be investigated further as an alternative to standard procedures.

EPT needs a standardised protocol, which would guide doctors to finding the right time to clamp the cord during treatment. In order to provide answers to this question the developed “cord clamping sheet” monitored the time after delivery until the cord was clamped. This new instrument led to create a reliable source of information about the timing of cord clamping with EPT. This is the first time data about this part of neonatal resuscitation was collected in a standardised way which represents a significant improvement for finding an internal workflow standard.

This study provided a systematic research and data collection concerning the procedure of EPT. It comprises a vital part in the investigation of a relatively new and not fully investigated procedure. Based on this research future randomised controlled trials on EPT will have to further investigate the impact of this procedure on extremely immature infants’ outcomes.

6. Bibliography

1. Eichenwald EC, Stark AR. Management and outcomes of very low birth weight. *New England Journal of Medicine* 2008; **358**(16): 1700-11.
2. Humberg A, Härtel C, Paul P, et al. Delivery mode and intraventricular hemorrhage risk in very-low-birth-weight infants: observational data of the German Neonatal Network. *European Journal of Obstetrics & Gynecology and Reproductive Biology* 2017; **212**: 144-9.
3. Mehler K, Grimme J, Abele J, Huenseler C, Roth B, Kribs A. Outcome of extremely low gestational age newborns after introduction of a revised protocol to assist preterm infants in their transition to extrauterine life. *Acta Paediatrica* 2012; **101**(12): 1232-9.
4. Kamper J, Jørgensen NF, Jonsbo F, Pedersen-Bjergaard L, Pryds O, Group DES. The Danish national study in infants with extremely low gestational age and birthweight (the ETFOL study): respiratory morbidity and outcome. *Acta Paediatrica* 2004; **93**(2): 225-32.
5. Hillman NH, Kallapur SG, Jobe AH. Physiology of transition from intrauterine to extrauterine life. *Clinics in perinatology* 2012; **39**(4): 769-83.
6. Göpel W, Kribs A, Ziegler A, et al. Avoidance of mechanical ventilation by surfactant treatment of spontaneously breathing preterm infants (AMV): an open-label, randomised, controlled trial. *The Lancet* 2011; **378**(9803): 1627-34.
7. Kribs A, Roll C, Göpel W, et al. Nonintubated surfactant application vs conventional therapy in extremely preterm infants: a randomized clinical trial. *JAMA pediatrics* 2015; **169**(8): 723-30.
8. Härtel C, Herting E, Humberg A, et al. Association of administration of surfactant using less invasive methods with outcomes in extremely preterm infants less than 27 weeks of gestation. *JAMA network open* 2022; **5**(8): e2225810-e.
9. Hooper SB, Polglase GR, Roehr CC. Cardiopulmonary changes with aeration of the newborn lung. *Paediatr Respir Rev* 2015; **16**(3): 147-50.
10. Bhatt S, Alison BJ, Wallace EM, et al. Delaying cord clamping until ventilation onset improves cardiovascular function at birth in preterm lambs. *J Physiol* 2013; **591**(8): 2113-26.
11. Hooper SB, Crossley KJ, Zahra VA, et al. Effect of body position and ventilation on umbilical artery and venous blood flows during delayed umbilical cord clamping in preterm lambs. *Arch Dis Child Fetal Neonatal Ed* 2017; **102**(4): F312-f9.
12. Fogarty M, Osborn DA, Askie L, et al. Delayed vs early umbilical cord clamping for preterm infants: a systematic review and meta-analysis. *American journal of obstetrics and gynecology* 2018; **218**(1): 1-18.
13. Seidler AL, Gyte GM, Rabe H, et al. Umbilical cord management for newborns< 34 weeks' gestation: a meta-analysis. *Pediatrics* 2021; **147**(3).
14. Katheria AC, Lakshminrusimha S, Rabe H, McAdams R, Mercer JS. Placental transfusion: a review. *J Perinatol* 2017; **37**(2): 105-11.
15. Dunn PM. The placental venous pressure during and after the third stage of labour following early cord ligation. *J Obstet Gynaecol Br Commonw* 1966; **73**(5): 747-56.
16. Rabe H, Mercer J. Knowledge gaps in optimal umbilical cord management at birth. Seminars in Perinatology; 2023: Elsevier; 2023. p. 151791.
17. Rabe H, Wacker A, Hulskamp G, et al. A randomised controlled trial of delayed cord clamping in very low birth weight preterm infants. *Eur J Pediatr* 2000; **159**(10): 775-7.
18. Salcido C, Shahidi SA, Poeltler DM, Gollin Y, Johnston LA, Katheria AC. Maternal bleeding complications and neonatal outcomes following early versus delayed umbilical cord clamping in cesarean deliveries for very low birthweight infants. *Journal of Perinatology* 2023; **43**(1): 39-43.

19. Dekker J, Martherus T, Cramer SJ, van Zanten HA, Hooper SB, te Pas AB. Tactile stimulation to stimulate spontaneous breathing during stabilization of preterm infants at birth: a retrospective analysis. *Frontiers in pediatrics* 2017; **5**: 61.
20. Backes CH, Rivera BK, Haque U, et al. Placental transfusion strategies in very preterm neonates: a systematic review and meta-analysis. *Obstet Gynecol* 2014; **124**(1): 47-56.
21. Mercer JS, Vohr BR, McGrath MM, Padbury JF, Wallach M, Oh W. Delayed cord clamping in very preterm infants reduces the incidence of intraventricular hemorrhage and late-onset sepsis: a randomized, controlled trial. *Pediatrics* 2006; **117**(4): 1235-42.
22. Yigit B, Tutsak E, Yildirim C, Hutchon D, Pekkan K. Transitional fetal hemodynamics and gas exchange in premature postpartum adaptation: immediate vs. delayed cord clamping. *Matern Health Neonatol Perinatol* 2019; **5**: 5.
23. Rabe H, Diaz-Rossello JL, Duley L, Dowswell T. Effect of timing of umbilical cord clamping and other strategies to influence placental transfusion at preterm birth on maternal and infant outcomes. *Cochrane Database Syst Rev* 2012; (8): Cd003248.
24. Chaparro CM, Neufeld LM, Alavez GT, Cedillo RE-L, Dewey KG. Effect of timing of umbilical cord clamping on iron status in Mexican infants: a randomised controlled trial. *The Lancet* 2006; **367**(9527): 1997-2004.
25. Van Rheenen P, Brabin BJ. Late umbilical cord-clamping as an intervention for reducing iron deficiency anaemia in term infants in developing and industrialised countries: a systematic review. *Annals of tropical paediatrics* 2004; **24**(1): 3-16.
26. Lozoff B, Beard J, Connor J, Felt B, Georgieff M, Schallert T. Long-lasting neural and behavioral effects of iron deficiency in infancy. *Nutrition reviews* 2006; **64**(suppl_2): S34-S43.
27. Armony-Sivan R, Eidelman AI, Lanir A, Sredni D, Yehuda S. Iron status and neurobehavioral development of premature infants. *Journal of perinatology* 2004; **24**(12): 757.
28. Mercer JS, Erickson-Owens DA, Vohr BR, et al. Effects of Placental Transfusion on Neonatal and 18 Month Outcomes in Preterm Infants: A Randomized Controlled Trial. *J Pediatr* 2016; **168**: 50-5.e1.
29. Hemmati F, Sharma D, Namavar Jahromi B, Salarian L, Farahbakhsh N. Delayed cord clamping for prevention of intraventricular hemorrhage in preterm neonates: a randomized control trial. *The Journal of Maternal-Fetal & Neonatal Medicine* 2022; **35**(19): 3633-9.
30. Robledo KP, Tarnow-Mordi WO, Rieger I, et al. Effects of delayed versus immediate umbilical cord clamping in reducing death or major disability at 2 years corrected age among very preterm infants (APTS): a multicentre, randomised clinical trial. *The Lancet Child & Adolescent Health* 2022; **6**(3): 150-7.
31. Nevill E, Meyer MP. Effect of delayed cord clamping (DCC) on breathing and transition at birth in very preterm infants. *Early Hum Dev* 2015; **91**(7): 407-11.
32. Katheria AC, Truong G, Cousins L, Oshiro B, Finer NN. Umbilical cord milking versus delayed cord clamping in preterm infants. *Pediatrics* 2015; **136**(1): 61-9.
33. Katheria A, Garey D, Truong G, et al. A randomized clinical trial of umbilical cord milking vs delayed cord clamping in preterm infants: neurodevelopmental outcomes at 22-26 months of corrected age. *The Journal of pediatrics* 2018; **194**: 76-80.
34. Shirk SK, Manolis SA, Lambers DS, Smith KL. Delayed clamping vs milking of umbilical cord in preterm infants: a randomized controlled trial. *American journal of obstetrics and gynecology* 2019; **220**(5): 482. e1-. e8.
35. March M, Hacker M, Parson A, Modest A, De Veciana M. The effects of umbilical cord milking in extremely preterm infants: a randomized controlled trial. *Journal of Perinatology* 2013; **33**(10): 763.

36. Al-Wassia H, Shah PS. Efficacy and safety of umbilical cord milking at birth: a systematic review and meta-analysis. *JAMA Pediatr* 2015; **169**(1): 18-25.
37. Katheria A, Reister F, Essers J, et al. Association of umbilical cord milking vs delayed umbilical cord clamping with death or severe intraventricular hemorrhage among preterm infants. *Jama* 2019; **322**(19): 1877-86.
38. Patel MN, Sundararajan S, Gopalakrishnan M. Impact of Delayed Cord Clamping on Red Blood Cell Transfusion in Very Low Birthweight Infants. *Pediatrics* 2022; **149**(1 Meeting Abstracts February 2022): 682-.
39. Backes CH, Rivera B, Haque U, Copeland K, Hutchon D, Smith C. Placental transfusion strategies in extremely preterm infants: the next piece of the puzzle. *Journal of neonatal-perinatal medicine* 2014; **7**(4): 257-67.
40. Dunn P. Premature delivery and the preterm infant. *Irish medical journal* 1976; **69**(10): 246-54.
41. Dunn PM. The importance of umbilical circulation in preventing respiratory distress syndrome following premature Caesarean delivery, 1957- 1973. *West of England Medical Journal* 2017; **116**.
42. Vardi P. Placental transfusion an attempt at physiological delivery. *The Lancet* 1965; **286**(7401): 12-3.
43. Kuehne B, Kirchgaessner C, Becker I, et al. Mask continuous positive airway pressure therapy with simultaneous extrauterine placental transfusion for resuscitation of preterm infants-a preliminary study. *Biomedicine Hub* 2018; **3**(2): 1-10.
44. Lin C-H, Lin S-Y, Yang Y-H, et al. Extremely preterm cesarean delivery "en caul". *Taiwanese Journal of Obstetrics and Gynecology* 2010; **49**(3): 254-9.
45. Apgar V. The newborn (Apgar) scoring system. *Pediatr Clin North Am* 1966; **13**(3): 645-50.
46. Cockburn F, Cooke R, Gamsu H, et al. The CRIB (clinical risk index for babies) score. *The Lancet* 1993; **342**(8865): 193-8.
47. Gagliardi L, Cavazza A, Brunelli A, et al. Assessing mortality risk in very low birthweight infants: a comparison of CRIB, CRIB-II, and SNAPPE-II. *Archives of Disease in Childhood-Fetal and Neonatal Edition* 2004; **89**(5): F419-F22.
48. Bell MJ, Ternberg JL, Feigin RD, et al. Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. *Annals of surgery* 1978; **187**(1): 1.
49. Papile L-A, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. *The Journal of pediatrics* 1978; **92**(4): 529-34.
50. Walsh MC, Yao Q, Gettner P, et al. Impact of a physiologic definition on bronchopulmonary dysplasia rates. *Pediatrics* 2004; **114**(5): 1305-11.
51. An international classification of retinopathy of prematurity. The Committee for the Classification of Retinopathy of Prematurity. *Arch Ophthalmol* 1984; **102**(8): 1130-4.
52. Aaberg T, Ben-Sira I, Charles S, et al. An international classification of retinopathy of prematurity: II. The classification of retinal detachment. *Archives of Ophthalmology* 1987; **105**(7): 906-12.
53. Farrar D, Airey R, Law GR, Tuffnell D, Cattle B, Duley L. Measuring placental transfusion for term births: weighing babies with cord intact. *Bjog* 2011; **118**(1): 70-5.
54. Kluckow M. Low systemic blood flow and pathophysiology of the preterm transitional circulation. *Early human development* 2005; **81**(5): 429-37.
55. Poryo M, Boeckh JC, Gortner L, et al. Ante-, peri-and postnatal factors associated with intraventricular hemorrhage in very premature infants. *Early human development* 2018; **116**: 1-8.

56. Alan S, Arsan S, Okulu E, et al. Effects of umbilical cord milking on the need for packed red blood cell transfusions and early neonatal hemodynamic adaptation in preterm infants born≤ 1500 g: a prospective, randomized, controlled trial. *Journal of pediatric hematology/oncology* 2014; **36**(8): e493-e8.
57. Eicher C, Seitz G, Bevot A, et al. The 'effects of transfusion thresholds on neurocognitive outcome of extremely low birth-weight infants (ettno)'study: Background, aims, and study protocol. *Neonatology* 2012; **101**(4): 301-5.
58. McLeod JS, Menon A, Matusko N, et al. Comparing mortality risk models in VLBW and preterm infants: systematic review and meta-analysis. *Journal of Perinatology* 2020; **40**(5): 695-703.
59. Lee HC, Subeh M, Gould JB. Low Apgar score and mortality in extremely preterm neonates born in the United States. *Acta Paediatrica* 2010; **99**(12): 1785-9.
60. Pediatrics AAo. The Apgar score. Advances in neonatal care: official journal of the National Association of Neonatal Nurses 2006; **6**(4): 220.
61. Iliodromiti S, Mackay DF, Smith GC, Pell JP, Nelson SM. Apgar score and the risk of cause-specific infant mortality: a population-based cohort study. *Lancet* 2014; **384**(9956): 1749-55.
62. Ong B, Cohen MM, Palahniuk RJ. Anesthesia for cesarean section--effects on neonates. *Anesthesia and analgesia* 1989; **68**(3): 270-5.
63. Rüdiger M, Küster H, Herting E, et al. Variations of Apgar score of very low birth weight infants in different neonatal intensive care units. *Acta paediatrica* 2009; **98**(9): 1433-6.
64. Laptook AR, Salhab W, Bhaskar B. Admission temperature of low birth weight infants: predictors and associated morbidities. *Pediatrics* 2007; **119**(3): e643-e9.
65. Ezz-Eldin ZM, Hamid TAA, Youssef MRL, Nabil HE-D. Clinical risk index for babies (CRIB II) scoring system in prediction of mortality in premature babies. *Journal of clinical and diagnostic research: JCDR* 2015; **9**(6): SC08.
66. Parry G, Tucker J, Tarnow-Mordi W, Group UNSSC. CRIB II: an update of the clinical risk index for babies score. *The Lancet* 2003; **361**(9371): 1789-91.
67. Bissinger RL, Annibale DJ. Thermoregulation in very low-birth-weight infants during the golden hour: results and implications. *Advances in Neonatal Care* 2010; **10**(5): 230-8.
68. Härtel C, Paul P, Hanke K, et al. Less invasive surfactant administration and complications of preterm birth. *Scientific reports* 2018; **8**(1): 8333.
69. Verder H, Bohlin K, Kamper J, Lindwall R, Jonsson B. Nasal CPAP and surfactant for treatment of respiratory distress syndrome and prevention of bronchopulmonary dysplasia. *Acta paediatrica* 2009; **98**(9): 1400-8.
70. Kribs A. How best to administer surfactant to VLBW infants? : BMJ Publishing Group; 2011.
71. Travers CP, Carlo WA, McDonald SA, et al. Mortality and pulmonary outcomes of extremely preterm infants exposed to antenatal corticosteroids. *Am J Obstet Gynecol* 2018; **218**(1): 130.e1-.e13.
72. Duong HH, Mirea L, Shah PS, Yang J, Lee SK, Sankaran K. Pneumothorax in neonates: Trends, predictors and outcomes. *J Neonatal Perinatal Med* 2014; **7**(1): 29-38.
73. Lee SM, Sie L, Liu J, Profit J, Lee HC. Evaluation of trends in bronchopulmonary dysplasia and respiratory support practice for very low birth weight infants: a population-based cohort study. *The Journal of Pediatrics* 2022; **243**: 47-52. e2.
74. Alejandre-Alcázar MA, Kwapiszewska G, Reiss I, et al. Hyperoxia modulates TGF-β/BMP signaling in a mouse model of bronchopulmonary dysplasia. *American Journal of Physiology-Lung Cellular and Molecular Physiology* 2007; **292**(2): L537-L49.

75. Bhutani VK, Wong RJ, Stevenson DK. Hyperbilirubinemia in Preterm Neonates. *Clin Perinatol* 2016; **43**(2): 215-32.
76. Rana A, Agarwal K, Ramji S, Gandhi G, Sahu L. Safety of delayed umbilical cord clamping in preterm neonates of less than 34 weeks of gestation: a randomized controlled trial. *Obstet Gynecol Sci* 2018; **61**(6): 655-61.
77. Watchko JF, Maisels MJ. Jaundice in low birthweight infants: pathobiology and outcome. *Arch Dis Child Fetal Neonatal Ed* 2003; **88**(6): F455-8.
78. Maisels M, Watchko J, Bhutani V, Stevenson D. An approach to the management of hyperbilirubinemia in the preterm infant less than 35 weeks of gestation. *Journal of Perinatology* 2012; **32**(9): 660.
79. Okuyama H, Kubota A, Oue T, Kuroda S, Ikegami R, Kamiyama M. A comparison of the clinical presentation and outcome of focal intestinal perforation and necrotizing enterocolitis in very-low-birth-weight neonates. *Pediatric surgery international* 2002; **18**(8): 704-6.
80. Shorter NA, Liu JY, Mooney DP, Harmon BJ. Indomethacin-associated bowel perforations: a study of possible risk factors. *Journal of pediatric surgery* 1999; **34**(3): 442-4.
81. Elgendy MM, Othman HF, Heis F, Qattea I, Aly H. Spontaneous intestinal perforation in premature infants: a national study. *Journal of Perinatology* 2021; **41**(5): 1122-8.
82. Patel RM, Knezevic A, Shenvi N, et al. Association of red blood cell transfusion, anemia, and necrotizing enterocolitis in very low-birth-weight infants. *Jama* 2016; **315**(9): 889-97.
83. Chen A-C, Chung M-Y, Chang JH, Lin H-C. Pathogenesis implication for necrotizing enterocolitis prevention in preterm very-low-birth-weight infants. *Journal of pediatric gastroenterology and nutrition* 2014; **58**(1): 7-11.
84. Ganesh A, Mitra S, Ganguly SS. Retinopathy of prematurity in VLBW and extreme LBW babies. *The Indian Journal of Pediatrics* 2003; **70**(4): 303-6.
85. Dani C, Reali M, Bertini G, Martelli E, Pezzati M, Rubaltelli F. The role of blood transfusions and iron intake on retinopathy of prematurity. *Early human development* 2001; **62**(1): 57-63.
86. Escobar GJ, Littenberg B, Petitti DB. Outcome among surviving very low birthweight infants: a meta-analysis. *Archives of disease in childhood* 1991; **66**(2): 204-11.
87. Stoll BJ, Hansen NI, Bell EF, et al. Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. *Pediatrics* 2010; **126**(3): 443-56.
88. Rysavy MA, Ehret DEY. Extremely Preterm Birth Outcomes in Sweden. *JAMA* 2019; **321**(12): 1163-4.
89. Ancel P-Y, Goffinet F, Group atE-W. Survival and Morbidity of Preterm Children Born at 22 Through 34 Weeks' Gestation in France in 2011: Results of the EPIPAGE-2 Cohort Study. *JAMA Pediatrics* 2015; **169**(3): 230-8.
90. McDonald SJ, Middleton P, Dowswell T, Morris PS. Effect of timing of umbilical cord clamping of term infants on maternal and neonatal outcomes. *Evidence-Based Child Health: A Cochrane Review Journal* 2014; **9**(2): 303-97.
91. Hutchon DJ, Wepster B. The estimated cost of early cord clamping at birth within Europe. *International Journal of Childbirth* 2014; **4**(4): 250-6.
92. WHO Guidelines Approved by the Guidelines Review Committee. Guidelines on Basic Newborn Resuscitation. Geneva: World Health Organization World Health Organization.; 2012.
93. Obstetricians ACo, Gynecologists, Practice CoO. Delayed umbilical cord clamping after birth: ACOG Committee Opinion, Number 814. *Obstetrics and gynecology* 2020; **136**(6): e100-e6.
94. Madar J, Roehr CC, Ainsworth S, et al. European Resuscitation Council Guidelines 2021: Newborn resuscitation and support of transition of infants at birth. *Resuscitation* 2021; **161**: 291-326.

95. Organization WH. WHO recommendations for care of the preterm or low-birth-weight infant: World Health Organization; 2022.
96. Hutchon D. Evolution of neonatal resuscitation with intact placental circulation. *Infant* 2014; **10**(2): 58-61.
97. Katheria A, Poeltler D, Durham J, et al. Neonatal Resuscitation with an Intact Cord: A Randomized Clinical Trial. *J Pediatr* 2016; **178**: 75-80.e3.
98. Winter J, Kattwinkel J, Chisholm C, Blackman A, Wilson S, Fairchild K. Ventilation of Preterm Infants during Delayed Cord Clamping (VentFirst): A Pilot Study of Feasibility and Safety. *Am J Perinatol* 2017; **34**(2): 111-6.
99. Brouwer E, Knol R, Vernooij ASN, et al. Physiological-based cord clamping in preterm infants using a new purpose-built resuscitation table: a feasibility study. *Arch Dis Child Fetal Neonatal Ed* 2019; **104**(4): F396-f402.
100. Duley L, Dorling J, Pushpa-Rajah A, et al. Randomised trial of cord clamping and initial stabilisation at very preterm birth. *Arch Dis Child Fetal Neonatal Ed* 2018; **103**(1): F6-f14.
101. Amendolia B, Kilic N, Afridi F, et al. Delayed Cord Clamping for 45 Seconds in Very Low Birth Weight Infants: Impact on Hemoglobin at Birth and Close to Discharge. *American Journal of Perinatology* 2022.
102. Meyer MP, Nevill E. Optimising cord clamping in preterm infants: one strategy does not fit all. *Frontiers in pediatrics* 2019; **7**: 46.
103. Mehler K, Oberthuer A, Keller T, et al. Survival among infants born at 22 or 23 weeks' gestation following active prenatal and postnatal care. *JAMA pediatrics* 2016; **170**(7): 671-7.

7. Appendix

7.1 Tables

7.2 Figures