Stimulating and maintaining spontaneous breathing of preterm infants at birth

Janneke Dekker

COLOFON

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ISBN: 978-94-632-3763-5
The research described in this thesis was supported by grants of Chiesi Pharmaceuticals BV, the Leiden University Fund, and the NWO innovational research incentives scheme (recipient A.B. te Pas: VIDI 91716428).
The printing of this thesis was financially supported by Willem-Alexander KinderZiekenhuis Leiden, Universiteitsbibliotheek Leiden, Concord Neonatal, AirMed Medical Supplies, Dräger, Chiesi Pharmaceuticals, ChipSoft, Advanced Life Diagnostics.
Cover layout: Sophie Cramer
Cover models: Simon & Anna-Sophie Dekker
Layout and printing: Gildeprint

STIMULATING AND MAINTAINING SPONTANEOUS BREATHING OF PRETERM INFANTS AT BIRTH

Proefschrift

Ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof.mr. C.J.J.M. Stolker,
volgens besluit van het College voor Promoties
ter verdedigen op dinsdag 10 september 2019
klokke 13:45 uur

door

Janneke Dekker
geboren te Alphen aan den Rijn
in 1988

Promotores:	Prof.dr. A.B. te Pas
	Prof.dr. S.B. Hooper
	(Monash University, Melbourne, Australia)
Leden promotiecommissie:	Prof.dr. E. Lopriore
	Prof.dr. I.K.M. Reiss
	(Erasmus MC-Sophia, Rotterdam, The Netherlands)
	Dr. H.L. Ersdal
	(University of Stavanger, Stavanger, Norway)

Nil volentibus arduum

(Amsterdams kunstgenootschap, 1669)

Niets is onmogelijk voor hen die willen

Voor mijn vader

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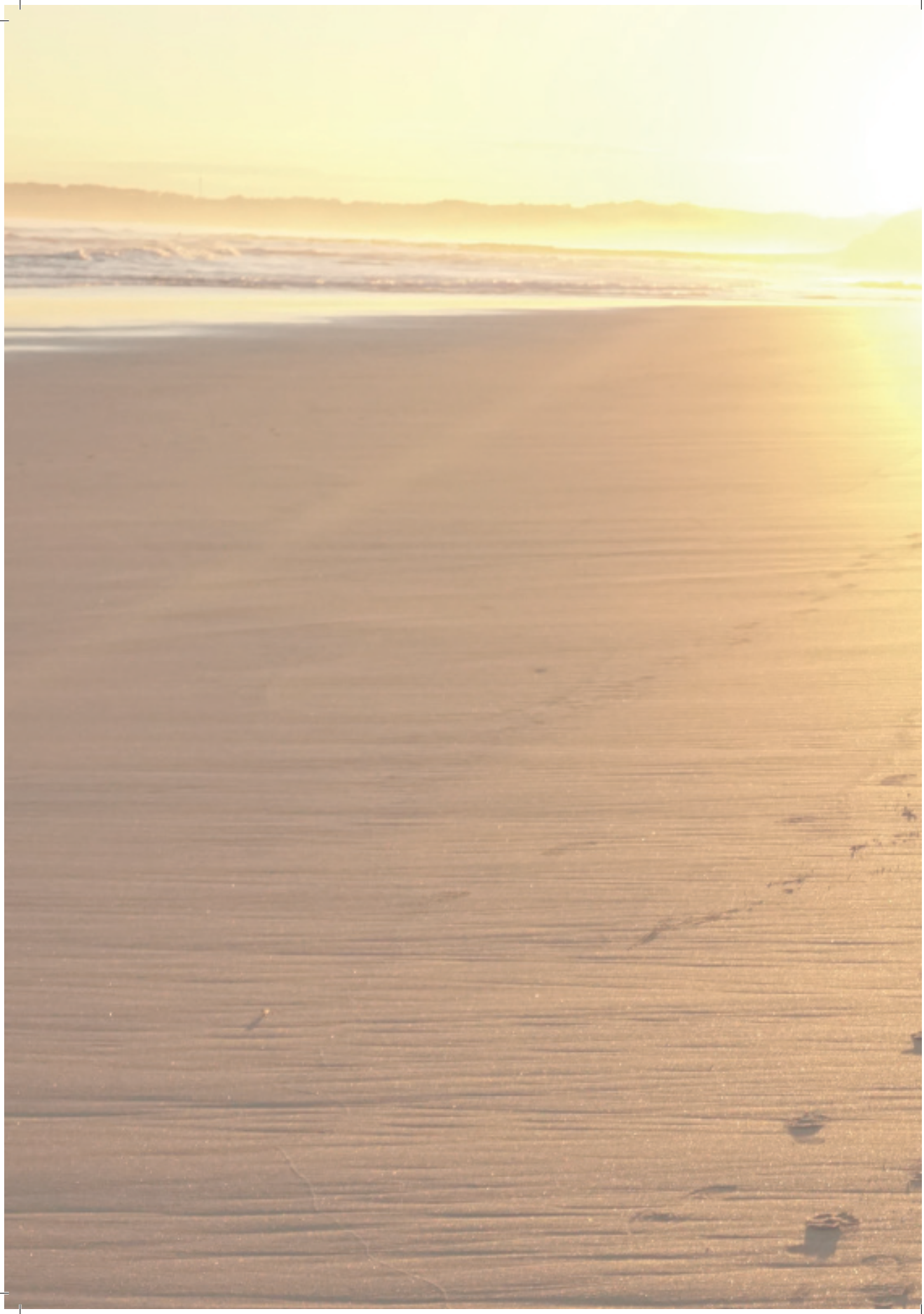
PART ONE

PREFACE AND GENERAL INTRODUCTION



PREFACE

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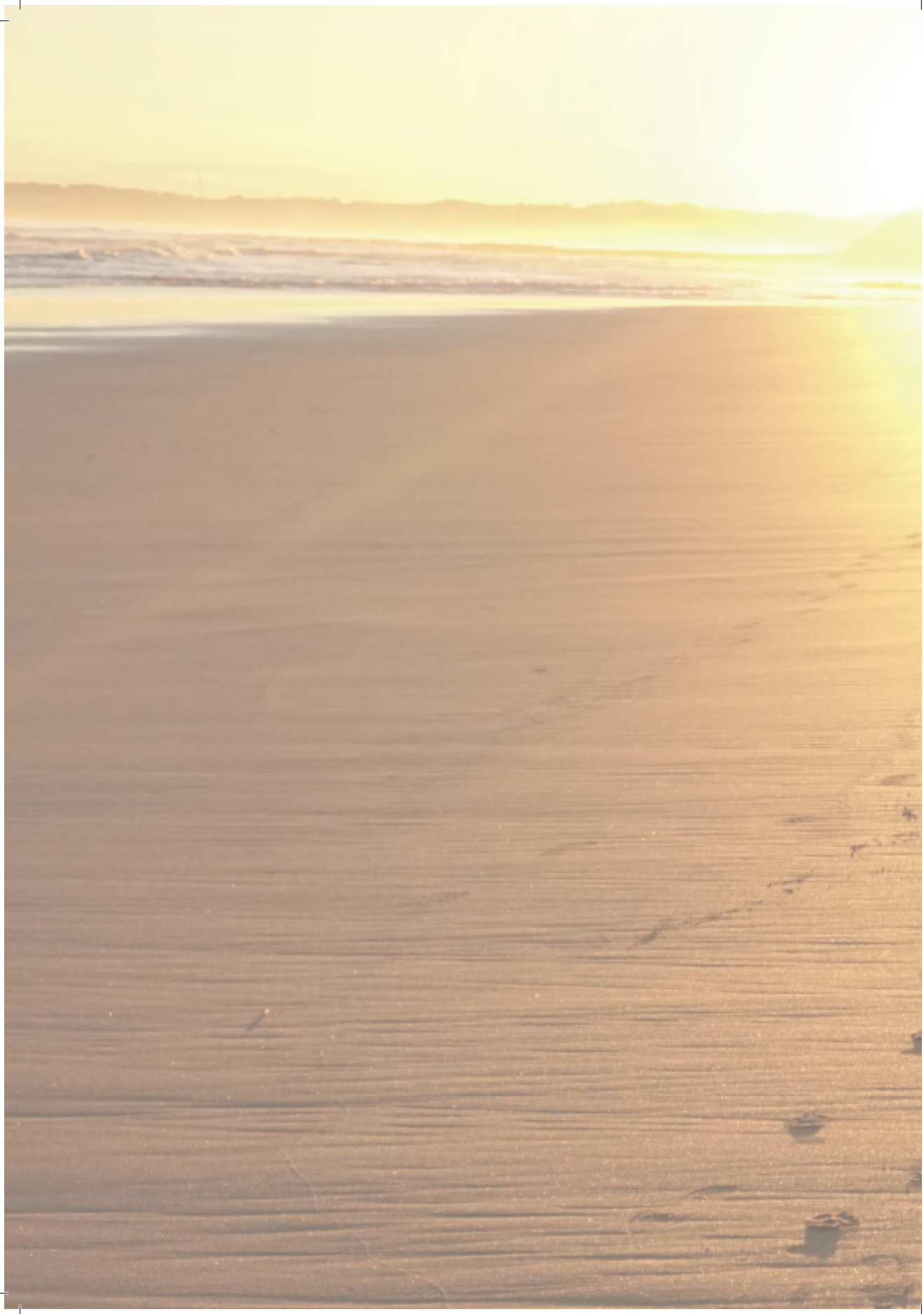
Friday morning, 09:30. The telephone of the paediatric resident rings. One of the obstetricians is calling to inform that an ambulance will arrive any minute, presenting a woman who has been pregnant for 25 weeks. This morning, she woke up having severe abdominal pain and backache, recurring every 30 minutes. To calm herself down she took a shower, but she noticed the pain only got worse. She contacted her midwife, who decided to visit her. After a physical examination, the midwife concluded that the pregnant woman was 8 cm diluted and the delivery was imminent. She called an ambulance and 15 minutes later they arrived at the obstetric ward of a hospital with a third-level Neonatal Intensive Care Unit (NICU).

Directly after the call of the obstetrician, the NICU resident contacts her supervising neonatal consultant and together with one of the NICU nurses they rush to the obstetric ward to prepare a resuscitation table. The NICU nurse pours water in the humidifier, turns up the heater above the resuscitation table and tees up the polyethylene wrap. Meanwhile, the consultant arrives and together with the resident they prepare the T-piece resuscitator and the intubation supplies. They just finish setting up the respiratory function monitor, when suddenly the door opens and the obstetric nurse comes through, carrying a small boy, that she places on the resuscitation table.

The NICU nurse starts the Apgar timer and the recording of the respiratory function monitor, and closes the polyethylene wrap around the little boy to keep him warm. Only his right hand protrudes the wrap so the nurse can attach the pulse oximeter to measure the heart rate and oxygen saturation of the infant. The young man who just became father comes over from the delivery suite to meet his newborn son. The NICU nurse encourages him to touch the boy and talk to him. After the initial assessment, the neonatal consultant places a face mask over the nose and mouth of the little boy to provide him with continuous positive airway pressure to support his respiratory transition. During the 30-second evaluation that follows, the consultant and resident do not notice any raising of the chest wall implicating spontaneous breathing, upon which they decide to administer a sustained inflation. During a second clinical evaluation, still no raising of the chest wall is visible. However, the respiratory function monitor shows an irregular undulatory pattern of the flow curve, possibly representing minor spontaneous respiratory effort of the infant. As the oxygen saturation does not reach the predefined target range, they decide to increase the fraction of inspired oxygen and grant the infant some more time to enhance his respiratory effort. Meanwhile, the NICU nurse starts tactile stimulation to encourage the infant to breathe. The respiratory effort

of the infant increases slowly, but oxygen saturations remain below target ranges. The infant is then provided with positive pressure ventilation by face mask, and the fraction of inspired oxygen is increased up to 1.0. Every 30 seconds, the infant is clinically evaluated, and the fraction of inspired oxygen is adjusted according to the oxygen saturations. Unfortunately, the need for ventilation persists and the infant is intubated 15 minutes after birth.

After this impressive start of this small boy's life, the NICU nurse deliberates with her colleagues: "Isn't there something else we could do to help these tiny babies breathe and avoid invasive ventilation?"



GENERAL INTRODUCTION

GENERAL INTRODUCTION

Most infants worldwide are born at term, defined as a gestation of a minimum of 37 weeks. Approximately 15 million infants are born prematurely every year.(1) Preterm birth is one of the leading causes of neonatal mortality, and premature infants are at risk of major morbidities, influencing both short- and long-term neurodevelopmental outcomes. The risk of complications is closely related to the gestational age at birth, with extreme preterm infants (born < 28^{th} week of gestation) having the greatest risk.(2)

As the incidence of prematurity continues to increase, considerable effort is being made to reduce the risk of adverse outcomes, even in the smallest infants. At birth, the transition from foetus to neonate requires possibly the most substantial physiological adjustments that occur in human life. This transition is complicated in preterm infants due to the immaturity of many organ systems. Most preterm infants therefore require support during their transition at birth.

Respiratory transition of the term infant at birth

While the foetus is *in utero,* gas exchange occurs across the placenta rather than the lungs. (3) The foetus shows breathing movements, but these are not aimed at gas exchange; instead, they enable small volumes of lung liquid to move in and out of the airways. The pattern of foetal breathing movements changes with the arousal state of the foetus. During the REM (rapid eye movement) sleep state, rhythmic breathing movements are shallow, becoming deeper in the non-REM state.(4, 5) During apnea the larynx remains closed, restraining the efflux of lung liquid.(5, 6) This process is necessary for lung development and growth.(7)

After birth, the site of gas exchange changes to the lungs, where oxygen and carbon dioxide are exchanged via the alveoli to the surrounding capillaries and vice versa.(3) However, to enable gas exchange the lung needs to be cleared of liquid. During labour, uterine contractions induce a change in the posture of the foetus, leading to an increase in abdominal pressure and together with the increase in transpulmonary pressure this results in the efflux of lung liquid.(3, 8) In addition, the increase in stress hormones during labour stimulates the uptake of Na^+ across the airway epithelium thereby changing the osmotic gradient, which leads to reabsorption of lung liquid.(9) Next, the remaining lung liquid needs to move across the distal wall of the airway. This appears during inspiration, as inspiratory muscles create a negative pressure by expanding the chest wall. The created pressure gradient between interstitial tissue and airway lumen results in the uptake of liquid from the airways into the interstitial tissue.(3) This movement of liquid appears to be rapid, resulting in the total clearance of lung liquid in seconds to minutes.(10, 11)

A liquid-filled lung generates a resistance around 100 times higher than that of the air-filled lung after transition.(12) The lung fills with air progressively during the first breaths after birth, which decreases the resistance and makes the lung more compliant.(12) However, the interstitial space fills with liquid, thereby increasing interstitial pressure. Until this liquid is cleared from the interstitial space and the pressure reduces again, this could lead to the re-entry of liquid into the airways.(3) In term infants, the pressure gradient that emerges during inspiration is much greater than the pressure gradient during expiration. The amount of liquid that re-enters the lung during expiration is therefore much smaller than the amount of liquid that is cleared during inspiration. (13, 14) In addition, surfactant present in the alveoli leads to a decrease in surface tension, reducing the pressure gradient between alveoli and interstitial space. This in turn reduces the potential for liquid re-entry.(15, 16)

Consequences of prematurity on respiratory transition

When an infant is born preterm, there are many obstacles to this process of respiratory transition. Firstly, it can be impacted by the structural immaturity of the lung. While alveolarization begins from a gestational age of 32 weeks and upwards, the lungs of preterm infants born earlier in gestation will be comprised mainly of sacculi. This compromises the surface available for gas exchange. In addition, infants born preterm will suffer from surfactant deficiency as the type-II alveolar epithelial cells responsible for the production of surfactant develop late in gestation.(17) This might lead to the collapse of the small airways at end-expiration, which also decreases the surface area available for gas exchange and leads to an increase in the effort it takes to breathe.

The preterm lungs have difficulty clearing airway liquid, as they do not have access to the Na^+ uptake mechanism which reverses the osmotic gradient leading to airway liquid reabsorption.(18) In addition, this process may be further negatively affected in the case of caesarean delivery, when less adrenalin is released.(19) The lower muscle tension of the preterm infant results in a smaller pressure gradient during inspiration. Lung liquid may therefore flow back into the airways from the interstitial space more easily than in term infants. Additionally, the ratio between the amount of liquid cleared during inspiration and liquid re-entering the lung during expiration appears to be smaller, leading to loss of functional residual capacity (FRC).(20, 21) The preterm infant tries to maintain FRC by increasing the airway pressure when breathing out by prolonging expiration time with expiratory braking maneuvers and grunting.(22)

Supporting the preterm infant in transitioning

While most interventions aimed at respiratory support of the preterm infant are performed postnatally, maturation of the lung – and thus the presence and amount of different surfactant-proteins - can be influenced prenatally. Maternal glucocorticoid treatment is often combined with beta-sympathomimetic drugs to stimulate secretion of the major component of surfactant and decrease the secretion rate of lung fluid.(23) In addition, the administration of these agents leads to a reduction in the gas diffusion distance.(15, 24)

Nevertheless, many preterm infants need respiratory support to facilitate respiratory transition at birth. While the lung undergoes different phases of lung liquid clearance (movement of liquid from proximal to distal airways, uptake of liquid in the interstitial space, clearance of liquid from the interstitial space), respiratory support should be adapted to these different phases to prevent the lung from injury. This is extremely difficult to achieve.(3) High pressures or long inflation times are necessary to move liquid through the lung to enter the interstitial space, but as soon as the liquid is cleared from the interstitial space these high pressures could lead to an overexpansion of the lung and cause injury.(3) It is difficult, if not impossible, to determine which phase of lung liquid clearance the infant has reached. In addition, the rate of lung aeration may not be equal in the different regions of the lung, increasing the complexity of respiratory support.(3)

To support the infant, positive pressure can be used to facilitate the change in pressure gradient between the airways and the interstitial pressure, leading to an uptake of lung liquid and prevention of liquid flowing back into the lung.(25) In addition, positive pressure can prevent the compliant chest wall from recoiling. However, it has been shown that intubation and subsequent invasive mechanical ventilation increase the risk of ventilator-induced lung injury.(26) Using nasal continuous positive airway pressure (CPAP) as respiratory support at birth enhances the aeration of the lung and decreases the work of breathing.(21) This technique of respiratory support can be used to prevent the need for intubation and subsequent mechanical ventilation, thereby decreasing the risk of ventilator-induced lung injury.(26, 27) Furthermore, non-invasive positive pressure ventilation can be used in case of apnea or insufficient spontaneous respiratory effort.

However, providing adequate ventilation using a face mask has been shown to be difficult due to mask leak or obstruction.(28, 29) While efforts have been made to decrease the incidence of these causes of ineffective non-invasive ventilation, the adducted larynx at birth has so far been overlooked as an important cause of

obstruction. Active larynx adduction promotes lung development in the foetus but prevents air from entering the lung after birth.(7, 30) This was recently demonstrated in a preterm rabbit model showing that the larynx is predominantly closed at birth during apnea, and opens only briefly when a breath is taken.(31) Applying positive pressure ventilation using a face mask results in distension of the upper airway instead of aeration of the lung.(32) Inflations administered without spontaneous breathing lead to ineffective ventilation.(33) The pattern of the mainly closed larynx only opening during spontaneous breathing changes once a stable breathing pattern is obtained; then the larynx remains mainly open.(31) It therefore seems logical that stimulating spontaneous breathing should result in more effective respiratory support, which would lead to an increase in oxygenation and a smoother respiratory transition.

Supporting the preterm infant after transition at birth

While the majority of preterm infants leave the delivery room supported by non-invasive ventilation, a proportion of them will suffer from severe respiratory distress syndrome (RDS) and administration of exogenous surfactant may be necessary. This was first described as beneficial in preterm infants by Fujiwara et al.(34) in 1980. Since then, many studies have focused on the dosage, timing and method of administration. Recent trials have demonstrated the feasibility and efficacy of administration of surfactant in a minimal invasive way, thereby omitting intubation.(35, 36) A stable respiratory drive is a prerequisite for making this procedure successful and avoiding intubation and mechanical ventilation. While stimulation of spontaneous breathing is an important focus during transition at birth, after birth maintenance of spontaneous breathing is essential. Interventions focusing on these aspects of neonatal care for preterm infants may have the potential to improve outcomes for infants born at the limit of viability.

AIM AND OUTLINE OF THE THESIS

The general aim of this thesis was to evaluate the effect of interventions performed in the delivery room and shortly after arrival at the neonatal intensive care unit on the respiratory effort of preterm infants at birth. This thesis comprises experimental, observational and randomized clinical trials. The interventions evaluated in this thesis are aimed at stimulation of spontaneous breathing (part two) and maintenance of spontaneous breathing (part three).

Part two of this thesis focuses on interventions that can be used to **stimulate spontaneous breathing** of preterm infants directly at birth. Tactile stimulation to

stimulate breathing of infants at birth has been common practice from earliest living memory and was recommended even before the first version of the resuscitation guidelines was developed. Although most interventions in the delivery room have been evaluated thoroughly, the use of tactile stimulation and its effect on respiratory effort in human preterm infants have not previously been described. **Chapter 1** describes an observational study performed at the Leiden University Medical Center (LUMC) evaluating the incidence, duration and timing of tactile stimulation during transition of preterm infants at birth, and differentiating the methods used for stimulation. While this study showed that stimulation could be applied either repetitively or based on clinical indication, we designed a randomized trial to assess the effect of these patterns of stimulation on the respiratory effort of preterm infants at birth. The results of this randomized trial conducted at the LUMC are presented in **Chapter 2**.

The second intervention evaluated in this thesis is the use of oxygen during stabilization at birth. Previous studies have shown that hypoxia inhibits the respiratory center in the foetal situation, causing larynx adduction.(37) Although this effect changes in the first days of life, it is likely to be present during transition at birth.(38) To stimulate spontaneous breathing, we should therefore focus on preventing hypoxia. In collaboration with Professor Stuart Hooper's research group, the effect of administering 100% oxygen was evaluated in an experimental setting, described in **Chapter 3**. By using phase contrast X-ray imaging and a custom-built mechanical ventilator to apply CPAP, the breathing pattern and aeration of the lung of preterm rabbits during transition could be visualized. This study compared the effect of 100% oxygen with 21% oxygen. During the stabilization of human preterm infants at birth, however, we use an oxygen blender that mixes oxygen with room air in order to enable titration of the fraction of inspired oxygen (FiO_2), after which the gas mixture is administered to the infant using a T-piece resuscitator. FiO_2 is titrated based on the oxygen saturation (SpO_2) target range described by Dawson et al.(39) to prevent the occurrence of prolonged hypoxia and hyperoxia. However, while the adjustment of FiO_2 is performed with the oxygen blender, it is unknown how long it takes for the gas mixture with the desired oxygen concentration to reach the infant at the distal part of the T-piece resuscitator. **Chapter 4** describes the evaluation of the duration between adjustment of FiO_2 at the oxygen blender and the achievement of the desired FiO_2 at the distal part of the T-piece resuscitator, which was assessed in a bench test and during clinical observations of the stabilization of preterm infants born at the LUMC. **Chapter 5** describes a study protocol for a multi-center randomized controlled trial. The aim of this trial was to compare the effect of initiating stabilization at birth with 100% O_2 to 30% O_2 on the respiratory effort of preterm infants. In both randomization arms, subsequent titration of FiO_2 will be performed to

target internationally recommended SpO_2 reference ranges. The results of this trial are presented in **Chapter 6**.

The third intervention evaluated in this thesis focusing on stimulating breathing of preterm infants in the delivery room is the administration of caffeine. Caffeine has been shown to be safe and effective in the prevention of apnea of prematurity. In recent years, the timing of administration of caffeine has shifted from the first days to the first hours after birth. As caffeine antagonizes adenosine at its receptor, its use might be extended to administration in the delivery room to stimulate breathing directly at birth. The randomized controlled trial conducted at the LUMC, which is described in **Chapter 7**, evaluated the effect of administration of caffeine in the delivery room on the respiratory effort of preterm infants at birth.

Part Three of this thesis then focuses on an intervention aimed at **maintenance of spontaneous breathing** after admission to the NICU. After transition has been successfully established and the infant is supported by CPAP, RDS can cause difficulty in reaching an appropriate level of oxygenation. A high oxygen requirement is one of the main causes of CPAP failure, which can be prevented by the application of exogenous surfactant while the infant remains on CPAP. Administering surfactant to a spontaneously breathing infant in a minimal invasive way still involves the use of a laryngoscope to visualize the trachea. As laryngoscopy has been shown to be uncomfortable, the use of propofol as premedication has been evaluated in Chapters 8 and 9. **Chapter 8** describes an observational study of comfort during minimal invasive surfactant therapy performed in the LUMC. Additionally, **Chapter 9** evaluates this practice using a randomized controlled trial.

In Part Four of this thesis, the main findings of the studies presented are discussed and future perspectives are considered. The thesis concludes with a summary of the studies, provided in English and Dutch.

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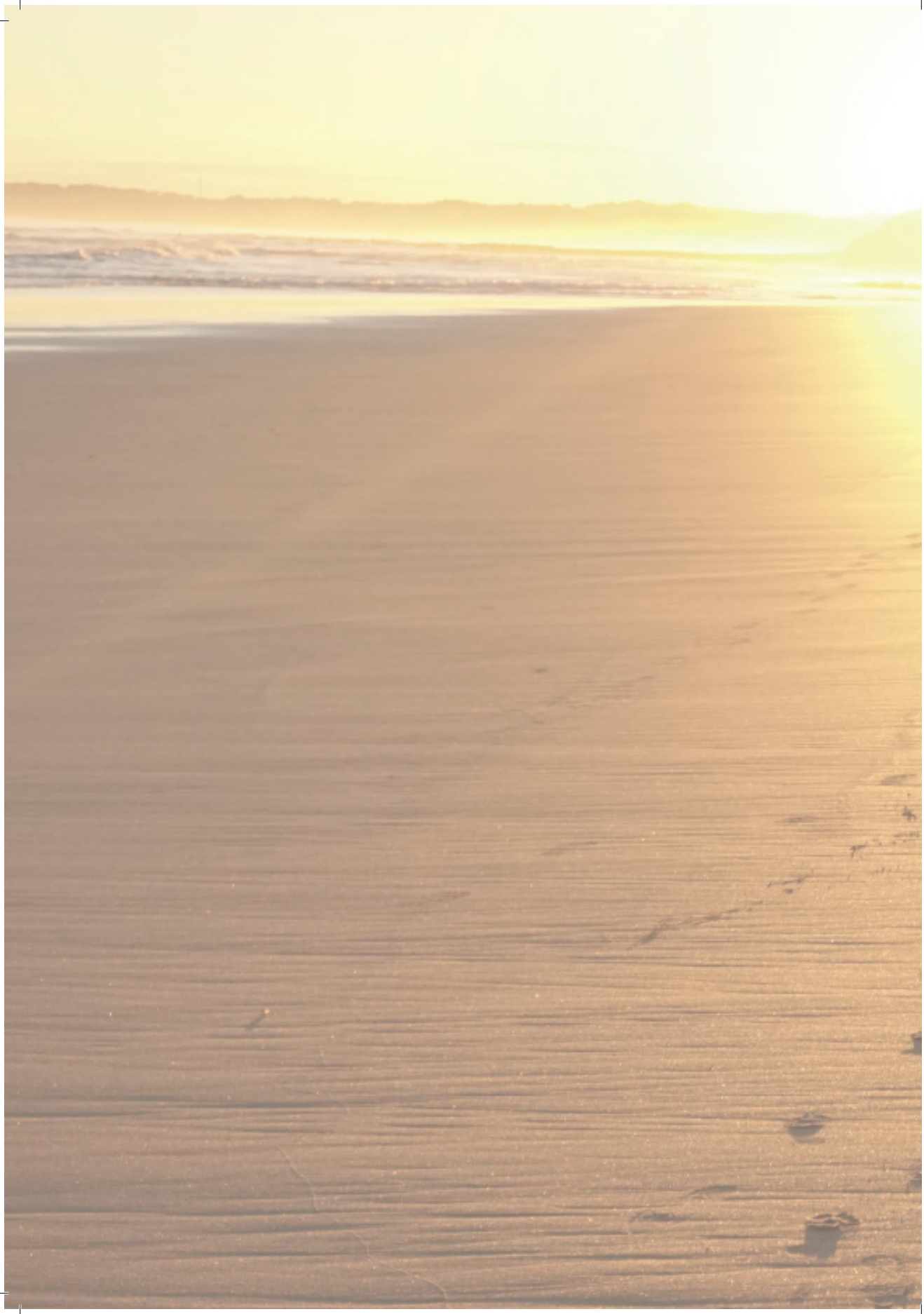
PART TWO

STIMULATING SPONTANEOUS BREATHING AT BIRTH



CHAPTER 1

Tactile stimulation to stimulate spontaneous breathing during stabilization of preterm infants at birth: a retrospective analysis

J Dekker
T Martherus
SJE Cramer
HA van Zanten
SB Hooper
AB te Pas

Frontiers in Pediatrics 2017;5:61

ABSTRACT

BACKGROUND

Tactile maneuvers to stimulate breathing in preterm infants are recommended during the initial assessment at birth, but it is not known how often and how this is applied. We evaluated the occurrence and patterns of tactile stimulation during stabilization of preterm infants at birth.

METHODS

Recordings of physiological parameters and videos of infants < 32 weeks gestational age were retrospectively analyzed. Details of tactile stimulation during the first 7 minutes after birth (timing, duration, type, and indication) were noted.

RESULTS

Stimulation was performed in 164/245 (67%) infants. The median (IQR) gestational age was 28^{+6} (27^{+2} – 30^{+1}) weeks, birth weight 1153 (880 – 1385) grams, Apgar score at 5 minutes was 8 (7 – 9). 140/245 (57%) infants were born after caesarean section, and 134/245 (55%) were male. There were no significant differences between the stimulated and the non-stimulated infants with regard to basic characteristics. In the stimulated infants, the first episode of stimulation was given at a median (IQR) of 114 (73 – 182) s after birth. Stimulation was repeated 3 (1 – 5) times, with a median (IQR) duration of 8 (4 – 16) s and a total duration of 32 (15 – 64) s. Modes of stimulation were: rubbing (68%) or flicking (2%) the soles of the feet, rubbing the back (12%), a combination (9%), or other (8%). In 67% of the stimulation episodes, a clear indication was noted (25% bradycardia, 57% apnea, 48% hypoxaemia, 43% combination) and an effect was observed in 18% of these indicated stimulation episodes. A total effect of all stimulation episodes per infant remains unclear, but infants who did not receive stimulation were more often intubated in the delivery room (14/79 (18%) vs 12/164 (7%), $p<0.05$).

CONCLUSION

There was a large variation in the use of tactile stimulation in preterm infants during stabilization at birth. In most cases, there was an indication for stimulation, but only in a small proportion an effect could be observed.

INTRODUCTION AND RATIONALE

Most preterm infants initiate breathing after birth, but their respiratory drive is weak and often insufficient.(1-5) However, in the last decade, the focus of respiratory care in the delivery room has shifted from intubation and mechanical ventilation toward non-invasive ventilation and supporting spontaneous breathing.(2, 6) Both local and international resuscitation guidelines recommend to assess respiratory effort, and if necessary, stimulate and support spontaneous breathing.(7-9)

Tactile stimulation (warming, drying, and rubbing the back or the soles of the feet) has been recommended in the guidelines to stimulate spontaneous breathing.(7-9) Although this is now a commonly accepted intervention, the effect remains unclear. Experimental studies have shown a positive effect of tactile stimulation on spontaneous breathing at birth(10, 11), but there is very little human data demonstrating the effect of stimulation, especially in preterm infants.

Although most interventions in the delivery room have been evaluated, frequency and method of tactile stimulation have not been evaluated objectively.(12-17) Besides the description of several studies that neonatal caregivers often defer from the resuscitation guidelines(12-17), the use of tactile stimulation might be influenced by the current practice that preterm infants are not dried but placed in a plastic wrap to prevent hypothermia.(18, 19)

We, therefore, evaluated the occurrence and methods of tactile stimulation of preterm infants directly after birth.

METHODS

In a retrospective study, we reviewed all neonatal stabilization procedures at birth of infants with a gestational age of < 32 weeks from January 2007 until June 2016 in the Leiden University Medical Center (LUMC). In this study, recordings of videos and physiological parameters of neonatal resuscitation in the delivery room were used. The recording of videos and physiological parameters of resuscitation in the delivery room for auditing is standard of care at the LUMC.

Videos and respiratory function monitoring (RFM), including heart rate, oxygen saturation, and fraction of inspired oxygen were recorded as soon as the infants' shoulder was

out during delivery. Respiratory parameters were recorded with either a Florian RFM (Acutronic Medical Systems AG, Hirzel, Switzerland), using a hot wire anemometer, or a New Life Box (Applied Biosignals, Weener, Germany) connected to a MRT-A RFM (Applied Biosignals, Weener, Germany), using a variable orifice pneumometer (AVEA Varflex Flow transdurer, Carefusion, Yorba Linda, CA, USA). Oxygen saturation and heart rate were recorded using a Masimo SET pulse oximeter (Masimo Radical, Masimo Corporation, Irvine, CA, USA). The pulse oximetry probe was placed around the infants' right wrist. In case the Florian RFM was used, gas flow, pressures given, tidal volume, oxygen saturation, heart rate, and breathing signals were digitized using the Spectra physiological software (Grove Medical Limited, Hampton, UK). Polybench software (Applied Biosignals, Weener, Germany) was used when making use of the New Life Box.

The videos and physiological parameters were independently reviewed and analyzed by two researchers involved in the study (Janneke Dekker and Tessa Martherus). In case of doubt, consensus was achieved with the help of a third researcher (Arjan B. te Pas), to guarantee objectivity of the analyzing process. The logging of the occurrence and methods of tactile stimulation was started after the infant was dried or put in the plastic wrap, the cap was put on, and the pulse oximeter and face mask were placed. For all included infants, we collected the following patient characteristics: gestational age at birth (GA), birth weight, gender, mode of delivery, Apgar score at 5 minutes after birth, antenatal corticosteroids, and intubation in the delivery room.

The main variable of interest was the occurrence of tactile stimulation of the neonate in the first 7 minutes after birth, as the majority of infants are being prepared for transport to the Neonatal Intensive Care Unit (NICU) after 7 minutes. We also noted the frequency and duration of tactile stimulation per infant, the time points and the method of stimulation (rubbing the back, rubbing the soles of the feet, flicking the soles of the feet, other). If stimulation was performed, we noted whether there was an indication for stimulation based on clinical signs such as apnea/irregular breathing, wherefore the infant needed positive pressure ventilation, bradycardia (a heart rate < 100 bpm), or hypoxia (oxygen saturation below the recommended target range described by Wyllie et al.(20)). In case of a clear clinical indication, we also noted the effect (recovery of heart rate > 100 bpm and/or regaining breathing/increased breathing effort). When tactile stimulation was not performed, we noted whether stimulation could have been indicated.

After all data of individual stimulation episodes was logged, a categorical scheme was drafted by two of the researchers (Janneke Dekker and Tessa Martherus) in which

patterns of stimulation were explicated. After this, all videos were reviewed and coded to one of the patterns.

This study was conducted according to the principles of the Declaration of Helsinki and in accordance with the Medical Research Involving Human Subjects Act (WMO). In the Netherlands, no ethical approval is required for anonymized studies with medical charts, and patient data that are used for daily care. The Research Ethics Committee issued a statement of no objection.

Statistical analysis

Results are presented as mean ± SD for normally distributed values or medians (IQR) for non-normally distributed values. The demographical data of stimulated infants were compared with non-stimulated infants using Student's t-test for parametric variables, the Mann-Whitney U test for non-parametric comparisons, and the χ^2 test for categorical variables. P-values <0.05 were considered statistically significant, reported p-values are two sided. Data analysis was performed using IBM SPSS Statistics version 23 (IBM Software, New York, NY, USA, 2012). Missing values were excluded case wise from the

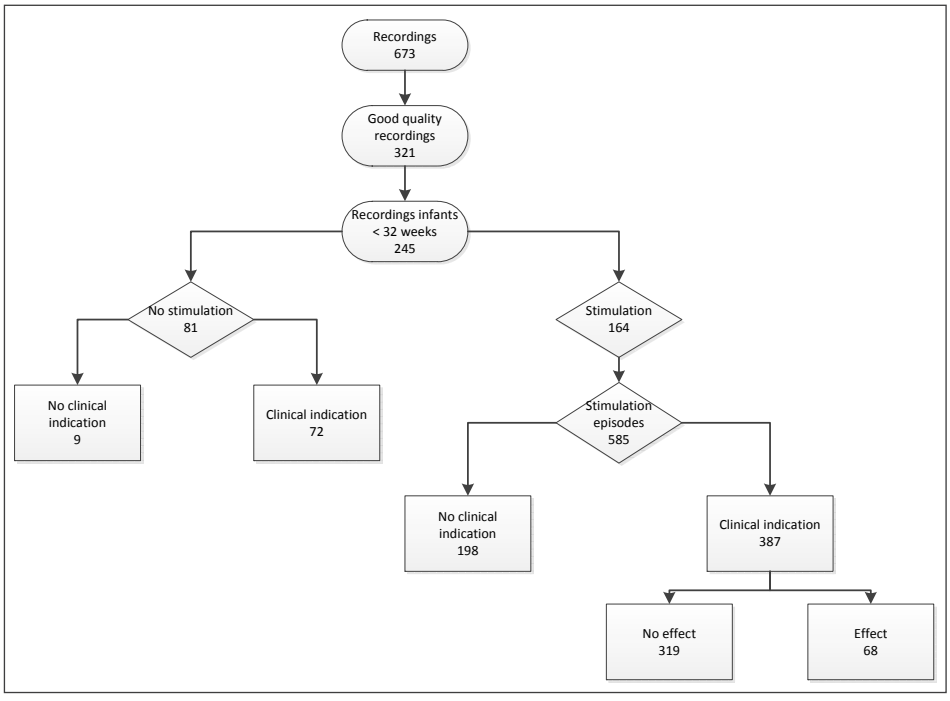


Figure 1 | Flowchart

analysis if they represented less than 5% of total values. Otherwise, multiple imputation was used. The remaining missing values were excluded case wise.

RESULTS

A total of 673 infants were recorded, of which 321 recordings were complete and of good quality. From these, 245 recordings included stabilization at birth of infants born with a GA < 32 weeks and were included in the analysis (Figure 1). The median (IQR) GA was 28^{+6} (27^{+2} – 30^{+1}) weeks, birth weight was 1153 (880 – 1385) grams, Apgar score at 5 minutes was 8 (7 – 9), 140/245 (57%) infants were born after caesarean section, 153/245 (62%) received antenatal corticosteroids, and 134/245 (55%) infants were male.

Tactile stimulation was performed in 164/245 (67%) infants. GA, birth weight, gender, mode of delivery, antenatal corticosteroids, and Apgar score did not differ significantly between stimulated and non-stimulated infants (Table 1). The first moment of stimulation started at median (IQR) 114 (73 – 182) s after birth. Each stimulation episode had a median (IQR) duration of 8 (4 – 16) s. The total time of stimulation was 32 (15 – 64) s. Four different stimulation patterns could be identified and categorized, based on information about indication and repetitiveness of stimulation (Table 2).

Table 1 | Demographical data

	Stimulated infants n = 164	Non-stimulated infants n = 81	*p*-value
Gestational age (weeks)[a]	29 0/7 (27 3/7 – 30 2/7)	28 4/7 (26 6/7 – 30 0/7)	0.298
Gender (% male)[b]	94/163 (58%)	40/80 (50%)	0.419
Birth weight (grams)[a]	1165 (875 – 1418)	1121 (880 – 1363)	0.543
Mode of delivery (% caesarean section)[b]	93/164 (57%)	47/81 (58%)	0.893
Antenatal corticosteroids (% full course)[b]	106/149 (71%)	47/70 (67%)	0.636
Apgar score at 5 minutes after birth[a]	8 (6 – 9)	8 (7 – 9)	0.669

Data are presented as median (IQR) for non-parametric data ([a]) and n (%) for categorical data ([b])

In the 164 infants that received stimulation, a total of 585 stimulation episodes were observed with median (IQR) 3 (1 – 5) stimulations per infant. Stimulation was performed most often by rubbing the soles of the feet (Table 3). In 387/578 (67%) of stimulation

episodes, a clear clinical indication for stimulation could be observed, which were bradycardia (25%), apnea/irregular breathing (57%), hypoxia (48%), or a combination of these (43%). An effect could be observed in 68/387 (18%) of these stimulation episodes.

Table 2 | Stimulation types

Stimulation type	Incidence, n (%)
Indicated repetitive stimulation	29/164 (18)
Non-indicated repetitive stimulation	2/164 (1)
Indicated non-repetitive stimulation	101/164 (62)
Non-indicated non-repetitive stimulation	32/164 (20)

Table 3 | Methods of stimulation

Method of stimulation	Incidence, n (%)
Rubbing the soles of the feet	400/585 (68)
Rubbing the back	70/585 (12)
Flicking the soles of the feet	10/585 (2)
Combination of abovementioned methods	55/585 (9)
Other (vigorous rubbing, drying, rubbing extended to the lower extremities)	49/585 (8)

Although 81/245 (33%) infants received no stimulation during resuscitation, a clinical indication during the resuscitation could be observed in 72/81 (89%) infants (bradycardia 67%, apnea 70%, hypoxia 70%, or a combination of these 69%).

A total of 26/243 (11%) infants were intubated in the delivery room. The incidence of intubation in the delivery room was significantly higher in infants who received no stimulation compared to the stimulated infants (14/79 (18%) vs 12/164 (7%), $p<0.05$). When comparing infants with a clinical indication for stimulation, the incidence of intubations was also significantly higher in non-stimulated infants (14/70 (20%) vs 12/130 (9%), $p<0.05$).

DISCUSSION

This is the first study in which recordings of video and physiological parameters were used to review the use of tactile stimulation in preterm infants at birth. In the majority of infants, tactile stimulation was applied during stabilization at birth. In most cases, there was a clinical indication for stimulation, although still a proportion of infants did not receive stimulation while this was indicated. In addition, the starting time, duration, and method of tactile stimulation varied between infants. Although we could not observe a clear direct effect of each stimulation episode in infants where stimulation was indicated, a total effect of all stimulation periods in an infant remains unclear. Indeed, when stimulation was indicated, infants who did not receive stimulation were more often intubated.

Tactile stimulation is suggested in international guidelines(7-9), but there are no clear recommendations on indication, timing, and method of tactile stimulation. This is probably due to the scarcity in data how tactile stimulation can be best used during stabilization of preterm infants at birth. The lack of a definition in the guidelines on timing and method of tactile stimulation probably explains the large variation we observed in practice.

Although the effect of tactile stimulation as single intervention has not been described in human preterm infants at birth, this has been demonstrated in animals. Faridy et al.(10) described that maternal rats perform rolling, licking, biting, and pushing of the newborn rat to stimulate its breathing. When newborn rats were removed from their mother directly after birth, they developed respiratory distress.(10) In apneic term lambs, spontaneous breathing commenced when they were stimulated with both mechanical and electrical stimuli.(11)

Breathing is initiated by the respiratory center in the medulla by different stimuli including hypoxia and hypercapnia.(21, 22) On the other hand, the respiratory center can be depressed by the level of adenosine present at birth, which may vary widely under different conditions such as mode of delivery and timing of cord clamping.(23-25) Breathing effort can be increased by changing the state of arousal of the infant by the use of tactile stimulation. The response on arousal may vary according to the location of the nerves that are stimulated.(26) Ioffe et al.(27) conducted a study in fetal lambs to test the respiratory response to somatic stimuli and found that the sleep state (NREM, REM, awake) changed when stimuli were given. According to this study, the respiratory response was greatest when fetal lambs were in REM sleep or awake at the end of stimulation.(27, 28)

However, the effect of tactile stimulation on breathing by means of arousal is not clear as infants are exposed to other stimuli at birth, which might change their arousal state, such as light, cold, and sound. In addition, most preterm infants receive respiratory support, which could also stimulate breathing effort.(29) In contrast, it could be possible that in the effort of applying tactile stimulation, the focus shifts away from other interventions during stabilization. In addition, vigorous stimulation could potentially lead to displacement of the mask.

In contrast to tactile stimulation at birth, stimulation to counteract apnea of prematurity after admittance to the NICU has been well studied in human infants, with stimulation performed in different ways. In the study of Kattwinkel et al.(30), when extremities were rubbed repetitively as method of tactile stimulation, the frequency of apnea was significantly reduced during repetitive stimulation in the 2 hours after the repetitive stimulation. In addition, the use of a stochastic resonance mattress is shown to reduce the incidence of apnea.(31, 32) However, other methods of stimulation like the use of kinaesthetic stimulation have not shown to be effective as treatment for apnea of prematurity in the NICU.(33) It is assumed that underdevelopment of the respiratory center in the medulla, hypoxia, or altered sensitivity of chemoreceptors to carbon dioxide or oxygen might be the cause of apnea of prematurity.(30) However, the mechanisms underlying apnea during prematurity might differ from the mechanisms of apnea at birth.

Although this study is limited by its retrospective design, objective parameters and videos could be used to observe the current practice in tactile stimulation as also the effect of stimulation. However, the decision to apply stimulation remains to the discretion of caregivers. Neither the motivation whether stimulation was performed nor the preferred methods were noted. Since we reviewed the recordings from 2007 to 2016, it is possible that the use of stimulation has changed since our practice has changed toward more support of spontaneous breathing. However, we could not observe a change along the timeline of analyzed recordings. As the video recordings were made anonymous with regard to the caregivers in charge of the stabilization procedure, we could not assess patterns in stimulation among different caregivers. Although there were no differences in characteristics between the groups, it is possible that other unmeasured variables besides stimulation influenced the intubation rate.

CONCLUSION

In summary, we observed that there was a large variability in the use of tactile stimulation during stabilization of preterm infants at birth. When stimulation was applied, most often this was indicated, while there was also often an indication when no stimulation was given at all. We could not observe a direct effect of stimulation in most occasions. While there is increasing awareness that most preterm infants breathe at birth and there is more emphasis on supporting this, stimulating breathing effort will also play an important role in this. The variation observed in current practice indicates that studies are warranted on duration and type of tactile stimulation leading to a better definition in guidelines when and how stimulation should be performed.

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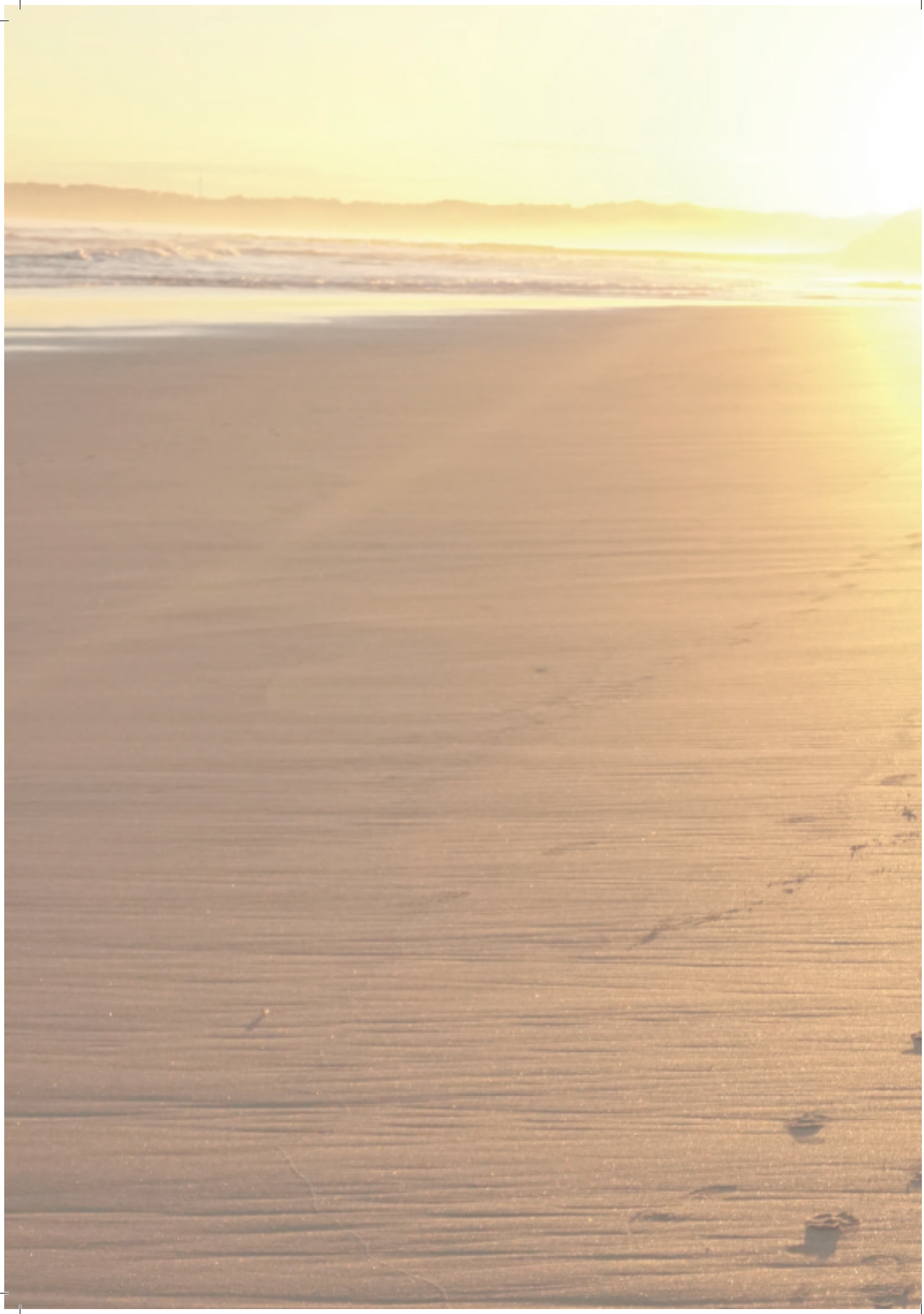

CHAPTER 2

Repetitive versus standard tactile stimulation of preterm infants at birth: a randomized controlled trial

J Dekker
SB Hooper
T Martherus
SJE Cramer
N van Geloven
AB te Pas

Resuscitation 2018;127:37-43

ABSTRACT

AIM

To evaluate the direct effect of repetitive tactile stimulation on breathing effort of preterm infants at birth.

METHODS

This randomized controlled trial compared the effect of repetitive stimulation on respiratory effort during the first 4 minutes after birth with standard stimulation based on clinical indication in preterm infants with a gestational age of 27 – 32 weeks. All details of the stimulation performed were noted. The main study parameter measured was respiratory minute volume, other study parameters assessed measures of respiratory effort; tidal volumes, rate of rise to maximum tidal volumes, percentage of recruitment breaths, and oxygenation of the infant.

RESULTS

There was no significant difference in respiratory minute volume in the repetitive stimulation group when compared to the standard group. Oxygen saturation was significantly higher (87.6 ± 3.3% vs 81.7 ± 8.7%, p=0.01) while the level of FiO_2 given during transport to the NICU was lower (0.28 (0.23 – 0.35) vs 0.34 (0.29 – 0.44), p=0.04). There was no significant difference in administration of positive pressure ventilation (52% vs 78%, p=0.13), or the duration of ventilation (median (IQR) time 8 (0 – 118) s vs 35 (13 – 131) s, p=0.23). Caregivers decided less often to administer caffeine in the delivery room to stimulate breathing in the repetitive stimulation group (10% vs 39%, p=0.036).

CONCLUSION

Although the increase in respiratory effort during repetitive stimulation did not reach significance, oxygenation significantly improved with a lower level of FiO_2 at transport to the NICU. Repetitive tactile stimulation could be of added value to improve breathing effort at birth.

INTRODUCTION AND RATIONALE

Most preterm infants need respiratory support for lung aeration during transition at birth.(1-3) In order to avoid injury to the still developing lungs and brain, the focus of respiratory support at birth has shifted from intubation and mechanical ventilation towards non-invasive ventilation.(4-7) However, there is still a high failure rate of continuous positive airway pressure (CPAP) in preterm infants after birth(4), which requires the infant to breath effectively. Studies have shown that most preterm infants breathe at birth(3), even during and in between positive pressure ventilation (PPV)(1, 2), but in the majority of infants their respiratory drive is weak and insufficient to aerate their lungs. Little attention has focussed on strategies that stimulate breathing during the immediate new-born period. Stimulating preterm infants to increase their respiratory effort could enhance the efficacy of CPAP support and might reduce the risk of CPAP failure. Thereby, it has been shown that preterm infants in whom mechanical ventilation is avoided after birth have better lung mechanics and decreased work of breathing at 8 weeks post-term.(8)

Currently, tactile maneuvers (warming, drying and rubbing the back or the soles of the feet) to stimulate breathing are recommended during the initial assessment of the infant at birth.(9, 10) This recommendation is largely based on many years of experience and expert opinion as at the time the recommendations were published, there were no studies available that specifically examined the effect of tactile stimulation of infants at birth. Only experimental studies, in apneic lambs and newborn rats after birth, have demonstrated increased spontaneous breathing after tactile stimulation.(11, 12)

We recently reported our current practice in tactile stimulation by reviewing previously recorded video and physiological parameters made in the delivery room. In this study we observed that duration and method of stimulation in preterm infants were variable and often not performed despite being clearly indicated.(13) Frequent omission of stimulation of infants at birth was also found by Gaertner et al.(14) Omitting stimulation could be related to the use of polyethylene wraps and the use of more extensive respiratory support.(14-16) The observed variation could be explained by the fact that the guidelines are not clear on how the stimulation should be used, possibly due to scarcity of data on this topic.

Our aim was to test the effect of systematically applied tactile stimulation at birth in a randomized controlled trial. We demonstrated in a recent observational study(13) that only 67% of preterm infants were stimulated and an effect could be observed in 18% of

stimulation episodes. However, it is difficult to interpret this result as we also observed that in 1/3 of episodes stimulation was not given while there was no indication and in 1/3 of infants, stimulation was not given while this was indicated. Colleagues of the neonatal team were still very reluctant to not stimulate infants as tactile stimulation is one of the most basic interventions during neonatal resuscitation. It would be therefore difficult to achieve clinical equipoise for a study in which tactile stimulation was compared to the omission of stimulation. In this recent observational study, we determined that stimulation could be applied either repetitively or when needed based on clinical indication.(13) It is possible that repetitive stimulation could improve the infant's breathing effort, as continuous stimulation might result in habituation.(17) We aimed to evaluate the direct effect of a standardized repetitive tactile stimulation on breathing effort of preterm infants at birth compared to standard stimulation based on clinical indication.

METHODS

A single blinded randomized controlled trial was conducted at the Leiden University Medical Center. Preterm infants with a gestational age between 27 and 32 weeks were included. Infants with congenital abnormalities or conditions that might have an adverse effect on breathing effort or ventilation were excluded. Infants were randomized using sequentially numbered opaque sealed envelopes to either be stimulated repetitively or to receive standard stimulation after birth. Allocation was stratified by gestational age (27^{+0} – 28^{+6} weeks vs 29^{+0} – 31^{+6} weeks), using variable block (4 – 6) sizes.

The interventions consisted of repetitive stimulation in the first 4 minutes after birth, defined as gently rubbing the back or the soles of the feet during 10 s, alternated with 10 s of rest. We have demonstrated in an observational study that stimulation was applied either repetitively or based on clinical indication.(13) For this reason we compared repetitive stimulation with standard stimulation, in conformation with the World Health Organization guidelines: gently rubbing the back or the soles of the feet when clinicians considered the breathing to be insufficient or absent.(7) After 4 minutes after birth, both groups received similar treatment, and stimulation was performed on discretion of the caregiver. The intervention was performed solely in the first 4 minutes after birth, as most preterm infants have a stable breathing pattern after this time interval.(18)

In both groups standard care was provided in the delivery room. Local resuscitation guidelines were followed using a Neopuff™ T-Piece Resuscitator, including delayed

cord clamping of 30 s, oxygen administration to target oxygen saturations based on the international normograms(19), and caffeine administration in the delivery room based on discretion of the caregiver, as previously described in the study of Dekker et al.(20). In both groups, physiological parameters, including respiratory function monitoring (RFM) were recorded during stabilization in the first 10 minutes of life. This RFM uses a small (dead space 1 mL) variable orifice anemometer to measure gas flow in and out of a face mask, thereby calculating inflation pressures, flow and tidal volumes. The difference between inspired and expired tidal volume equals the leak from the face mask. The minute volume (MV), rate of rise to maximum tidal volumes (RoR) and time of positive pressure ventilation (PPV) given were calculated. Oxygenation and heart rate were measured with the Masimo SET pulse oximeter. A pulse oximetry probe was placed around the ulnar aspect of the infant's right wrist. Fraction of inspired oxygen (FiO_2) was measured with a Teledyne oxygen analyzer inserted into the inspiratory limb of the Neopuff™ circuit. All signals measured were recorded at 200 Hz using the New Life Box physiological recording system with Polybench software (Advanced Life Diagnostics, Weener, Germany). Pulmochart software (Advanced Life Diagnostics, Weener, Germany) was used for analysing recorded data.

The main study parameter was the average MV at 1 – 4 minutes after birth. Other respiratory effort parameters which were blindly assessed, were: MV in the first 7 minutes after birth, tidal volumes, RoR, respiratory rate, percentage of tidal volumes > 4 mL/kg or 8 mL/kg (recruitment breaths), respiratory support given (CPAP vs PPV) and caffeine administration in the delivery room. Time of PPV, oxygen delivery, oxygen saturation and heart rate were also compared between groups. The following demographic data were collected: gestational age (GA), birth weight, gender, Apgar score, antenatal use of corticosteroids, mode of delivery. Short-term clinical outcomes were noted: intraventricular hemorrhage, intubation during resuscitation or within the first 24 hours after birth and need for surfactant.

Mian et al.(21) measured an average MV of 150 ± 70 mL/kg/min over the first 100 breaths in preterm infants < 33 weeks GA. The study of Huberts et al.(18) shows that MV in spontaneous breathing preterm infants increases with 60% from minute 2 to minute 5, but this increase was lower in infants receiving PPV. We therefore considered an increase of 40% in average MV at 1 – 4 minutes clinically relevant, for this a sample size of 44 infants would be needed (α of 0.05 and power (1 – β) of 0.8, 2-sided t-test).

The ethical committee of the LUMC approved the study protocol. Informed parental consent was obtained antenatally when possible. Consent was asked retrospectively in

case of an emergency situation (e.g. mother in full labour or when immediate delivery was necessary) or when obtaining antenatal consent was inappropriate (e.g. if the condition of the mother did not allow for proper consideration on participation). This study was registered in www.trialregister.nl, with registration number NTR6021.

Statistical analysis was performed with SPSS software version 23.0 (SPSS, Chicago, Illinois). The parameters of both groups were assessed for normality using the Kolmogorov-Smirnov and Shapiro-Wilkinson test. Demographics of the repetitive and standard stimulation groups were compared by χ^2 test for categorical variables, Student's t-test for normally distributed data, or Mann-Whitney U test for non-normally distributed data. Parameters of respiratory effort were compared between the groups over time using a linear mixed-effect regression model after appropriate transformation to meet the normality assumption, in which the time after birth, stratification group and randomization group were entered. We allowed for interaction between time and randomization group. Study parameters that were only assessed once over time were compared by a two-way factorial ANOVA, including both the randomization and stratification group. Categorical outcomes were assessed by Fisher's exact test.

For the calculation of parameters of respiratory effort, tidal volumes during inspiration were used, as this is the active part of a breath and represents effort, expired tidal volumes represent expiration and are passive, which does not completely represent respiratory effort. However, when there is mask leak, inspired tidal volumes cannot be used; the best approximation to measure effort is then only expired tidal volume. Whenever there was a mask leakage of 100%, MV was calculated for that minute using only tidal volumes recorded without 100% mask leakage, and dividing it by the number of seconds for which data was present. Calculations of the other variables were made based on available data. Two-sided p-values <0.05 were considered statistically significant.

RESULTS

A total of 75 eligible infants were born in the LUMC between September 2016 and April 2017. During this period 24 infants could not be included due to inclusion in other studies, antenatal consent was refused or due to logistical reasons. Thus, 51 infants were randomized to receive either repetitive or standard stimulation, but 7 infants were excluded from the analysis because there was no retrospective consent, there was no recording obtained or the recording could not be analyzed because of persistent 100% leak (Figure 1).

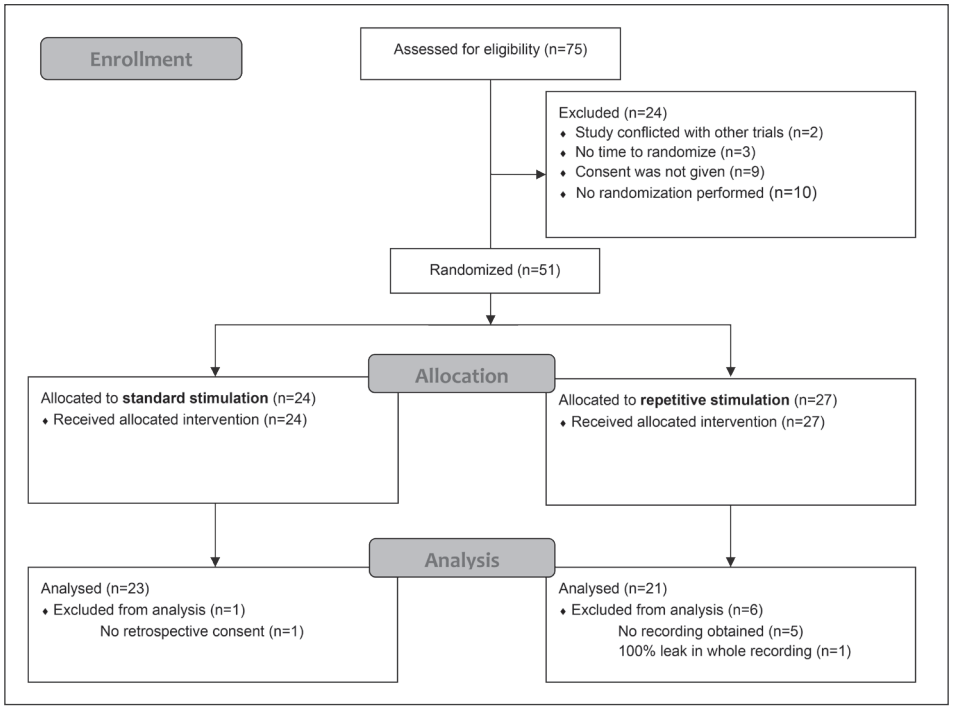

Figure 1 | CONSORT flow diagram

There were no significant differences between the repetitive and standard stimulation groups with regard to GA, birth weight, gender, Apgar score at 1, 5 and 10 minutes, mode of delivery, and full dose of antenatal steroids (Table 1).

Table 1 | Demographical data

	Standard stimulation n = 23	Repetitive stimulation n = 21	*p*-value
Gestational age (weeks)[a]	29^{+0} (27^{+5} – 31^{+0})	29^{+5} (28^{+1} – 30^{+6})	0.487
Gender (% male)[b]	14/23 (61%)	11/21 (52%)	0.570
Birth weight (grams)[a]	1252 (1050 – 1388)	1350 (1073 – 1580)	0.597
Apgar score at 1 minutes[c]	6 ± 3	6 ± 2	0.897
Apgar score at 5 minutes[a]	8 (7 – 9)	8 (7 – 9)	0.655
Apgar score at 10 minutes[a]	9 (8 – 9)	9 (9 – 9)	0.492
Mode of delivery (% caesarean section)[b]	17/23 (74%)	12/21 (57%)	0.241
Antenatal corticosteroids (% full course)[b]	14/23 (61%)	13/21 (62%)	0.944

Data are presented as median (IQR) for non-parametric data ([a]), n (%) for categorical data ([b]) and mean ± SD for parametric data ([c])

Stimulation performed

At least one tactile stimulation episode was performed in all infants in the repetitive stimulation group and in 22/23 (96%) infants in the standard stimulation group. Infants in the repetitive stimulation group were stimulated significantly more often (8 (7 – 10) vs 3 (3 – 6) episodes, p<0.001), although there were no significant differences in starting time, episode duration, or total stimulation time (Table 2). According to the study protocol, the standard stimulation group received significantly more episodes of stimulation based on clinical indication (46% vs 63%, p=0.009) (Table 2).

Table 2 | Stimulation performed

	Standard stimulation n = 23	Repetitive stimulation n = 21	*p*-value
Stimulation performed (%)[a]	22/23 (96%)	21/21 (100%)	1.000
Time stimulation started (s after birth)[b]	74.5 ± 42.9	71.3 ± 34.1	0.794
Duration of stimulation episodes (s)[c]	12 (4 – 24)	10 (9 – 11)	0.076
Total stimulation time (s)[c]	59 (24 – 120)	86 (63 – 105)	0.388
Number of stimulation episodes[c]	3 (3 – 6)	8 (7 – 10)	<0.001
Type of stimulation[a]			0.271
Rubbing the back	4/92 (4%)	2/168 (1%)	
Rubbing soles of the feet	84/92 (91%)	160/168 (95%)	
Both	4/92 (4%)	6/168 (4%)	
Clinical indication for stimulation[a]	57/91 (63%)	76/166 (46%)	0.009
Type of indication[a]			0.048
Bradycardia	3/57 (5%)	4/76 (5%)	
Apnea	14/57 (25%)	36/76 (47%)	
Hypoxia	15/57 (26%)	11/76 (15%)	
Combination	25/57 (44%)	25/76 (33%)	

Data are presented as n (%) for categorical data ([a]), mean ± SD for parametric data ([b]) and median (IQR) for non-parametric data ([c]).

Effect of tactile stimulation on breathing effort

There was no significant difference in the median (IQR) MV at minutes 1 – 4 and 1 – 7 (1 – 4 minutes: 51.5 (5.3 – 114.2) mL/kg vs 69.2 (11.5 – 153.9) mL/kg, p=0.439, 1 – 7 minutes: 89.6 (21.4 – 141.7) mL/kg vs 110.5 (42.3 – 166.8) mL/kg, p=0.324). In addition, there were no significant differences in respiratory rate, tidal volume and RoR of spontaneous breaths on CPAP in the repetitive stimulation group when compared to the standard stimulation group during the intervention (1 – 4 minutes) and during the total period (1 – 7 minutes) (Table 3, Figure 2). The period of mask ventilation did not significantly differ between the groups either (median (IQR) time 8 (0 – 118) s vs 35 (13 – 131) s, p=0.231). There were no differences in percentage of tidal volumes > 4 mL/kg or > 8 mL/kg (Table 3).

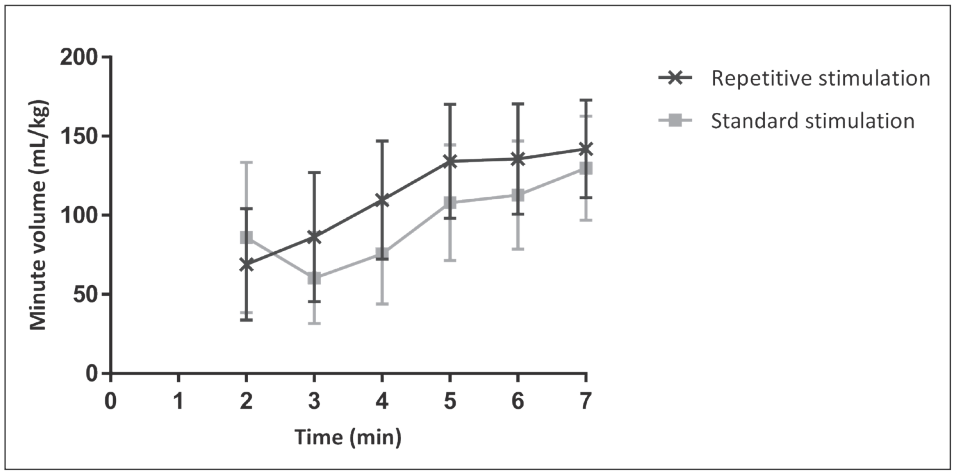

Figure 2 | Average minute volume of spontaneous breaths (observed means)

There were no significant differences between the groups in pulse rate but the average oxygen saturation was significantly higher in the repetitive stimulation group (87.6 ± 3.3% vs 81.7 ± 8.7%, p=0.007), while there was no significant difference in the FiO_2 given at each minute (p=0.121) (Figure 3). While the maximum FiO_2 during stabilization was not different, the requirement for a lower FiO_2 extended well beyond the 7 minutes stabilization period. This was reflected by a significantly lower FiO_2 requirement at the time of transport to the NICU in the repetitive stimulation group (0.28 (0.23 – 0.35) vs

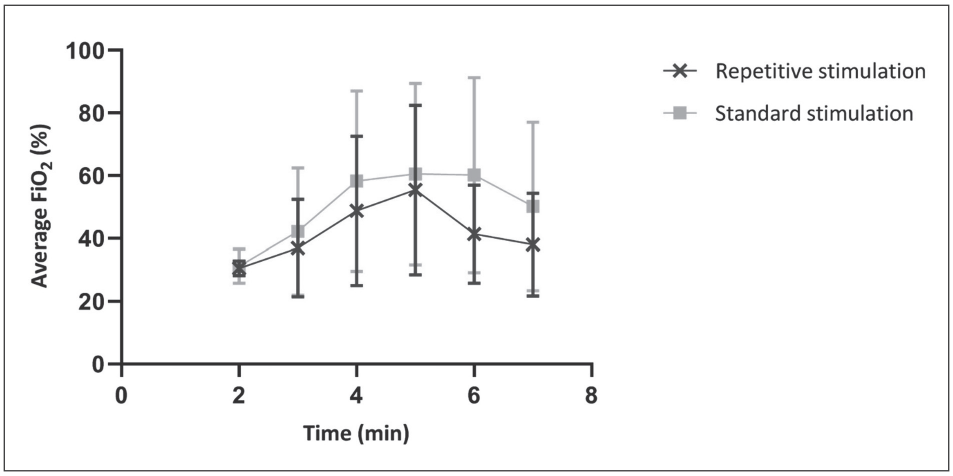

Figure 3 | Average FiO_2 over time

0.34 (0.29 – 0.44), p=0.036), while there was no difference in the time at which infants were transported (Table 3). None of the infants were intubated in the delivery room. There was no significant difference in administration of PPV (48% vs 22%, p=0.130) or the duration of PPV administered (16 (0 – 118) s vs 35 (13 – 131) s, p=0.231). Caregivers decided less often to administer caffeine in the delivery room to stimulate breathing in the repetitive stimulation group (10% vs 39%, p=0.036) (Table 3). Caffeine was administered at a mean ± SD time point of 06:25 ± 01:13 minutes after birth.

Effect of tactile stimulation on clinical outcomes

There were no significant differences in incidence of intraventricular hemorrhage (IVH) or administration of surfactant during NICU admission (Table 3).

Table 3 | Effect of tactile stimulation on breathing effort

	Standard stimulation n = 23	Repetitive stimulation n = 21	*p*-value
Tidal volume at 1 – 4 minutes after birth (mL/kg)[a]	2.7 (1.0 – 5.7)	3.6 (1.7 – 6.3)	0.131
Tidal volume at 1 – 7 minutes after birth (mL/kg)[a]	2.9 (1.3 – 5.4)	3.7 (2.1 – 5.9)	0.134
Rate of rise to maximum tidal volumes at 1 – 4 minutes after birth (mL/kg/s)[a]	7.4 (3.7 – 13.6)	10.3 (4.5 – 19.3)	0.213
Rate of rise to maximum tidal volumes at 1 – 7 minutes after birth (mL/kg/s)[a]	8.4 (4.5 – 14.1)	10.8 (6.0 – 17.5)	0.219
Respiratory rate/min at 1 – 4 minutes after birth[a]	23 (7 – 36)	24 (8 – 45)	0.795
Respiratory rate/min at 1 – 7 minutes after birth[b]	32 ± 19	35 ± 19	0.627
Oxygen saturation (%)[b]	81.7 ± 8.7	87.6 ± 3.3	0.007
Pulse rate[b]	143 (133 – 150)	138 (133 – 151)	0.581
Percentage of tidal volumes > 4 mL/kg (%)[b]	39.7 ± 21.2	47.1 ± 25.0	0.315
Percentage of tidal volumes > 8 mL/kg (%)[b]	5.0 (2.0 – 14.0)	6.0 (1.5 – 22.5)	0.673
Time of mask ventilation (s)[b]	35 (13 – 131)	16 (0 – 118)	0.231
Maximum FiO_2 during resuscitation[b]	0.93 (0.49 – 1.0)	0.62 (0.35 – 0.99)	0.110
Time at which infant is transported to NICU (min:sec)[b]	15:06 (10:34 – 19:09)	12:32 (9:24 – 16:21)	0.258
FiO_2 at start of transport to NICU[b]	0.34 (0.29 – 0.44)	0.28 (0.23 – 0.35)	0.036
Caffeine administered during stabilization at birth[c]	9/23 (39%)	2/21 (10%)	0.036
Time after birth of caffeine administration (min)[b]	06:32 (05:25 – 07:46)	06:00 (04:10 – 06:00)	0.661
Respiratory support after birth (% CPAP only)[c]	5/23 (22%)	10/21 (48%)	0.112
Intraventricular hemorrhage ≥ grade 3[c]	0/23 (0%)	1/21 (5%)	0.477
Surfactant administration at NICU[c]	4/23 (17%)	5/21 (24%)	0.716

Data is presented as median (IQR) or mean ± SD of the raw data; p-values are presented of the linear mixed model (a). Data is presented as median (IQR) or mean ± SD of the raw data; p-values are presented of the two-way factorial ANOVA (b). Data is presented as n (%) or categorical data, p-values are presented of the Fisher's exact test (c).

DISCUSSION

This randomized trial is the first study quantifying the respiratory minute volume as a direct effect of tactile stimulation. Tactile stimulation to stimulate breathing of infants at birth has been common practice before living memory and has been recommended even before the first version of the resuscitation guidelines.(22) Sharing the observations of the retrospective study and the lack of clinical equipoise in the neonatal team in "not to stimulate" could have resulted in bias prior to the start of the trial. For this reason, we tested the effect of tactile stimulation by comparing standard stimulation, which is dependent upon discretion of the caregiver, with an unambiguous protocol of repetitive stimulation. Performing a trial of this nature could have influenced the caregiver's performance in all patients (Hawthorne effect). We considered this to be unavoidable and for this reason we noted all details of the tactile stimulation performed.

While there was no significant difference in MVs, tidal volumes and RoR, all respiratory effort values were increased in the repetitive stimulation group and thus all point in the same direction. Therefore, although no significant statistical differences were observed, the findings might be clinically relevant. This is also expressed by a significant increase in oxygen saturations. Also, fewer infants in the repetitive stimulation group received caffeine at birth when compared to the standard stimulation group. As caffeine was administered at the discretion of the caregiver, this indicates that infants in the repetitive stimulation group were assessed to have a better respiratory effort. These results indicate that repetitive tactile stimulation at birth has value in improving breathing efforts in preterm infants at birth and should not be overlooked as an important contributor to the provision of respiratory support in the delivery room.

Respiratory care at birth has shifted towards non-invasive ventilation and the need to avoid intubation and mechanical ventilation.(2, 5, 23, 24) However, it has become clear that mask ventilation is often inadequate and ineffective(1) and that the larynx is mostly closed at birth in apneic newborns and only opens during a spontaneous breath, which is demonstrated in newborn sheep.(25) The larynx must be open to ventilate the lung non-invasively, and the best way to do so is to stimulate breathing. We recently demonstrated that caffeine at birth increases respiratory effort(20) and we now observed similar positive effects with tactile stimulation. While we were unable to achieve statistical significance in the respiratory parameters examined, the higher oxygenation state indicates that respiratory function was substantially better in the repetitive stimulation group.

Although the effect of tactile stimulation has been described previously in animal models(11, 12), the effect in human preterm infants has only just recently gained attention.(13, 14) In these studies, the incidence of stimulation was much lower than the high incidence of stimulation we observed in both stimulation groups in this trial. Initiating this trial therefore substantially increased awareness on and the incidence of tactile stimulation. Indeed, while infants in the standard stimulation group would normally only be stimulated when there was a clinical indication, we found that only 63% of stimulations were clinically indicated. This could have resulted in reducing the difference in primary outcome between the two groups. As a result, together with a higher than expected between patient variability in the primary outcome, a larger sample size would be needed to demonstrate statistical differences between the groups. Nevertheless, we demonstrated significant differences in oxygenation between the groups, indicating that the non-statistical differences in respiratory function between the two groups were biologically significant.

The most frequently used method of stimulation in this randomized trial was rubbing the soles of the feet, thereby activating proprioceptors which are known to reduce breathing pauses in preterm infants with apnea of prematurity(26) and increase the frequency of breathing.(27) In contrast, in the study of Gaertner et al.(14), infants were more often stimulated by chest or back rub, and they noted a larger increase in crying, compared to stimulation by rubbing the soles of the feet. Stimulation by chest or back rub might affect the respiratory center via somatic or visceral mechanoreceptors in the thorax region.(28, 29) However, Binks et al.(30) showed that this could also inhibit inspiration, as vibration of the thoracic surface could also excite intrapulmonary receptors and suggesting that the lung volume is already increased. More data is needed on the effect of different stimulation locations to inform caregivers in what is the best and most effective way of stimulating preterm infants to enhance respiratory efforts. Although we do not introduce a new intervention, merely a repetition, caution should be taken to ensure the stimulation is performed gently and with care.

CONCLUSION

In this randomized trial, there were no differences in respiratory parameters between the repetitive tactile stimulation and standard tactile stimulation groups. Nevertheless, tactile stimulation overall improved oxygenation with a lower maximum FiO_2 level, lower level of FiO_2 at transport to the NICU and the need for less post transport caffeine administration. This suggests tactile stimulation improves respiration function. Future larger studies are required to confirm this finding and identify the best method for tactile stimulation.

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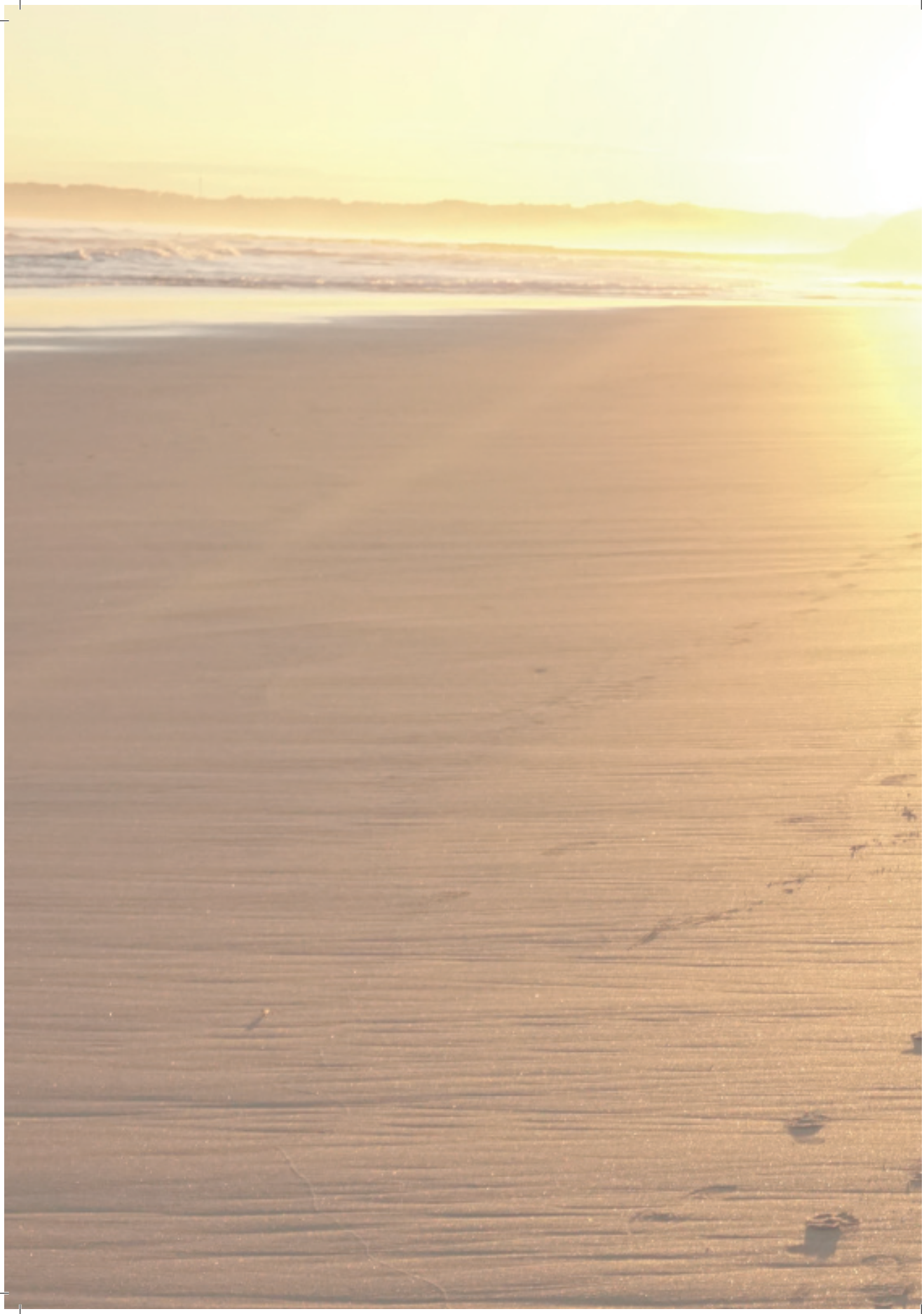

CHAPTER 3

Increasing respiratory effort with 100% oxygen during resuscitation of preterm rabbits at birth

J Dekker
M Croughan
KJ Crossley
MJ Wallace
EV McGillick
MJ Kitchen
P DeKoninck
M Thio
T Martherus
G Ruben
CC Roehr
SJE Cramer
A Flemmer
L Croton
AB te Pas
SB Hooper

Manuscript submitted

ABSTRACT

BACKGROUND

Spontaneous breathing is essential for the success of non-invasive respiratory support at birth. As hypoxia is a potent inhibitor of spontaneous breathing, using a high O_2 concentration at birth might lead to higher respiratory effort.

METHODS

Preterm rabbits (29 days gestation, term ~32 days) received continuous positive airway pressure containing either 21% O_2 or 100% O_2, based on randomization. If apnea occurred, positive pressure ventilation with either 21% O_2 or 100% O_2 was applied. Respiratory rate and variability in inter-breath interval were measured using an oesophageal tube, functional residual capacity was deduced of phase-contrast X-ray images.

RESULTS

Apnea occurred more frequently in kittens who started breathing in 21% O_2 compared to 100% O_2 (11/12 vs 1/8, p=0.001). Kittens starting in 21% O_2 had a significantly higher inter-breath interval at the start of the experiment and after rescue from apnea (start: 68.5 ± 11.9% vs 40.1 ± 4.2%, p=0.042, after rescue: 83.6 ± 32.2% vs 9.5 ± 1.3%; p=0.014). Similarly, respiratory rate/minute was significantly lower in the 21% O_2 group at both timepoints. FRC values did not differ between study groups at both timepoints.

CONCLUSION

Initiating resuscitation with a high O_2 concentration might result in increased respiratory effort thereby positively influencing respiratory transition at birth.

INTRODUCTION AND RATIONALE

Most preterm infants breathe at birth, but struggle to achieve sufficient pulmonary gas exchange due to a weak respiratory drive, low respiratory muscle strength and immaturity of the lung.(1-5) As a result, non-invasive respiratory strategies are commonly used to assist preterm infants, which mostly focus on supporting the mechanics of breathing. For instance, continuous positive airway pressure (CPAP) is applied to support the infant's breathing efforts, or if these are absent (i.e. during apnea), positive pressure ventilation (PPV) is used to replace them.(6, 7) However, several studies have demonstrated that non-invasive ventilation using a face mask is often inadequate due to mask leakage or airway obstruction.(1, 2) In particular, it has recently been demonstrated that larynx adduction can prevent air from entering the lung during non-invasive mask ventilation. (8, 9) Larynx adduction during apnea plays an important role in lung growth and development before birth, a process which persists well after birth.(1, 2, 8, 10) Thus, as the larynx needs to be open after birth to allow lung aeration, spontaneous breathing is now considered essential for the success of non-invasively applied respiratory support in very preterm infants. Indeed, several studies have now shown that lung aeration only occurs when newborns are breathing spontaneously.(2, 9)

It is now becoming increasingly clear that non-invasive respiratory strategies should not only focus on supporting the mechanics of breathing, but should also focus on stimulating spontaneous breathing. Spontaneous breathing can be stimulated by a number of mechanisms, including tactile stimulation, CPAP and caffeine.(11-17) However, as hypoxia is a potent inhibitor of spontaneous breathing, these interventions may not be sufficient to stabilize infants after birth when using an inspired gas with low oxygen concentrations. Historically, 100% O_2 was used to resuscitate preterm infants without titrating the inspired oxygen content or consistently monitoring oxygen saturation (SpO_2) levels.(4, 18) However, this practice changed when it became clear that hyperoxia can be toxic to the lungs(19, 20) and instead, current guidelines are now heavily focused on prevention of hyperoxia. This led to the recommendation of starting resuscitation with a low O_2 concentration, and then titrating based on the infants SpO_2. (21-24) As a result, we now accept a longer duration of hypoxia after birth, without questioning the impact of this.

In very preterm infants, when hypoxia is present at 5 minutes after birth, it is associated with a lower heart rate, a higher mortality before hospital discharge and a higher risk of intraventricular hemorrhage.(25) In addition, it is well established that, in the foetus and newborn, hypoxia is a potent inhibitor of the respiratory center, causing a suppression

of breathing and adduction of the larynx.(26) Although this inhibitory effect of hypoxia gradually changes during the first few weeks after birth, it must be present at birth. (27) As a result, avoiding levels of hypoxia that inhibit breathing would appear to be an important component of any strategy designed to stimulate spontaneous breathing at birth. However, translating this knowledge into decisions as to what O_2 levels should be used to achieve an adequate oxygenation of the infant at birth, is extraordinarily complex. This is because the presence of airway liquid greatly reduces the surface area for gas exchange, necessitating the use of a higher partial pressure gradient for O_2 diffusion. However, as the surface area increases exponentially with lung aeration, the need for this higher partial pressure gradient dramatically decreases, necessitating a rapid reduction in the required O_2 concentration to avoid hyperoxia.

As partial lung aeration is common after birth in very preterm neonates, we hypothesized that the use of a high O_2 concentration at birth reduces the risk of a hypoxia-induced inhibition of breathing, leading to a higher respiratory rate and a more stable breathing pattern. Furthermore, we hypothesized that a more stable breathing pattern will lead to better aeration of the lung (measured as functional residual capacity; FRC), leading to a reduced need for O_2.

METHODS

All animal procedures were approved by the SPring-8 Animal Care and Monash University's Animal Ethics Committees. The study was conducted in experimental hutch 3 of beamline 20B2 in the Biomedical Imaging Center at the SPring-8 synchrotron, Japan.

Experimental procedure

Pregnant New Zealand White rabbits at 29 days gestation (term ~ 32 days) were sedated using propofol (8 mg/kg iv bolus, Rapinovet, Merck Animal Health), followed by a maintenance dose of 50 mg/kg/h. A 22G epidural catheter (BD 405254) was then inserted into the epidural space on the lower spine to administer 2% lignocaine (4 mg/kg) + 0.5% bupivacaine (1 mg/kg). After establishing a spinal anaesthesia, the propofol infusion ceased, but sedation continued with an infusion of butorphanol (0.5 mg/kg/h) and midazolam (1.0 mg/kg/h). The rabbit's reflexes, respiratory rate, oxygen saturation and heart rate were constantly monitored. A caesarean section was performed to deliver rabbit kittens one at a time. An oesophageal tube was inserted and a face mask applied before the umbilical cord was cut. The kittens were weighed and a dose of caffeine

base (20 mg/kg), naloxone (1 mg/kg) and anexate (10 µg/kg) were administered intraperitoneally. The kittens were placed laterally (right side) on a heated platform in the expected path of the X-ray beam. Electrocardiogram leads were attached and the face mask was connected to a custom-built mechanical ventilator(28) to apply CPAP. All kittens started on CPAP as means of respiratory support, commencing with a pressure of 15 cm H_2O, which was titrated down to 8 cm H_2O, with a rate of 2 cm/30 seconds. A gas blender attached to the ventilator was used to regulate the O_2 concentration. Kitten heart rate and face mask and oesophageal pressures were recorded using Labchart (Powerlab, ADInstruments, Sydney, Australia).

The experiment commenced when the kitten was attached to the equipment and was breathing spontaneously in order to determine the effect of the O_2 concentration on the breathing pattern. The experiment consisted of two phases. Kittens were initially divided into two groups and commenced breathing in either 21% O_2 or 100% O_2 (phase 1), with the breathing pattern being monitored. If kittens became apneic, a rescue intervention was performed with PPV using a ventilation rate of 60 breaths per minute, peak inflation pressure of 25 cmH_2O and PEEP of 8 cmH_2O. The O_2 concentration during the rescue ventilation depended on the group. Kittens that commenced in 21% O_2 received either 21% O_2 or 100% O_2, whereas kittens that commenced in 100% O_2 remained in 100% O_2. Whenever ventilation alone was not sufficient for regaining a stable breathing pattern, physical stimulation was applied. When breathing was restored, breathing pattern was again analyzed (phase 2). Kittens were randomly allocated prior to delivery for starting the experiment in 21% O_2 or 100% O_2, and for rescue intervention in case of apnea with 21% O_2 or 100% O_2 (Figure 1).

All animals were humanely euthanized at the end of the experiment using pentobarbitone sodium (> 100 mg/kg) administered intravenously in the doe and intraperitoneally in the kittens.

Phase-contrast X-ray imaging

High resolution phase-contrast X-ray imaging was used to measure lung gas volumes, as described previously by Kitchen et al.(29), using a Hamamatsu ORCA flash C11440-22C detector (effective pixel size 15.2 µm). A synchrotron source tuned to 24 keV was used, with the X-ray source-to-kitten distance was ~210 m, and the kitten-to-detector distance was 2 m.

Data analysis

Data on stability of spontaneous breathing during the total experiment and during both phases (at start of the experiment and after rescue intervention) were gathered and compared between the randomization groups. We aimed to compare the stability of breathing at phase 2 (after rescue intervention), but we could not predict if apnea would occur evenly in the randomization groups. We measured the timepoints at which apnea occurred and when a stable breathing pattern was regained, which was considered as the start of phase 2. The averages of these timepoints were considered as the start of phase 2 in the non-apneic kittens as well, in order to compare the breathing variability and breathing rate at phase 2 in all kittens.

Stability of breathing was assessed during the different phases of the experiment using respiratory rate and inter-breath interval variability from oesophageal pressure recordings. The timepoint of occurrence of apnea, duration of apnea and amount of rescue intervention needed (none/PPV/PPV and stimulation/could not be rescued) were also compared.

Imaging was used to determine the average FRC during phase 1 (stable breathing in 21% O_2 or 100% O_2) and during phase 2 (breathing pattern after rescue intervention with either 21% O_2 or 100% O_2). In addition, the differences between the FRC at the start and end of each phase were compared between the groups.

Statistical analysis

Statistical analysis was performed with SPSS software version 23.0 (SPSS, Chicago, Illinois, USA). When outcomes were assessed to be normally distributed, groups were compared using either a Student's t-test or one-way ANOVA. When the data were assessed as being non-parametric we used either a Mann-Whitney U test or Kruskal-Wallis test. Fisher's exact test was used to test categorical variables. Two-sided p-values <0.05 were considered statistically significant.

RESULTS

A total of 26 kittens were randomized to start the experiment in 21% O_2 or in 100% O_2 (Figure 1); 16 in the 21% O_2 group and 10 in the 100% O_2 group. 6 kittens were excluded before the experiment commenced because a stable breathing pattern was not reached, allowing the onset of imaging. There were no differences between randomization groups with regard to birth weight or caffeine dose administered (Table 1).

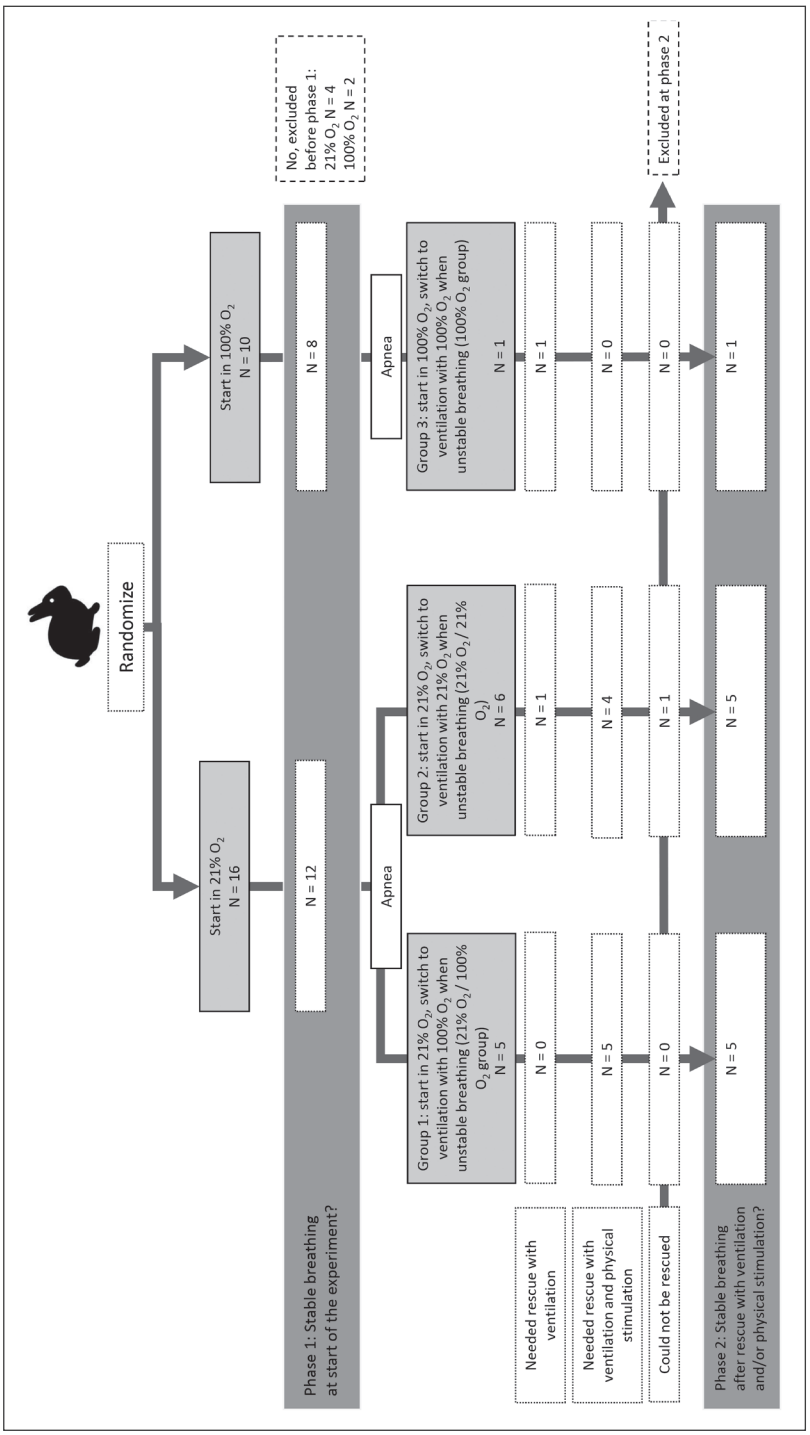

Figure 1 | Flow diagram of allocation of kittens

Table 1 | Demographics

	21% O_2 group n = 12	100% O_2 group n = 8	*p*-value
Birthweight (grams)	34.6 ± 4.5	31.8 ± 5.7	0.229
Caffeine dose (mg/kg)	18.7 ± 1.4	19.2 ± 2.9	0.593

Data is presented as mean ± SD.

Effect of tactile stimulation on breathing effort

Apnea

11/12 kittens who started breathing in 21% O_2 became apneic and needed rescue intervention, compared to 1/8 kittens in the 100% O_2 group (p=0.001). The time point (3.7 minutes) at which apnea occurred in the 1 kitten in the 100% O_2 group that became apneic was not different from the mean ± SD time that kittens started in 21% O_2 became apneic (3.2 ± 0.5 minutes, p=0.802).

Of 11 kittens that became apneic after starting the experiment in 21% O_2, 5 received rescue intervention with 100% O_2 and 6 were allocated to rescue intervention with 21% O_2 (Figure 1). Rescue intervention was started after kittens became apneic at a median (IQR) time of 2.8 (8.4 – 30.2) s. Additional physical stimulation was needed in 5/5 kittens who were rescued with 100% O_2 and 4/6 kittens who were rescued with 21% O_2. Rescue failed in 1 kitten that received 21% O_2 as the rescue treatment (Table 2). The duration of rescue intervention was comparable, whether this was performed with 21% O_2 or 100% O_2 (206.5 ± 31.7 s vs 186.2 ± 23.6 s).

The only kitten that became apneic after starting the experiment in 100% O_2, also received rescue intervention with 100% O_2. This rescue intervention started 58 seconds after initiation of apnea and consisted solely of PPV. After 91.6 seconds, the rescue intervention was discontinued as the kitten had regained a spontaneous breathing pattern.

Stability of breathing

During the total experiment, the median (IQR) respiratory rate was significantly different between all groups (21% O_2/100% O_2 group 42.7 (33.1 – 52.2) breaths/minute, 21% O_2/21% O_2 group 29.9 (22.0 – 37.2) breaths/minute, 100% O_2 group 46.4 (37.0 – 54.3) breaths/minute, p<0.001)(Figure 2, Figure 3).

Table 2 | Effect of tactile stimulation on breathing effort

	21%/100% O_2 group n = 5	21%/21% O_2 group n = 6	100% O_2 group N = 1	p-value
Duration of apnea (minutes)[a]	3.0 ± 0.8 (n = 5)	2.9 ± 0.6 (n = 5)	2.4 (n = 1)	0.725
Duration of rescue therapy (s)[a]	206.5 ± 31.7	186.2 ± 32.6	91.6	0.299
Rescue therapy[c]				0.106
PPV	0 (0%)	2 (33%)	1 (100%)	
PPV & physical stimulation	5 (100%)	3 (50%)	0 (0%)	
Could not be rescued	0 (0%)	1 (17%)	0 (0%)	
Variability of inter-breath interval (%)[a]	9.5 ± 1.3	83.6 ± 32.2	27.2 ± 6.1	0.014
Average respiratory rate/min[b]	43 (33 – 46)	27 (18 – 31)	45 (39 – 50)	0.007

Data is presented as mean ± SE (a), median (IQR) (b) or n (%) (c).

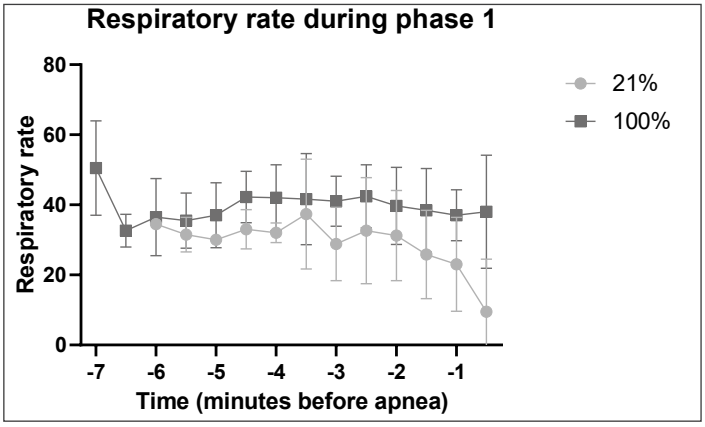

Figure 2 | Respiratory rate during phase 1 of the experiment

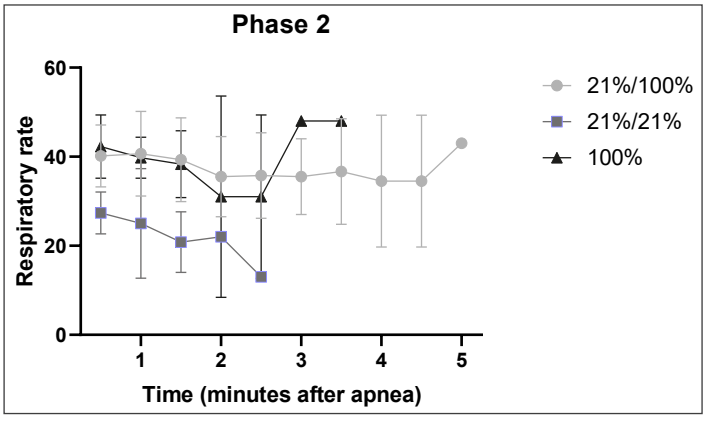

Figure 3 | Respiratory rate during phase 2 of the experiment

During phase 1 at the beginning of the experiment, the variability in inter-breath interval was significantly higher in kittens who started in 21% O_2 compared to kittens started in 100% O_2 (68.5 ± 11.9% vs 40.1 ± 4.2%, p=0.042). The average respiratory rate per minute was significantly lower in the 21% O_2 group (29 ± 4 vs 42 ± 3, p=0.038).

During phase 2 (following the restoration of breathing) the variability of inter-breath interval was markedly higher in kittens that received rescue intervention with 21% O_2 (21% O_2/21% O_2 group; 83.6 ± 32.2 %) vs kittens that were rescued with 100% O_2 (21% O_2/100% O_2 group; 9.5 ± 1.3 %; p=0.014) (Table 2). In addition, the average respiratory rate was significantly lower in kittens in the 21% O_2/21% O_2 group compared to kittens in the 100% O_2 group (27 (18 – 31) breaths/minute vs 45 (39 – 50) breaths/minute; p=0.007) (Table 2).

Imaging

At the start of the experiment (phase 1), FRC values were not different between the study groups (Table 3). Similarly, FRCs measured at the start and end of this phase did not significantly differ between study groups (p=0.876) (Figure 4).

Table 3 | FRC data phase 1

	21%/100% O_2 group n = 6	21%/21% O_2 group n = 6	100% O_2 group N = 8	*p*-value
Average FRC at start of experiment (phase 1) (mL/kg)	22.68 ± 2.35	21.87 ± 5.50	15.19 ± 10.40	0.381
FRC difference between start and end of phase (mL/kg)	0.81 ± 0.60	1.45 ± 1.62	0.50 ± 4.74	0.876

Data is presented as mean ± SD, p-values are reported of Kruskal-Wallis test.

In the kittens who started resuscitation in 21% O_2 and became apneic, FRC levels were again measured once they returned to a stable breathing pattern after the rescue intervention (phase 2). The average FRC during this phase did not differ between kittens receiving rescue intervention with either 21% O_2 or 100% O_2 (Table 4). Again, the difference between FRC at the start and end of this phase did not significantly differ between the study groups (p=0.915) (Table 4, Figure 4).

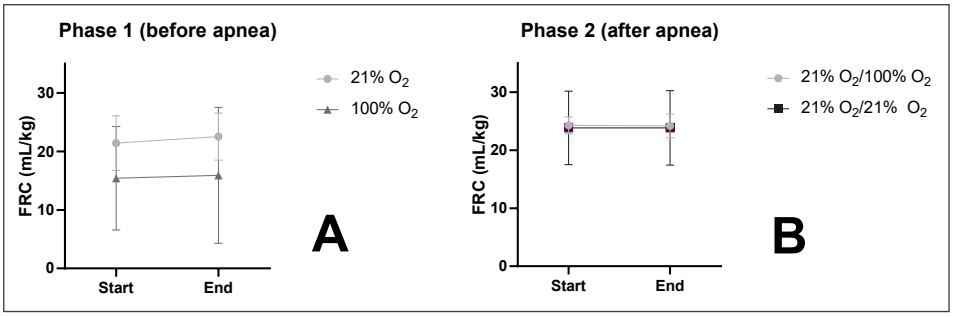

Figure 4 | FRC at start and end of phase 1

Table 4 | FRC data phase 2

	21%/100% O_2 group n = 6	21%/21% O_2 group n = 6	*p*-value
Average FRC after rescue intervention (phase 2) (mL/kg)	24.21 ± 1.64	23.87 ± 6.24	0.908
FRC difference between start and end of phase (mL/kg)	-0.08 ± 0.74	-0.02 ± 1.14	0.915

Data is presented as mean ± SD, p-values are reported of Student's t-test.

DISCUSSION

We have shown that initiating resuscitation of preterm kittens with 21% O_2 resulted in a more unstable breathing pattern (higher variability in inter-breath interval), a lower respiratory rate and a higher incidence of apnea compared to kittens starting resuscitation in 100% O_2. Furthermore, the kittens who were rescued with 21% O_2 after apnea, also had a higher inter-breath variability and lower respiratory rate than kittens rescued with 100% O_2. On the other hand, being resuscitated or rescued from apnea with 21% O_2 vs 100% O_2 did not significantly affect the degree of lung aeration as assessed by measuring FRC. These data indicate that oxygenation is the predominant factor determining the stability of breathing and the avoidance of apnea in the newborn immediately after birth. As the degree of lung aeration was similar between the two groups, despite a much higher respiratory activity, apparently respiratory activity alone is not the primary determinant of lung aeration during transition after birth.

It is well accepted that hypoxia is a potent inhibitor of breathing movements in the fetus and this inhibitory effect of hypoxia persists well into the newborn period. However, the application of this knowledge has been overwhelmed by concerns of hyperoxia and

because it is widely considered that PPV can replace spontaneous breathing if the infant is apneic. However, we now know that the presence of spontaneous breathing is essential for the success of non-invasive respiratory support at birth, as it ensures an open larynx. As a result, a focus on supporting breathing has become a priority. We found that resuscitation of preterm rabbits with 21% O_2 resulted in an unstable breathing pattern and a markedly lower breathing rate than resuscitation with 100% O_2. As partial lung aeration reduces the surface area for gas exchange, it is likely that breathing air was insufficient to sustain the oxygen needs of our preterm kittens, initially resulting in an unstable breathing pattern that eventually led to apnea in 11/12 kittens. However, increasing O_2 to 100%, increased the partial pressure gradient for oxygen diffusion, which likely overcame the surface area limitation, thereby increasing oxygenation levels. This was reflected by a greater breathing stability and a higher breathing rate, with only 1/8 kittens resuscitated with 100% oxygen becoming apneic.

Our study also showed that, following apnea, kittens rescued with 100% O_2 had a more stable breathing pattern (lower inter-breath variability) and a higher respiratory rate than kittens rescued with 21% O_2. These findings are consistent with those of van Vonderen et al.(30), who showed a similar increase in respiratory effort in preterm infants after O_2 was increased from 21% to 100%. By increasing the fraction of inspired oxygen, the level of hypoxia was reduced, thereby increasing breathing stability. These findings are consistent with the concept that hypoxia at birth leads to respiratory instability and/or suppression, which compromises lung aeration and causes the larynx to open only during a breath.(9) This in turn leads to persisting hypoxia and the onset of a vicious cycle that opposes successful transition and necessitates escalated interventions that increase the risk on adverse outcomes. This scenario is consistent with the findings of Oei et al.(25), who showed that initiating resuscitation with a lower O_2 concentration (< 30%) resulted in a higher risk of maintaining a SpO_2 < 80% at 5 minutes after birth. This in turn was associated with an increased risk of intraventricular hemorrhage, neurodevelopmental impairment or death.(25, 31)

We found that FRC was similar in all groups, indicating that the degree of lung aeration is not necessarily linked to the level of breathing activity in preterm newborns as we hypothesized. However, this study is limited by the difference in time after birth at which imaging commenced, which in turn is used to measure FRC. As the kittens are very fragile, providing them with medication, an oesophageal tube, a face mask and attaching them to the ventilator is a delicate process, which can vary the delay between birth and imaging onset. However, while this delay is not well defined in our study, human preterm infants likely experience similar variations in the delay between birth

and resuscitation onset. Indeed, the start of resuscitation depends on infant vitality and the moment of cord clamping. Nevertheless, we predicted that increased breathing activity would be associated with higher FRC levels, but the two were clearly unrelated in this study. We have previously demonstrated that the pressure gradients generated by inspiration are responsible for a large proportion (>95%) of lung aeration (airway liquid clearance) in near term rabbit kittens. As a result, an FRC of ~15 mL/kg can rapidly (3 - 5 breaths) accumulate in the absence of any end-expiratory pressure. However, the inability of some preterm kittens to accumulate an appropriate FRC, despite ongoing spontaneous breathing, indicates that in preterm neonates, spontaneous breathing by itself is insufficient to facilitate lung aeration. Other interventions to improve aeration could possibly comprise higher pressure levels of non-invasive respiratory support, although this has not yet been confirmed.

An observational study investigating SpO_2 and heart rate changes at birth demonstrated that, using current guidelines, infants ≥ 25 weeks of gestation had values similar to the reference ranges reported by Dawson et al.(24, 32) On the other hand, using current resuscitation guidelines, infants < 25 weeks of gestation had lower SpO_2 values and a higher incidence of bradycardia, despite all efforts to improve their clinical situation.(32) However, an oxygen titration protocol was used that commenced with a O_2 concentration of 30% and incorporated O_2 increments of 10 – 20% every 30 - 60 seconds. This incremental increase in oxygenation was likely insufficient to overcome the limitation associated with a reduced surface area for gas exchange, leading to progressive hypoxia and a suppression of breathing. In addition, it has been recently shown that, when using a T-piece ventilator, there is a delay apparent between the moment of titration of oxygen at the oxygen blender and the moment at which the infant receives the oxygen at the distal part of the circuit.(33) It is still not clear which titrating protocol results in optimizing oxygenation directly at birth. However, the current study suggests that initiating resuscitation with a higher O_2 concentration might lead to better oxygenation, thereby reducing the hypoxia-inhibiting effect on breathing which might result in better aeration.

This study is also limited by our inability to accurately measure the level of oxygenation in our preterm kittens. While we administered 21% O_2 or 100% O_2, we do not know what PaO_2 and oxygen saturation levels were achieved and, therefore, to what extent oxygenation of the kittens influenced the results. It is clear that hyperoxia is detrimental for preterm infants as it increases free radical production and can overwhelm their immature antioxidant capacity, causing organ damage.(34-36) On the other hand, we have now shown that using too little oxygen leads to breathing instability, a lower

breathing rate and a high risk of apnea that will necessitate a rapid escalation in the level and type of intervention. Thus, while the administration of 100% O_2 has been actively discouraged for the last few years (since 2010), it is time to question whether this is appropriate. Indeed, with appropriate monitoring the risk of hyperoxia can be minimized by titrating the O_2 concentration to keep SpO_2 values within defined ranges, bearing in mind that the required O_2 concentration depends upon the gas exchange surface area. However, the optimal oxygen saturation range that we should aim for is not clear in those extreme preterm infants, as the internationally recommended target ranges are based on healthy term and preterm infants not requiring any resuscitation.(24) Trials are underway to test the effect of initiating resuscitation with O_2 concentrations of 100% on breathing effort, while avoiding both hypoxia and hyperoxia, as well as determining the optimal oxygen saturation target range for extreme preterm infants.

CONCLUSION

Administration of 100% O_2 to preterm rabbit kittens at birth increased both the stability of breathing and respiratory rate when given immediately after birth or as a rescue treatment following apnea. The results of this study provide evidence that initiating resuscitation with a high O_2 concentration might result in an increase of respiratory effort thereby positively influencing respiratory transition at birth. However, further studies in human preterm infants are mandatory to confirm the effect of 100% O_2 on respiratory effort and oxygenation. In addition, it needs to be demonstrated to what extent hyperoxia can be avoided after initiation of resuscitation with 100% oxygen, using a titration protocol based on oxygen saturation.

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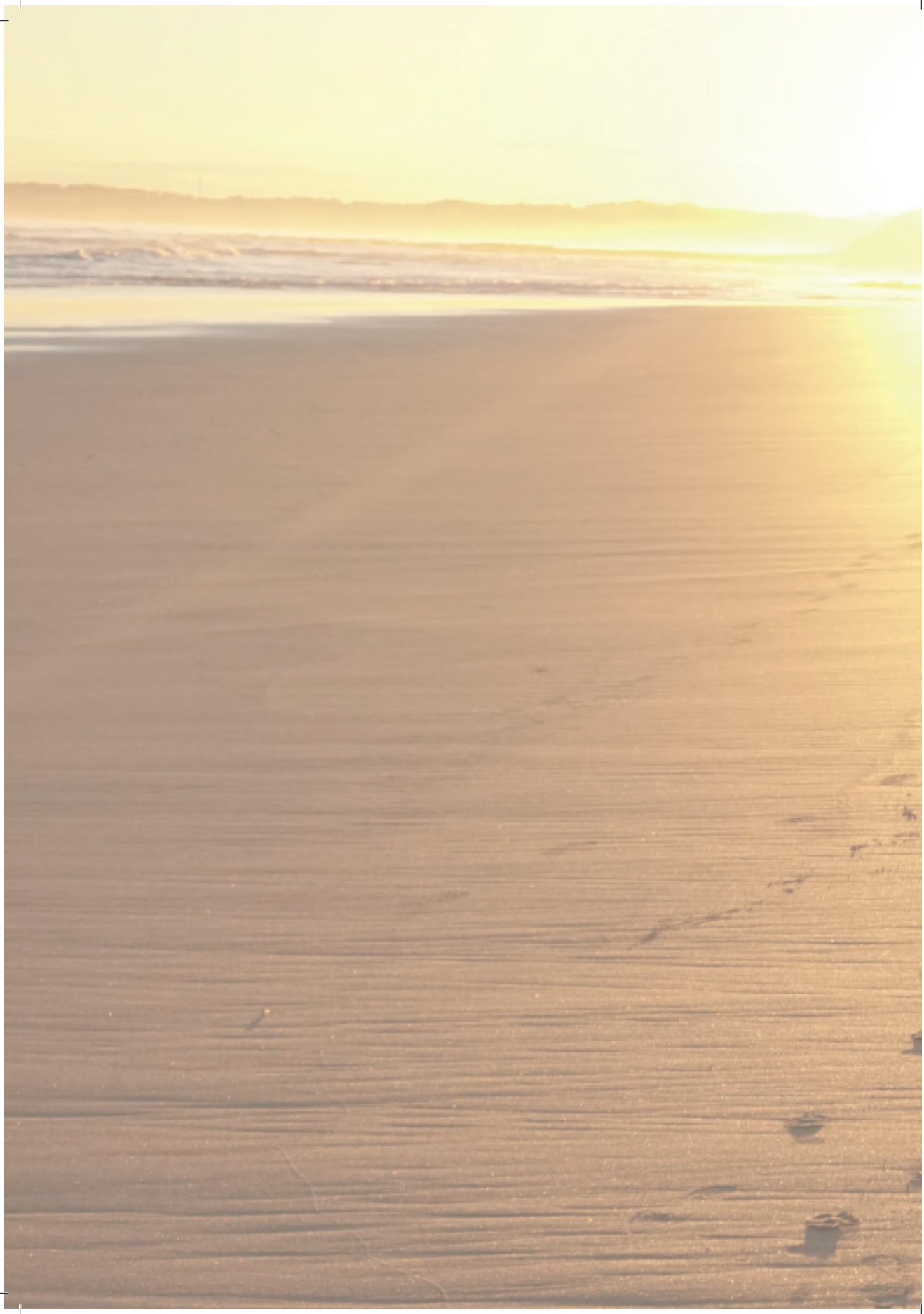

CHAPTER 4

Time to achieve desired fraction of inspired oxygen using a T-piece ventilator during resuscitation of preterm infants at birth

J Dekker
FJ Stenning
LJFB Willms
T Martherus
SB Hooper
AB te Pas

Resuscitation 2019;136:100-104

ABSTRACT

AIM

To determine the time between adjustment of fraction of inspired oxygen (FiO_2) at the oxygen blender and the desired FiO_2 reaching the preterm infant during respiratory support at birth.

METHODS

This observational study was performed using a Neopuff™ T-piece Resuscitator attached to either a test lung (during initial bench tests) or a face mask during the stabilization of infants at birth. FiO_2 was titrated following resuscitation guidelines. The duration for the desired FiO_2 to reach either the test lung or face mask was recorded, both with and without leakage. A respiratory function monitor was used to record FiO_2 and amount of leak.

RESULTS

In bench tests, the median (IQR) time taken to achieve a desired FiO_2 was 34.2 (21.8 – 69.1) s. This duration was positively associated with the desired FiO_2 difference, the direction of titration (upwards) and the occurrence of no leak (R^2 0.863, F 65.016, $p<0.001$). During stabilization of infants (median (IQR) gestational age 29^{+0} (28^{+2} – 30^{+0}) weeks, birthweight 1290 (1240 – 1488) grams), the duration (19.0 (0.0 – 57.0) s) required to reach a desired FiO_2 was less, but still evident. In 27/55 (49%) titrations, the desired FiO_2 was not achieved before the FiO_2 levels were again changed.

CONCLUSION

There is a clear delay before a desired FiO_2 is achieved at the distal end of the T-piece resuscitator. This delay is clinically relevant as this delay could easily lead to over- and under titration of oxygen, which might result in an increased risk for both hypoxia and hyperoxia.

INTRODUCTION AND RATIONALE

The transition to life after birth is hampered in preterm infants due to the immature respiratory system expressed by poor compliance of the lungs, weakness of the respiratory muscles and an immature respiratory center with a weak respiratory drive. (1) Most preterm infants need respiratory support after birth to aerate their lungs and gain adequate oxygenation.(2) A T-piece ventilator that administers continuous positive airway pressure (CPAP) or positive pressure ventilation (PPV) via face mask is currently the method of first choice in order to minimize lung injury.(2-4) Additional oxygen is often used to reach and maintain adequate oxygenation.(5)

During stabilization after birth, additional oxygen needs to be titrated based on recommended oxygen saturations (SpO_2) to decrease the risk of hypoxia and hyperoxia. (3, 6) Hypoxia may lead to delayed cellular damage, as during hypoxia the production of free radicals will be provoked by an elevated level of hypoxanthine.(7, 8) On the other hand, free radical production (associated with both oxidative and nitrosative stress) also increases during hyperoxia, which can overwhelm the relatively immature antioxidant capacity of the preterm infant.(9-12) The excess of free radicals in turn may cause wide-spread damage to cells, enzymes, lipids, DNA and proteins.(9-12)

A time-dependent oxygen saturation range is targeted during stabilization, that is based on previously described international normograms.(6) To achieve oxygen saturations in this range, neonatal resuscitation guidelines recommend commencing resuscitation with a fraction of inspired oxygen (FiO_2) of 0.21 - 0.3.(3) The desired oxygen concentration is achieved by mixing oxygen and room air using an oxygen blender, from which the gas mixture is administered to the neonate via the T-piece ventilator. During stabilization after birth, infants are evaluated every 30 s, which guides the amount of support and titration of additional oxygen.(3) The study of Goos et al.(5) showed that the FiO_2 needed during stabilization at birth is highly variable between infants, with a reported range of 0.21 – 0.99, with 7 (3 – 10) adjustments of FiO_2.

While the adjustment of FiO_2 is performed at the oxygen blender, this is located upstream of the T-piece resuscitation device and simply alters the concentration of oxygen entering the device. However, it is unknown how long it takes for the gas mixture with the desired oxygen concentration to reach the infant at the distal part of the circuit. As such, further titration could take place before the original desired FiO_2 of gas entering the infant has been achieved. This, in turn, could lead to an increased risk of hypoxia or hyperoxia.

The aim of this study was therefore to determine the time between adjustment of FiO_2 at the oxygen blender and the desired FiO_2 reaching the preterm infant during respiratory support at birth.

METHODS

An observational study was conducted at the Neonatal Intensive Care Unit of the Leiden University Medical Center. The study consisted of two parts: a bench test and clinical observations of neonatal stabilization at birth.

The resuscitation of preterm infants at birth was replicated during a bench test, using a Neopuff™ T-piece Resuscitator (Fisher & Paykel Healthcare, Auckland, New Zealand) attached to a 50 mL test lung (Dräger, Lübeck, Germany). The circuit was set up with a flow of 8 L/minute, positive end expiratory pressure (PEEP) of 8 cm H_2O, and a peak inspiratory pressure (PIP) of 20 cm H_2O. FiO_2 was measured at two distinct positions at the circuit, using oxygen analyzers (Teledyne, Analytical Instruments, USA): proximally - at the outlet of the Neopuff™, and distally – between the T-piece of the Neopuff™ and the test lung (Figure 1). In the clinical part of the study, the same circuit was connected to a face mask (Fisher & Paykel Healthcare, Auckland, New Zealand) instead, which was placed over the mouth and nose of a preterm infant.

During the bench test, FiO_2 was increased from 0.3 to 0.5 to 1.0, and decreased to 0.8, 0.5, 0.3 and 0.21. The time needed to reach the desired FiO_2 at the test lung was recorded, whereby a margin of 0.05 difference between FiO_2 at the proximal and distal part of the circuit was accepted. Mask ventilation without any leakage is difficult to accomplish in clinical practice and leak percentages of 50% are not uncommon.(13) As the amount of flow passing the Neopuff™ circuit and reaching the test lung might be influenced by the amount of leak of the circuit, we performed the bench test both with a leakage of 50% and without any leakage. Leak was created by using a small tube through which some of oxygen/air mixture could flow away. This tube was placed between the Neopuff™ circuit and the test lung. The amount of leakage was ascertained by using a Respiratory Function Monitor (RFM). Both tests (with and without leak) were performed 6 times.

During stabilization of infants, local resuscitation guidelines were followed for increasing FiO_2, using the same increments as those used during the bench test: FiO_2 was started at 0.3, and increased to 0.5 and 1.0 if SpO_2 remained below international normograms.

(6) In addition, FiO_2 was decreased whenever SpO_2 exceeded 90% to 0.8, 0.5, 0.3 and 0.21. Again, we analyzed the time delay between titration of FiO_2 at the proximal part of the circuit and the desired FiO_2 being achieved at the distal part, accepting a difference of 0.05 between those measurements. The amount of leakage was taken into consideration when analyzing the results.

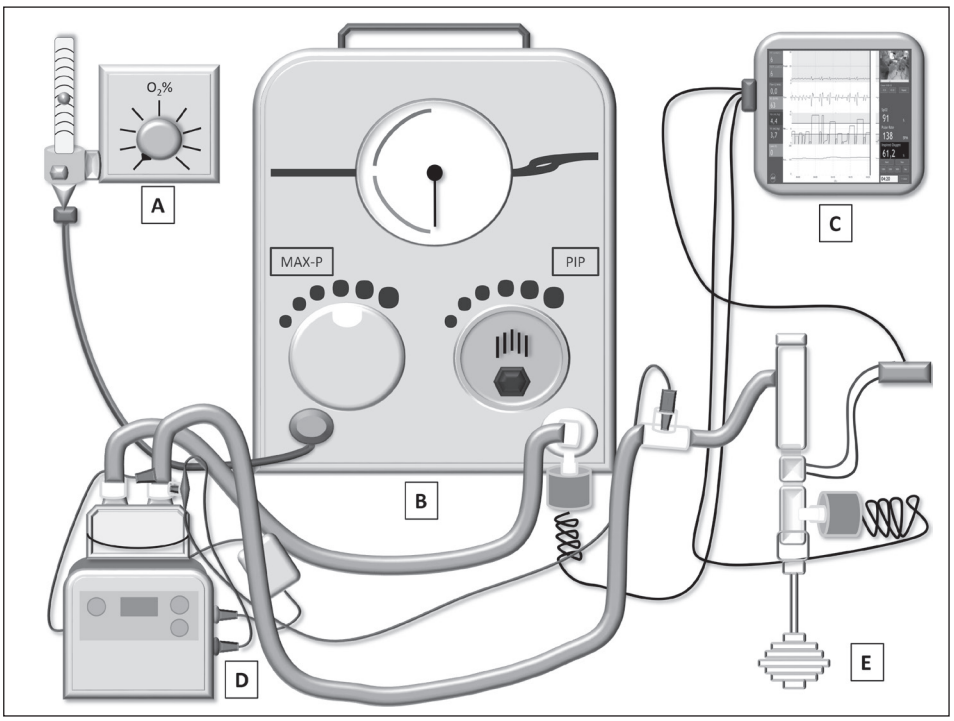

Figure 1 | Set-up bench test

In both parts of the study, a RFM was used for recording FiO_2 at both positions within the circuit, as well as clinically relevant physiological parameters such as oxygen saturation and respiratory function. The RFM uses a small (dead space 1 mL) variable orifice flow sensor (Avea Varflex Flow Transducer, Carefusion, Yorba Linda, CA, USA) to measure gas flow in and out of a face mask, thereby calculating inflation pressures, flow and tidal volumes. The difference between inspired and expired tidal volume estimates leak from the face mask, which was also recorded. Oxygen saturations and heart rates were measured with a Masimo SET pulse oximeter (Masimo SET, Masimo Corporation, Irvine, California, USA). A pulse oximetry probe was placed around the

ulnar aspect of the infant's right wrist. All signals measured were recorded at 200 Hz using the NewLifeBox-R physiological recording system with Polybench software (Applied Biosignals, Weener, Germany). Oxygen analyzers were calibrated at the start of every test and clinical observation.

Data of both parts of the study was analyzed using Microsoft Excel (Microsoft Corporation, Washington, United States). During stabilization of infants, FiO_2, SpO_2 and respiratory function were recorded at 0.5 second intervals. Significant leak was accepted as being > 50% face mask leak. In the event that titration proceeded before the desired FiO_2 from the previous titration (set at the proximal part of the Neopuff™ circuit) had reached the infant (at the distal part of the circuit), the time between titrations was recorded as duration of achieving the desired FiO_2.

Statistical analysis was performed with SPSS version 23.0 (IBM software, NY, USA, 2015). Data is presented as mean ± SD of parametric outcome parameters, non-parametric outcome parameters are presented as median (IQR). Assessment of the relationship between duration of achievement of desired FiO_2, with the difference in FiO_2 that was aimed for during titration and the occurrence of leak, was determined using multiple regression. P-values <0.05 were considered statistically significant. Reported p-values are two-sided.

This was an observational study and as such under the Dutch law on Medical Research in Humans, no specific consent from an ethics committee was required. Nevertheless, the Research Ethics Committee of the Leiden University Medical Center issued a statement of no objection.

RESULTS

Bench test

When adjusting the FiO_2, the oxygen analyzer at the proximal part of the circuit reached the desired oxygen concentration within 3 ± 1 seconds, independent of the amount of leakage in the circuit. There was a delay in time to achieve desired FiO_2 at the distal part of the circuit (median (IQR) duration 34.2 (21.8 – 69.1) s).

Without mask leak, the median (IQR) duration of the FiO_2 to reach the desired concentration at the distal part of the Neopuff™ circuit during up-titration was 58.2 (35.7 - 86.1) s. The largest delay in reaching the desired concentration during up-

titration was found in the titration from FiO_2 of 0.5 to 1.0 (Table 1). The median (IQR) duration of the FiO_2 to reach the desired concentration at the distal part of the circuit during down-titration was 70.7 (39.9 – 88.8) s. The largest delay in reaching the desired concentration during down-titration was found in the titration from FiO_2 0.8 to 0.5 (Table 1).

With a leak of 50%, up-titration took a median (IQR) duration of 20.0 (17.8 – 28.6) s, with again the largest delay in titration of FiO_2 from 0.5 to 1.0 (Table 1). Down-titration with 50% mask leakage resulted in a median (IQR) duration of 22.1 (20.9 – 28.7) s for the FiO_2 to reach the desired concentration at the distal part of the circuit. The largest delay was found during down-titration of FiO_2 from 0.8 to 0.5 (Table 1).

Table 1 | Duration to achieve desired FiO_2 in bench test

Change in FiO_2	Duration (s) 0% leak n = 6	Duration (s) 50% leak n = 21
Up titration		
FiO_2 0.3-0.5	40.9 ± 10.8	17.9 ± 0.2
FiO_2 0.5-1.0	80.8 ± 17.0	26.6 ± 4.2
Down titration		
FiO_2 1.0-0.8	65.0 ± 19.6	21.1 ± 0.4
FiO_2 0.8-0.5	100.6 ± 10.6	34.6 ± 6.0
FiO_2 0.5-0.3	69.3 ± 2.4	24.5 ± 3.9
FiO_2 0.3-0.21	32.6 ± 8.2	19.7 ± 2.4

Data are presented as mean ± SD

There was a positive association between the duration to achieve the desired FiO_2 at the distal part of the Neopuff™ circuit and the desired FiO_2 difference (longer duration when aiming for a larger FiO_2 difference), the direction of titration (up instead of down) and the occurrence of no leak (R^2 0.863, F 65.016, p<0.001, Table 2).

Table 2 | Multiple linear regression analysis for the association between duration to achieve desired FiO_2 and direction of titration, occurrence of leak and desired FiO_2 difference

Variables	Regression coefficient β (95% CI)	Standardized β	t	P-value
Occurrence of leak[a]	-38.317 (-44.973 – 31.662)	-0.782	-11.743	< 0.001
Direction of titration[b]	-23.973 (-32.540 – 15.405)	-0.464	-5.707	< 0.001
Desired FiO_2 difference	1.220 (0.900 – 1.539)	0.634	7.789	< 0.001

Reference is the occurrence of no leak (a) and titration downwards (b)

Stabilization of infants

Data was recorded from the stabilization of eight preterm infants. Included infants had a median (IQR) gestational age of 29^{+0} (28^{+2} – 30^{+0}) weeks and birth weight of 1290 (1240 – 1488) grams. All except one infant needed PPV during resuscitation. A total of 55 FiO_2 titrations occurred in these infants, with a median (IQR) of 7 (5 – 9) per infant. The median (IQR) desired FiO_2 difference was 0.16 (0.09 – 0.21). Median (IQR) time between titration episodes was 52.5 (27.0 – 103.0) s. In 17/55 (31%) titration episodes, the time until the next titration was less than 30 s.

Overall, there was a variable delay in time to achieve desired FiO_2 at the distal part of the circuit (median (IQR) duration 19.0 (0.0 – 57.0) s). FiO_2 at the distal part of the Neopuff™ circuit changed during titration with a median (IQR) speed of 0.75% (0.42 – 1.28) per s.

There were only four titration episodes in which the average amount of leak was > 50%. We observed a difference in median (IQR) speed with which FiO_2 at the distal part of the Neopuff™ changed: 0.7(0.42 – 1.09) % per s with an average mask leak of 0 – 49 %, compared to 2.47 (0.26 – 3.57) % per s with an average mask leak of > 50 %.

The desired FiO_2 was never reached before the titration of FiO_2 proceeded in 27/55 (49%) of titrations. This was not influenced by the occurrence of mask leakage (no leak: 24/48 (50%) vs leak: 3/7 (43%), p=1.000).

DISCUSSION

This study shows a clear delay in obtaining the desired oxygen concentration at the distal part of the Neopuff™ circuit in both the bench test and during stabilization of preterm infants at birth. As the international resuscitation guideline prescribes evaluation periods of 30 s, the clinical evaluation of the infant and physiological parameters might precede the effect of the performed intervention (e.g. titration of oxygen).(3) This is demonstrated by the finding that in half of all titration episodes, the desired FiO_2 was not yet reached at the distal part of the Neopuff™ circuit. In addition, the time between two titration episodes was less than 30 s in 31 % of titrations performed in this study.

Our results confirm the findings of the study of Follett et al.(14) who observed that the achievement of desired oxygen concentration at a test lung was delayed. However, the titration of oxygen in their study was slightly different than our study, and it is not clear

what the time intervals between titration steps were. Follett et al.(14) also used different ventilators and different lengths of ventilation circuits, while we used the equipment that we use in the clinical setting. However, while both the current trial and the trial of Follett et al.(14) demonstrated that there appears to be a delay in achievement of desired FiO_2, the exact duration of delay might be dependent on multiple variables, including type of ventilator and length of ventilation circuits. In addition, the rate with which the desired FiO_2 is reached in the infant might also be dependent of the volume containing the FiO_2 that needs to be replaced, which is influenced by the pressure administered (PIP and PEEP), respiratory rate and type of respiratory support (CPAP vs PPV vs sustained inflation).

We observed a higher duration to obtain the desired FiO_2 in the bench test, compared to the clinical stabilization procedures. As the desired FiO_2 was not reached in 49 % of all titrations, the duration to obtain the desired FiO_2 was based on the time between titrations and represents an underestimation of the actual duration. Therefore, the delay in obtaining the desired FiO_2 may be closer to the values seen in the bench test.

In addition, the lower duration to reach the desired FiO_2 during stabilization of preterm infants could also be due to the occurrence of leak, as the duration increases when there is no leak. In this study, only 4/55 titration episodes occurred during ventilation with an average of > 50 % leak. One should aim for a minimum level of leakage between the face mask and the face of the infant to achieve adequate ventilation, which, in clinical practice is shown to be difficult to accomplish with a high variability in percentage of leak.(13) These findings indicate that, when ventilating adequately (thus without mask leakage), following an adjustment in FiO_2 there could be a delay in obtaining the desired oxygen concentration at the infant. Furthermore, it is likely that this delay will reduce as the amount of leak increases. However, when mask leakage is variable, it is unclear when the desired oxygen concentration will be reached, possibly leading to over- and under-titration and an increased risk of hypoxia and hyperoxia.

The explanation of the delay in obtaining the desired oxygen concentration is still not clear. We have contacted the manufacturer of the Neopuff™ Infant Resuscitator (Fisher & Paykel Healthcare Auckland, New Zealand), and they acknowledge the results of the study, but the explanation for this delay still remains unclear. They recommend that clinicians should be aware of this when titrating oxygen to achieve target oxygen saturations and allow time for the system to equilibrate. We speculate that the delay might be influenced by not having a continuous flow through the circuit, particularly during inflations. Most T-piece resuscitators like the Neopuff™ have the PIP valve

located inside the box at the proximal end of the circuit and a PEEP valve located at the distal, infant end of the circuit. During an inflation, the PEEP valve is closed (by placing a finger on it) and the circuit, which includes the infants lungs, is pressurized by the flow of gas from the Neopuff™ down the circuit and into the infant. In the absence of leak, once the set PIP pressure is reached, the PIP valve opens and so gas stops flowing down the circuit and instead begins to flow out of the PIP valve, located upstream of the circuit, in the box. As such, during inflations, when the PIP pressure is reached, very little gas will flow down the circuit to the infant, thereby greatly increasing the time for the desired FiO_2 to reach the infant. In case of leak, gas will continue to flow down the circuit, particularly if the set PIP is not reached, thereby reducing the duration to reach the desired FiO_2. During lung deflation, lifting the finger from the PEEP valve allows expired gas from the infant to flow out through the PEEP-valve. In addition, the decrease in pressure allows the PIP valve to close which redirects gas flow down the circuit and out through the PEEP valve. Thus, at end-expiration all gas flowing into the Neopuff™ flows down the ventilation circuit and out the PEEP valve, allowing the set air-oxygen mixture to rapidly reach the distal (infant end) of the circuit.

However, if correct, the variability in time for the desired FiO_2 to reach the infant end of the circuit may be explained by the degree of leak and the relative amount of time spent at either PIP or PEEP.

CONCLUSION

In summary, there is a clear delay in set up of FiO_2 at the proximal part of the T-piece ventilator and achievement of desired FiO_2 at the distal part. This delay is clinically relevant when aiming for adequate mask ventilation, whereby over- and under titration of oxygen might result in an increased risk of hypoxia and hyperoxia.

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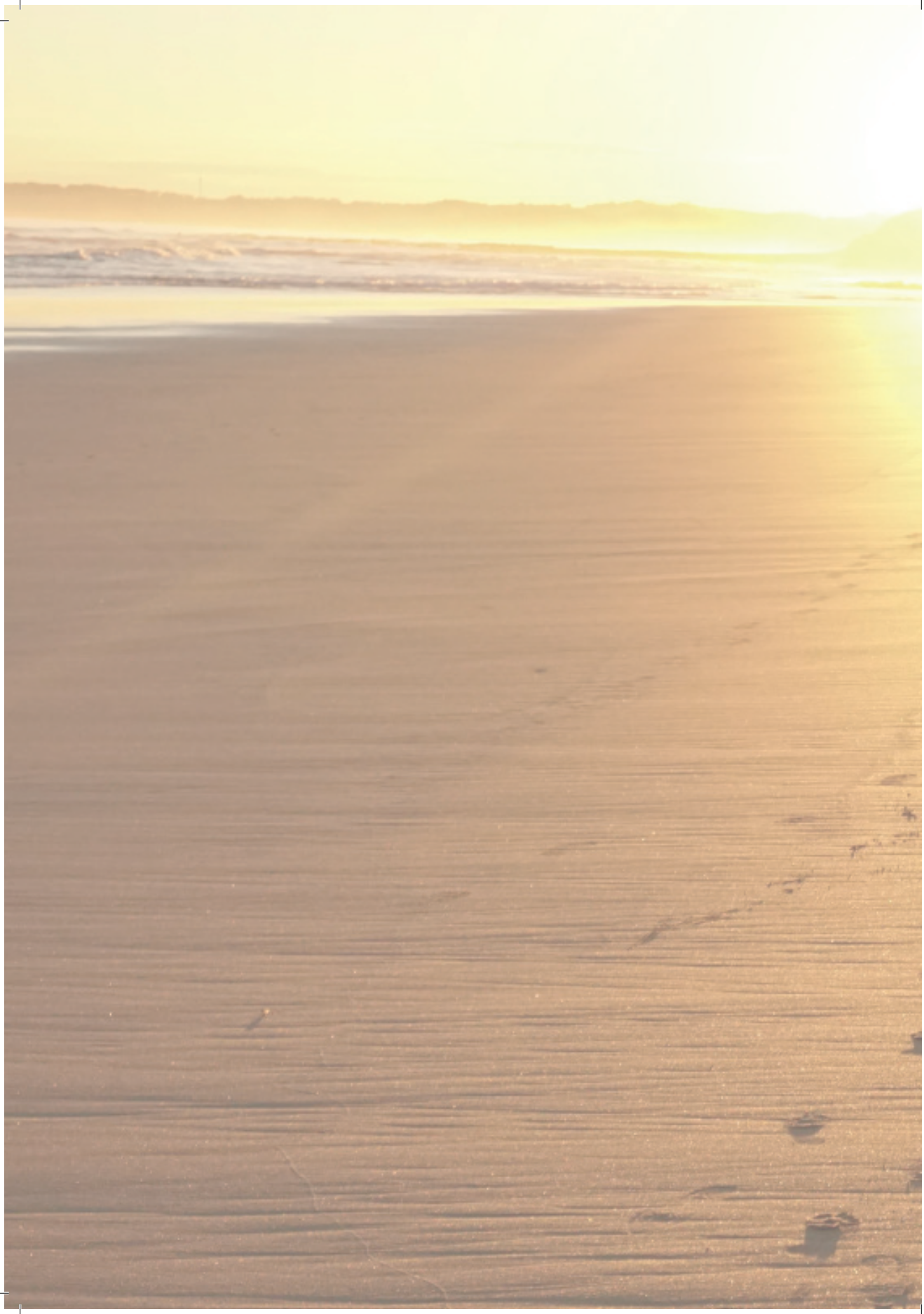

CHAPTER 5

High versus low initial oxygen to improve the breathing effort of preterm infants at birth: study protocol for a randomized controlled trial

J Dekker
SB Hooper
M Giera
EV McGillick
GJ Hutten
W Onland
AH van Kaam
AB te Pas

Frontiers in Pediatrics 2019;7:179

ABSTRACT

BACKGROUND

Although most preterm infants breathe at birth, their respiratory drive is weak and supplemental oxygen is often needed to overcome hypoxia. This could in turn lead to hyperoxia. To reduce the risk of hyperoxia, currently an initial low fraction of inspired oxygen (FiO_2) (0.21 – 0.3) is recommended during stabilization at birth, accepting the risk of a hypoxic period. However, hypoxia inhibits respiratory drive in preterm infants. Starting with a higher level of oxygen could lead to a shorter duration of hypoxia by stimulating breathing effort of preterm infants, and combined with subsequent titration based on oxygen saturation (SpO_2), prolonged hyperoxia might be prevented.

METHODS

This multi-center randomized controlled trial will include 50 infants with a gestational age between 24 - 30 weeks. Eligible infants will be randomized to stabilization with an initial FiO_2 of either 1.0 or 0.3 at birth. Hereafter, FiO_2 will be titrated based on the oxygen saturation target range. In both groups, all other interventions during stabilization and thereafter will be similar. The primary outcome is respiratory effort in the first 5 minutes after birth expressed as average minute volume/kg. Secondary outcomes include inspired tidal volumes/kg, rate of rise to maximum tidal volume/kg, percentage of recruitment breaths with tidal volumes > 8mL/kg, duration of hypoxia and hyperoxia and plasma levels of markers of oxidative stress (8-iso-prostaglandin F2α).

DISCUSSION

Current resuscitation guidelines recommend oxygen titration if infants fail to achieve the 25^{th} centile of the SpO_2 reference ranges. It has become clear that, using this approach, most preterm infants are at risk for hypoxia in the first 5 minutes after birth, which could suppress the breathing effort. In addition, for compromised preterm infants who need respiratory support at birth, higher SpO_2 reference ranges in the first minutes after birth might be needed to prevent prolonged hypoxia. Enhancing breathing effort by achieving an adequate level of oxygenation could potentially lead to a lower incidence of intubation and mechanical ventilation in the delivery room, contributing to a lower risk on lung injury in high-risk preterm infants. Measuring 8-iso-prostaglandin F2α could lead to a reflection of the true amount of oxygen exposure in both study groups.

INTRODUCTION AND RATIONALE

Although most preterm infants breathe at birth, their respiratory drive is weak and often insufficient to aerate their lungs and establish gas exchange.(1-3) Historically, these infants were intubated and mechanically ventilated, which is associated with a higher risk of lung and brain injury.(4) To minimize injury, this approach is now avoided and the focus of respiratory care has shifted to support the mechanics of breathing by providing a continuous positive airway pressure (CPAP) by face mask.(5, 6) Despite this approach, there is still a high failure rate of CPAP in preterm infants in case of a weak respiratory drive, associated with higher mortality and major morbidities.(7) It has become clear that the best way to facilitate air entry into the lungs using non-invasive respiratory support is to stimulate spontaneous breathing(8, 9), which is also likely to be the most gentle and effective way of providing respiratory care without causing injury**.** Therefore, the focus of respiratory care should include both support of the mechanics of breathing and stimulation of spontaneous breathing. Currently, international guidelines recommend tactile stimulation and some local guidelines additionally advocate the use of caffeine in the delivery room to stimulate breathing in addition to the use of non-invasive ventilation.(5, 10, 11)

Even with these techniques to support and stimulate spontaneous breathing, achieving adequate oxygenation with a low initial FiO_2 during stabilization has been shown to be difficult.(12, 13) Most preterm infants born with a gestational age < 32 weeks are therefore at risk for hypoxia.(14) This, in turn leads to inhibition of respiratory drive because the inhibitory effect of hypoxia on fetal breathing movements persists well into newborn life.(2) Increasing the FiO_2 can reduce the level of hypoxia leading to an increase of respiratory effort in preterm infants. This was demonstrated in an observational study where an increase in respiratory drive was observed after switching FiO_2 from 0.21 to 1.0 at some point during stabilization at birth.(15)

The optimal initial oxygen concentration for stabilizing infants after birth is still a subject of debate, as both hypoxia and hyperoxia can cause damage to multiple organ systems.(16, 17) Hypoxia may lead to delayed cellular damage(18), as during hypoxia the production of free radicals will be provoked, by an elevated level of hypoxanthine. (19) On the other hand, high oxygen levels increase the biosynthesis of prostaglandins which are an additional mechanism inhibiting chemoreceptors in the respiratory center. (20) In addition, free radical production (associated with both oxidative and nitrosative stress) increases during hyperoxia, which can overwhelm the relatively immature antioxidant capacity of the preterm infant. The resulting excess of free radicals cause wide-spread damage to cells, enzymes, lipids, DNA and proteins.(21-24)

The recent meta-analysis of Oei et al.(18) showed a higher mortality rate in the group of preterm infants (< 32 weeks of gestation) resuscitated with an initial FiO_2 of 0.21 compared to the group resuscitated with an initial FiO_2 of 1.0. Furthermore, when stabilization of preterm infants after birth was started with low oxygen concentrations (FiO_2 0.21-0.3), SpO_2 and heart rates increased more slowly than the SpO_2 references outlined in the international guidelines, despite the use of respiratory support. This indicates that when starting respiratory support with lower oxygen concentrations preterm infants are at a higher risk for hypoxia.(25) Indeed, this was shown in the study of Goos et al.(12) and White et al.(13).

Regardless of the initial FiO_2 level, in all studies most infants ended up with a FiO_2 between 0.4 and 0.6 after 5 minutes to reach SpO_2 target ranges. Currently, to prevent hyperoxia and achieve normoxia in newborns, international guidelines recommend to start stabilization after birth in preterm infants with low FiO_2 (0.21 - 0.3)(26), after which FiO_2 should be titrated based on SpO_2 target ranges.(16, 17, 27) In this recommendation, a period of low oxygen saturation is accepted and the effect of hypoxia on respiratory effort seems to be disregarded.(28)

We hypothesize that with an initial FiO_2 of 1.0, infants will reach SpO_2 target ranges more rapidly, leading to less hypoxia and an increase in respiratory effort. With careful titration of FiO_2 based on SpO_2 target ranges, we hypothesize that the occurrence of hyperoxia will be minimized.

The aim of this current study is to determine the effect of an initial FiO_2 of 1.0 compared to standard management of an initial FiO_2 of 0.3 on breathing effort of preterm infants immediately after birth.

STUDY DESIGN AND POPULATION

Study design

The design of this multi-center study is a randomized clinical trial. Eligible infants between 24^{+0} - 29^{+6} weeks of gestation will be randomized to either start stabilization with an initial FiO_2 of 1.0 or 0.3.

Study population

The study will take place at the neonatal intensive care unit (NICU) of the Leiden University Medical Center (LUMC) and the NICU of the Amsterdam UMC (AUMC), both

located in the Netherlands. Infants with a gestational age between 24^{+0} - 29^{+6} weeks, born at the LUMC and the AUMC will be eligible for inclusion. Infants with a congenital abnormality or condition that might have an adverse effect on breathing or ventilation will be excluded, such as congenital diaphragmatic hernia, trachea-oesophageal fistula or cyanotic heart disease.

Primary outcome

The primary outcome is respiratory effort, expressed as average minute volume normalized for body weight in the first 5 minutes after birth.

Secondary outcomes

Secondary outcomes include:

- Average tidal volumes in the first 5 minutes after birth
- Average rate of rise to maximum tidal volumes in the first 5 minutes after birth
- Percentage of recruitment breaths with tidal volumes above 8 mL/kg in the first 5 minutes after birth
- Duration of hypoxia (defined as oxygen saturation < 25^{th} percentile of the target ranges defined by Dawson et al.(27)) in the first 10 minutes after birth
- Duration of hyperoxia (defined as oxygen saturation > 95%) in the first 10 minutes after birth
- Changes in diaphragmatic activity in the first 5 minutes after birth
- Incidence of intubation in the delivery room
- Concentration of 8-iso-prostaglandin F2α(29), an oxidative stress metabolite associated with hyperoxia in blood.

Volumes measured will be normalized for body weight. In addition, the interaction between those primary and secondary outcomes and the time after birth will be assessed.

Other outcomes

Other outcomes that will be collected include:

- Percentage of time of face mask ventilation applied during stabilization at birth
- Average oxygen saturation and heart rate in the first 5 minutes after birth
- Oxygen saturation and heart rate *per minute* in the first 5 minutes after birth
- Percentage of spontaneous breaths with tidal volumes above 4 mL/kg
- Percentage of time that oxygen saturation is within 90-95% range in the first 5 minutes after birth

- Percentage of time that oxygen saturation is within 90-95% range between 5 and 10 minutes after birth
- Percentage of time that oxygen saturation is within target ranges defined by Dawson et al.(27) in the first 10 minutes after birth

In addition, we will collect basic characteristics such as gestational age, birth weight, mode of delivery, incidence of complications in pregnancy, use of antenatal corticosteroids, maternal medication use, and Apgar score 1 and 5 minutes after birth. This study is not designed to demonstrate any differences in clinical short- and long-term outcomes. However, we will record study parameters focussing on short-term clinical outcomes, such as incidence of intubation in the delivery room and in the first 24 hours after birth, pneumothorax, intraventricular hemorrhages grade 3 or more and neonatal death.

Sample size calculation

There is no data available on measurements of respiratory effort in the first 5 minutes after birth during stabilization with a FiO_2 of 1.0 compared to 0.3. In a recent study, the average minute volume of preterm infants over the first 100 breaths was 150 ± 70 mL/kg/min.(30) In a retrospective observational study, we observed an 80% relative increase in respiratory drive when FiO_2 was switched from 0.21 to 1.0, in the first minute after FiO_2 was increased (from 134 mL/kg/min to 240 mL/kg/min). Other parameters of respiratory effort increased as well: average expired tidal volumes (Vt) increased by 37% (from 4.9 mL/kg to 6.7 mL/kg), and rate of rise to maximum tidal volumes increased by 32% (13.8 mL/kg/s to 18.2 mL/kg/s).(15)

However, since we investigate a FiO_2 of 0.3 instead of 0.21 in the lower oxygen arm, we expect half of the effect (40% relative increase) in respiratory drive than demonstrated in the study of van Vonderen et al.(15). Therefore, to detect an increase in average minute volume in the first 5 minutes after birth from 150 to 210 mL/kg/min, using a standard deviation of 70 mL/kg/min, with a power of 80% and an α error of 5% (two tailed test), 22 infants are required for each arm. Given the fact that parents may withdraw their consent or decline deferred consent after the infant is included in the study, or that the recording of physiological measurements fails in 10% of all infants, a total of 50 infants will be recruited (25 in each arm).

TREATMENT OF SUBJECTS

Randomization

Allocation will be stratified by gestational age. Infants of 24^{+0} - 26^{+6} weeks and 27^{+0} - 29^{+6} weeks will be included using variable block (4-6) sizes. Concealment of allocation will be ensured by using the randomization process of Castor EDC (Amsterdam, The Netherlands), an electronic data capture system. The neonatal fellow or neonatal consultant in charge of the procedure will randomize using Castor EDC.

Study procedures

Infants will be randomized to start stabilization after birth with an initial FiO_2 of either 1.0 or 0.3, after which FiO_2 will be titrated following target SpO_2 recommended in our local guidelines on stabilization after birth, which are based on the target ranges described by Dawson et al.(27) In both groups, all other procedures in the delivery room and NICU will be performed according to the local and international guidelines, only the initial FiO_2 setting will be different. Cord clamping will be performed after 30 seconds in case of an apneic infant, and after 60 seconds if the infant is breathing at birth.

After the initial FiO_2 setting, titration will be performed according to the local protocol:

- When SpO_2 is below target ranges defined by Dawson et al.(27), FiO_2 will be titrated up to 0.5 and subsequently to 1.0, when the initial FiO_2 setting was 0.3. In case of an initial FiO_2 setting of 1.0, FiO_2 will be maintained at 1.0.
- When SpO_2 is above target ranges, FiO_2 will be titrated down to 0.5 and subsequently to 0.3 and 0.21. If SpO_2 is above target ranges when stabilization is initiated with FiO_2 0.3, FiO_2 will be titrated down to 0.21 directly.

Measurements

Measurement of respiratory function

In both groups, standard care will be provided in the delivery room and NICU and local guidelines on stabilization after birth will be followed, using a Neopuff™ infant T-piece resuscitator (Fisher & Paykel Healthcare, Auckland, New Zealand) or a Giraffe Star System (Anandic Medical Systems, Feuerthalen, Switzerland). Respiratory function monitoring at birth will be recorded during stabilization in the first 10 minutes of life, which is standard practice in our units. The respiratory function monitor will be used to measure inflation pressures, flow and tidal volumes. This can be used to measure average minute volume, average rate of rise to maximum tidal volumes, and the total time of ventilation given. It uses a small variable resistance anemometer to measure

gas flow in and out of a face mask or endotracheal tube. This signal is automatically integrated to provide inspired and expired tidal volumes. The difference equals the leak from the face mask or endotracheal tube.

Breathing effort will also be measured by activity of the diaphragm using electromyography (EMG) with the Dipha-16 (Demcon, Groningen, The Netherlands). The Dipha-16 is used to record spontaneous breathing (EMG of diaphragm) and heart rate (ECG). Both parameters are used for audit of stabilization, which is standard care in the NICU of the LUMC. Three EMG electrodes connected to the Dipha amplifier (similar to ECG electrodes) will be placed. Two are placed bilateral at costo-abdominal margin in the nipple line, and the common electrode will be placed on one of the legs.

To record SpO_2 and heart rate, a Masimo SET pulse oximeter probe (Masimo Radical, Masimo Corporation, Irvine, California, USA) or Nellcor™ pulse oximeter probe (Covidien, Dublin, Ireland) is placed around the right wrist or hand of the infant. The FiO_2 is measured using a portable oxygen analyzer AX300-I (Teledyne Analytical Instruments, CA, USA), and the airway pressures are registered by a variable orifice flow sensor (Avea Varflex Flow Transducer, Carefusion, Yorba Linda, CA,USA) connected to the face mask measuring the flow in and out the infant. The signals are digitized at 200 Hz using the NewLifeBox-R physiological recording system (Advanced Life Diagnostics, Weener, Germany) and all signals are recorded by the NewLifeBox Neo-RSD computer system (Advanced Life Diagnostics, Weener, Germany) supported by Polybench physiological software (Applied Biosignals, Weener, Germany). Pulmochart software (Applied Biosignals, Weener, Germany) is used to analyze primary and secondary outcomes.

Measurement of metabolites of oxidative stress in blood

Chemical oxidation products of polyunsaturated fatty acids, formed by peroxidation initiated by free radicals, are shown to be the best biomarkers to assess oxidative stress in humans. Of oxidized fatty acids, the best indicator for oxidative stress is 8-iso-prostaglandin F2α(31) Cord blood 8-iso-prostaglandin F2α is a stable marker of lipid peroxidation at birth, and will therefore be analyzed as baseline value for oxidative stress obtained after birth.(32)

Measurement time points:

- Cord blood will be drawn directly after birth as a baseline value.
- At approximately 1 hour after birth, a blood sample will be taken together with the sample normally taken for measuring standard analyses; this ensures that the infant

is not exposed to an extra venepuncture or capillary puncture. The total amount of inspired oxygen given from birth will be recorded from birth until the sample is taken.
- At 24 hours after birth, again together with measurement of standard blood analyses.

For every sample, a total of 300 µL blood will be used to measure 8-iso-PGF2α. A preterm infant of 500 gram has ~40 mL of total blood volume, whereas an infant of 1000 gram has ~80 mL. As only 2 samples will be taken from the preterm infants, only 600 µL of blood will be withdrawn, which accounts for 0.75-1.5% of total blood volume.

PROCEDURES

Recruitment and consent

In addition to the initial FiO_2 setting, standard care is provided in both groups. Respiratory data is collected as standard clinical care. As the starting FiO_2 is randomly assigned, we will ask for parental consent. Antenatal consent will be obtained before birth if the mother is hospitalized in the LUMC or AUMC with imminent preterm labour before 30 weeks of gestation. Whenever antenatal consent is obtained, but the mother passes the term of 30 weeks of gestation, the infant will not be included in this study.

In case the condition of the mother and/or fetus suddenly deteriorates, resulting in an emergency situation with immediate delivery, it may not be possible to ask for antenatal consent from either parent. Also, when the mother arrives in hospital in full labour and tocolysis is not an option, it may not be possible, or appropriate to ask for antenatal consent. Emergency situations after birth cannot always be predicted and the neonatologist may have to act upon the infant's interest without being able to discuss the treatment plan with the parents, let alone ask for study consent. The worsening of the condition of these infants, who are often also not pre-treated with antenatal steroids, will increase the chances for respiratory insufficiency after birth and the need for respiratory support in the delivery room. Exclusion of this group of infants will therefore cause an important selection bias.

For the infants born in the following situations, retrospective consent will be used:
- In case of an emergency situation (e.g. the mother is in full labour or immediate delivery is necessary), when there is not enough time for antenatal consent.
- When obtaining antenatal consent is inappropriate (e.g. the condition of the mother does not allow for proper consideration of both parents on whether or not to participate in a trial). In this case there is enough time, though trying to obtain informed consent in this situation would be inappropriate.

This study fulfils the criteria for a waiver of informed consent in Europe (Human Medicines Evaluation Unit 1995), Canada (Natural Sciences and Engineering Research Council of Canada 2006) and the United States (USA Food and Drug Administration 1999) and Australian NHMRC guidelines for studies in emergency medicine (National Health and Medical Research Council 2002). For these infants, retrospective consent will be sought from the parents to use data obtained. For this important study to be feasible, and enrol a representative sample, a waiver of informed consent is appropriate when consent cannot be obtained prior to delivery.

The parents will be informed as soon as possible after the study and asked to consent to data being collected on their infant. There are many precedents of randomized trials conducted with such a consent waiver in emergency situations in neonatal stabilization after birth and adult resuscitation.

While undertaking research studies without prior informed consent needs careful consideration, studies of emergency medical interventions, such as neonatal stabilization after birth, are exceptionally difficult if prior consent is required. Arguably, it is more unethical to use treatments that have not been studied than to evaluate them in a carefully controlled manner.(23, 33)

Withdrawal of individual subjects

Parents can withdraw their consent at any time for any reason if they wish to do so without any consequences. When parents want to withdraw their infant for any reason, this infant will be handled as drop-out. As we included an additional 10% of infants in the sample size calculation, this will not influence the power calculation.

(Serious) adverse events

Adverse events are defined as any undesirable experience occurring to a subject during the study, considered related to the initial FiO_2 setting of 1.0. All adverse events observed by the investigator or the staff will be recorded and noted to the medical ethical committee of the LUMC.

This study population (preterm infants) has a high risk of serious complications, which are inherent to their vulnerable condition and unrelated to the intervention that is under evaluation in this trial. These complications (pneumothorax, bronchopulmonary dysplasia (BPD), retinopathy of prematurity (ROP), intraventricular hemorrhage (IVH) grade 3 or more, cystic periventricular leukomalacia (PVL), necrotizing enterocolitis (NEC) grade 2 or more, death) are not expected to be related to the intervention

(starting stabilization after birth with a FiO_2 of 1.0 versus 0.3) as the intervention will be performed only at the start of stabilization, after which in both groups titration will be performed. In light of the above, immediate and individual reporting of all these conditions related complications will not enhance the safety of the study, and therefore these complications will be handled as adverse events.

Statistical analysis

Data will be presented as median (interquartile range (IQR)), mean ± standard deviation (SD) or number (percentage) where appropriate. Data will be checked for meeting the assumption of normality first by making a frequency distribution per minute. Outliers will be identified and handled according to the data (outliers will be deleted if there are arguments to do so, or transformed if not). Intention-to-treat will be employed. Statistical analysis will be performed with SPSS software version 23.0 (SPSS, Chicago, Illinois, 2012).

Differences in average respiratory minute volume in the first 5 minutes after birth between the study groups will be tested with a Student's t-test if the data are found to be parametric, or with Mann-Whitney U test when the data are non-parametric.

The interaction of respiratory minute volume, tidal volumes, rate of rise to maximum tidal volumes and diaphragm activity in the first 5 minutes after birth with time will be analyzed with a linear mixed model, in which both study group (treatment) and time will be taken in consideration. Differences in average tidal volumes, average rate of rise to maximum tidal volumes, and percentage of recruitment breaths will be tested with a Student's t-test if the data is found to be parametric, or with Mann-Whitney U test when the data is non-parametric. The concentration of 8-iso-prostaglandin F2α will be analyzed with a linear mixed model, in which both study group (initial FiO_2 0.3 vs 1.0) and time will be taken in consideration.

Other study parameters and basic characteristics will only be explored to test for differences between the study groups. If there are found to be significant differences with clinical relevance for the primary outcome, this will be taken into account when analyzing the data by incorporating these differences in the linear mixed model.

Ethical considerations

The study will be conducted according to the principles of the Declaration of Helsinki and in accordance with the Medical Research Involving Human Subjects Act (WMO).

The study protocol is approved by the ethical committee of the LUMC and AUMC.

Dissemination of results

Results of the study will contribute to a manuscript, which will be submitted for publication to a peer-reviewed international medical journal, and will be presented at national and international conferences.

DISCUSSION

Most preterm infants need additional oxygen during stabilization at birth to achieve appropriate SpO_2 target ranges.(34) Results of a recent meta-analysis demonstrated that persisting hypoxia at 5 minutes after birth is associated with a higher risk of mortality and the development of an intraventricular hemorrhage, which we should try to avoid.(14) Although a recent systematic review showed no differences in percentage of infants achieving SpO_2 target ranges between infants initiating stabilization with high or low FiO_2, the level of evidence of these results is low.(35) It is therefore still unclear whether the initial FiO_2 level influences breathing effort, thereby influencing the capacity of preterm infants to reach the target range. However, it has recently became clear that oxygenation can only be influenced in infants supported by non-invasive ventilation in case of presence of spontaneous breathing, as then the larynx opens and an open airway is achieved.(9) Before focussing on achievement of SpO_2 target ranges, we should look at the effect of initiation stabilization with a high FiO_2 on breathing effort.

The current SpO_2 target ranges used during stabilization at birth are based on the reference values described by Dawson et al.(27) While these target ranges have been based on data from healthy term and preterm infants, those values might not apply to infants requiring more respiratory assistance at birth. In the current resuscitation guidelines, oxygen titration is recommended if infants fail to achieve the 25^{th} centile of the SpO_2 reference ranges and are thus hypoxemic in the first minutes after birth.(14, 36) Because hypoxia is known to provide inhibitory input into the respiratory center, this effect on respiratory effort has been overlooked with regard to the importance of oxygen titration. It is possible that the compromised preterm infant requiring respiratory support during transition would benefit by higher SpO_2 reference ranges during stabilization at birth. By initiating stabilization at birth with a FiO_2 of 1.0, we hypothesize that infants will achieve higher SpO_2 values in the first minutes after birth, leading to an increase in respiratory effort at birth.

A FiO_2 of 1.0 should be administered with caution to avoid the detrimental effects of hyperoxia.(21, 23, 24) However, in this study the amount of time that infants receive pure oxygen is very limited since FiO_2 will be titrated according to SpO_2 values. To evaluate pure oxygen exposure, both the amount of time that infants spent above SpO_2 target range and the level of 8-iso-prostaglandin F2α indicating oxidative stress will be measured.

While low SpO_2 values reflect a low oxygenation level in arterial blood, known as hypoxaemia, this does not necessarily represent low oxygen uptake and usage by tissues, particularly cerebral tissues. While pO_2 gradients drive oxygen diffusion into tissues and pO_2 levels in blood are related to blood SpO_2 levels, there are many factors that determine oxygen uptake and usage by tissues. For instance, organs can increase blood flow to maintain oxygen delivery and/or increase oxygen extraction when blood oxygen content decreases. As such, SpO_2 alone provides limited information on oxygen consumption by tissues at high or low blood oxygen levels. In addition, individual patients might differ from one another in their ability to cope with free radicals released in response to inflammation, hyperoxia and hypoxia before or after birth.(21) Although the study is not powered to detect a difference in oxidative stress, we decided to objectively measure this using 8-iso-prostaglandin F2α as marker of oxidative stress, which indicates the true amount of oxygen exposure.

The reported trials comparing high versus low levels of oxygen did not report on how oxygenation levels influence respiratory effort. Although the level of respiratory support during stabilization at birth was reported in most trials and shown to be not significantly different, the effectiveness of spontaneous breathing in both infants receiving higher or lower FiO_2 was not evaluated.(18, 37-39, 43) Gaining an adequate level of spontaneous breathing during stabilization at birth to improve the success of non-invasive respiratory support could potentially lead to a lower incidence of intubation and mechanical ventilation in the delivery room, contributing to a lower risk of lung injury in high-risk preterm infants.

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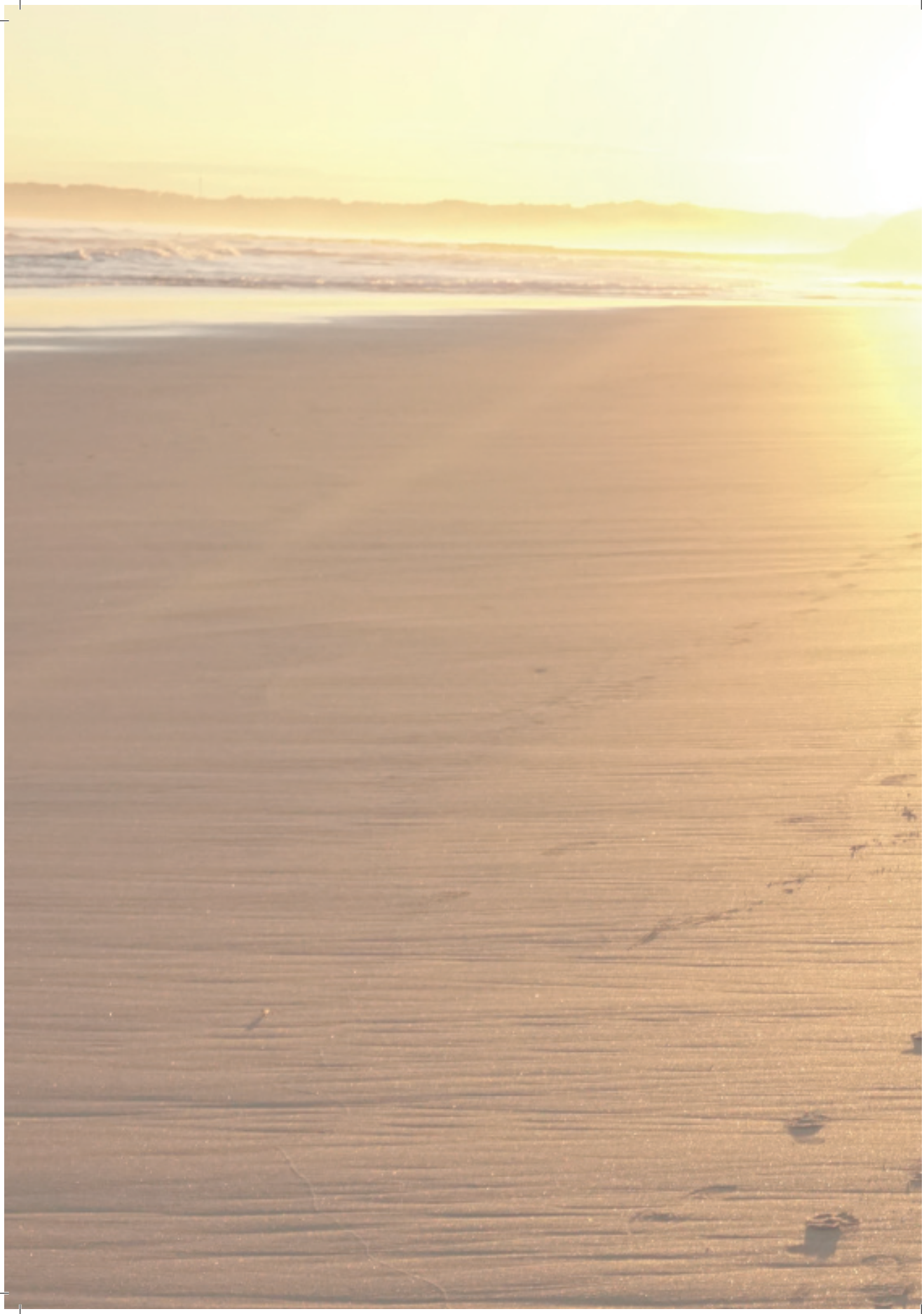

CHAPTER 6

The effect of initial high versus low FiO_2 on breathing effort in preterm infants at birth: a randomized controlled trial

J Dekker
T Martherus
E Lopriore
M Giera
EV McGillick
GJ Hutten
RW van Leuteren
AH van Kaam
SB Hooper
AB te Pas

Manuscript in preparation

ABSTRACT

BACKGROUND

Non-invasive ventilation in preterm infants at birth is hampered by closure of the glottis between breaths when breathing is intermittent. Infants are currently stabilized with initial low fraction of inspired oxygen (FiO_2) (0.21 - 0.3) which increases the risk of hypoxia and suppression of breathing in the first minutes after birth. We hypothesized that stabilization of preterm infants at birth with an initial high FiO_2 (1.0), followed by titration, would improve breathing effort when compared to an initial low FiO_2.

METHODS

In a bi-center randomized controlled trial, infants < 30 weeks gestation were stabilized at birth with an initial FiO_2 of 0.3 or 1.0, after which oxygen was titrated using the reference ranges described by Dawson et al.(12). Primary outcome was minute volume of spontaneous breathing. We also assessed tidal volumes, rate of rise to maximum tidal volumes (RoR) and respiratory rate with a respiratory function monitor. Pulse oximetry was used to measure heart rate and oxygen saturation (SpO_2). Hypoxia was defined as SpO_2 < 25th percentile and hyperoxia as SpO_2 > 95%. Differences in breathing effort and oxygenation parameters were tested with a Student's t-test or Mann-Whitney U test, depending on the distribution. The interaction of breathing effort parameters with time was analyzed with a linear mixed model.

RESULTS

52 infants were randomized (26 in 100% O_2-group, 26 in 30% O_2-group) and recordings were obtained in 44 infants (20 infants in 100% O_2-group, 24 infants in 30% O_2-group). Minute volumes were significantly higher in the 100% O_2-group (146.34 ± 112.68 mL/kg/min) compared to 30% O_2-group (74.43 ± 52.19 mL/kg/min) (p=0.014). Average tidal volumes and RoR in the first 5 minutes after birth were significantly higher in the 100% group, while the duration of mask ventilation given was significantly shorter. Oxygenation was significantly higher in infants in the 100% O_2-group (85 (64 – 93) %) compared to the 30% O_2-group (58 (46 – 67) %) (p<0.001) in the first 5 minutes after birth. The duration of hypoxia was significantly shorter in the 100% O_2-group, while the duration of hyperoxia was not different between groups.

CONCLUSION

Initiating stabilization of preterm infants at birth with 100 % oxygen led to a higher breathing effort, improved oxygenation, and a shorter duration of mask ventilation as compared to 30 % oxygen, without increasing the risk for hyperoxia.

INTRODUCTION AND RATIONALE

Spontaneous breathing movements are present even before birth, enabling small volumes of liquid to move in and out of the airways. During apnea, the glottis is predominantly closed, restraining the efflux of lung liquid.(1-3) This process is essential in allowing the lung to grow and develop. After birth, the purpose of breathing movements changes to gas exchange, which necessitates the lungs to fill with air instead of liquid. However, the function of the larynx present in the fetal situation appears to persist in newborn life. During apnea after birth, is has been shown that the glottis remains predominantly closed and opens only briefly in case of spontaneous breathing.(4-6)

While most preterm infants need respiratory support at birth because of their low muscle strength and structural and biochemical immaturity of the lungs, this is now provided non-invasively instead of invasively to reduce the risk for lung and brain injury. However, applying non-invasive respiratory support necessitates an open airway in order to aerate the lungs and support the infant in transitioning. A closed glottis during apnea hampers the effectiveness of non-invasive ventilation, demonstrated in the study of van Vonderen et al.(5) in which a non-invasively applied sustained inflation to preterm infants at birth was only effective in case of concurrent spontaneous breathing. Interventions aimed at improving effectiveness of non-invasive ventilation should therefore focus on stimulation of spontaneous breathing in order to open the glottis.

One of the factors influencing breathing activity is oxygenation. Hypoxia leads to suppression of fetal breathing movements before birth, and it is now well established that this inhibitory effect of hypoxia on breathing persists for days-weeks after birth.(7) Avoiding persisting hypoxia at birth might result in enhancing spontaneous breathing and opening of the glottis, allowing non-invasive respiratory support to be effective. This, in turn, might reduce the need for intubation and mechanical ventilation and thereby reduces the risk of lung and brain injury.

Oxygenation is largely defined by the surface area available for gas exchange and the diffusion distance as well as the partial pressure gradient for oxygen between the alveoli and adjacent capillaries. It has become clear that in most very preterm infants, clinicians fail to create adequate lung aeration and thus have to use a higher FiO_2 to compensate for the suboptimal surface area created for gas exchange. However, the 2015 versions of the international resuscitation guidelines recommend to start resuscitation with a low FiO_2 in order to reduce the risk of hyperoxia, which is associated with an increased production of oxygen free radicals leading to tissue damage in infants with an immature

antioxidant capacity.(8-11) After this initial FiO_2, oxygen is recommended to be titrated based on the 25th percentile of the oxygen saturation target ranges defined by Dawson et al.(12), which has been based on data of healthy term and preterm infants. Using this approach, we accept that infants will be at risk for hypoxia, thereby overlooking the subsequent effect on breathing effort. A number of trials have recently been performed to assess the effect of initiating stabilization of preterm infants at birth with either a high or low FiO_2. In the largest randomized controlled trial so far, recruitment had to be ceased because of a higher incidence of neonatal mortality in the low FiO_2-group.(13) However, results of the meta-analysis performed by Welsford et al.(14) were inconclusive about the ideal initial FiO_2 in relationship with clinical outcomes, due to a very low overall level of evidence. Still, it has been shown that persisting hypoxia at 5 minutes after birth is associated with a higher risk of mortality and the development of intraventricular hemorrhage.(15) This might be caused by hypoxia-induced respiratory depression, leading to a higher risk on intubation and mechanical ventilation. So far, the effect of high versus low initial FiO_2 at birth on breathing effort and oxygenation of preterm infants has not been evaluated.

We aimed to evaluate the effect of reducing hypoxia by using an initial high vs low FiO_2 (1.0 vs 0.3) on breathing effort of preterm infants at birth. We thereby hypothesize that initiating resuscitation of preterm infants at birth with a high FiO_2 will increase breathing effort.

METHODS

A single blinded randomized controlled trial was conducted at the Leiden University Medical Center (LUMC) and the Amsterdam University Medical Center (AUMC), both located in the Netherlands. Preterm infants with a gestational age between 24^{+0} - 29^{+6} weeks were included. Infants with congenital abnormalities or conditions that might have an adverse effect on breathing effort of ventilation were excluded. Infants were randomized for resuscitation with an initial FiO_2 of 0.3 (30% O_2-group) or 1.0 (100% O_2-group) using Castor EDC (Amsterdam, the Netherlands), an electronic data capture system. Allocation was stratified by gestational age (24^{+0} – 26^{+6} weeks and 27^{+0} – 29^{+6} weeks), using variable block sizes (4-6).

After the initial FiO_2 setting, FiO_2 was titrated following SpO_2 target ranges recommended in our local guidelines on stabilization after birth, which are based on the target ranges described by Dawson et al.(12) Cord clamping was performed after 30 seconds in

case of an apneic infant, and after 60 seconds if the infant was breathing at birth. In both groups, respiratory support was provided using a Neopuff™ infant T-piece resuscitator (Fisher & Paykel Healthcare, Auckland, New Zealand) or a Giraffe Star System (Anandic Medical Systems, Feuerthalen, Switzerland). Respiratory function monitoring at birth was recorded during stabilization in the first 10 minutes of life, which is standard practice in our units.

The respiratory function monitor was used to measure inflation pressures, flow and tidal volumes. The measurements were used to calculate average minute volume, RoR, and the total time of ventilation given. It uses a small variable resistance anemometer to measure gas flow in and out of a face mask or endotracheal tube. This signal is automatically integrated to provide inspired and expired tidal volumes. The difference equals the leak from the face mask or endotracheal tube. To record SpO_2 and heart rate a Masimo SET pulse oximeter probe (Masimo Radical, Masimo Corporation, Irvine, California, USA) or Nellcor™ pulse oximeter probe (Covidien, Dublin, Ireland) was placed around the right wrist or hand of the infant. The FiO_2 was measured using a portable oxygen analyzer AX300-I (Teledyne Analytical Instruments, CA, USA), and the airway pressures were registered by a variable orifice flow sensor (Avea Varflex Flow Transducer, Carefusion, Yorba Linda, CA,USA) connected to the face mask measuring the flow in and out the infant. The signals were digitized at 200 Hz using the NewLifeBox-R physiological recording system (Advanced Life Diagnostics, Weener, Germany) and all signals were recorded by the NewLifeBox Neo-RSD computer system (Advanced Life Diagnostics, Weener, Germany) supported by Polybench physiological software (Applied Biosignals, Weener, Germany). Pulmochart software (Applied Biosignals, Weener, Germany) was used to analyze primary and secondary outcomes.

The main study parameter was breathing effort, expressed as average minute volume normalized for body weight in the first 5 minutes after birth. Other parameters of breathing effort that were assessed, are: breathing rate, tidal volumes and RoR in the first 5 minutes after birth as well as the duration of ventilation given via face mask in the first 10 minutes after birth. Since Apgar scores are composed by the neonatologist often retrospectively after stabilization at birth has been completed, these could have been influenced by the received intervention. Therefore, both Apgar scores at 1 and 5 minutes after birth are considered outcomes. In addition, oxygenation of the infants was assessed by measuring SpO_2 values in the first 10 minutes and determining the amount of time that infants were considered to be hypoxic (SpO_2 < 25th percentile of the target ranges defined by Dawson et al.(12)) or hyperoxic (SpO_2 > 95%). The following demographic data were collected: gestational age, birth weight, gender, antenatal use

of corticosteroids, mode of delivery and maternal medication use. Short-term clinical outcomes were noted: intraventricular hemorrhage ≥ grade III, intubation during resuscitation or within 24 hours after birth and neonatal mortality.

In a recent study, the average minute volume of preterm infants over the first 100 breaths was 150 ± 70 mL/kg/min.(16) The study of van Vonderen et al.(17) shows an 80% relative increase in respiratory drive when FiO_2 was switched from 0.21 to 1.0, in the first minute after FiO_2 was increased (from 134 mL/kg/min to 240 mL/kg/min). However, since we investigate a FiO_2 of 0.3 instead of 0.21 in the lower oxygen arm, we expected half of the effect (40% relative increase) in respiratory drive than demonstrated in the study of Van Vonderen et al.(17) For this, a sample size of 44 infants would be needed (α of 0.05 and power $(1 - \beta)$ of 0.8, 2-sided). To anticipate a 10% fail of recording of physiological measurements, and an additional risk for withdrawment or decline of consent after inclusion of an infant, 52 infants were recruited.

The ethical committees of the LUMC and AUMC approved the study protocol. Informed parental consent was obtained antenatally when possible. In case of an emergency situation (e.g. mother in full labour or when immediate delivery was necessary) or when obtaining antenatal consent was inappropriate (e.g. if the condition of the mother did not allow for proper consideration on participation), consent was asked retrospectively. This study was registered in www.trialregister.nl, with registration number NTR6878. The study protocol is published in Frontiers in Pediatrics: https://doi.org/10.3389/fped.2019.00179.

Statistical analysis was performed with SPSS software version 23.0 (SPSS, Chicago, Illinois, 2012). Differences in parameters of breathing effort in the first 5 minutes after birth between the study groups were tested with a Student's t-test if the data are found to be parametric, or with Mann-Whitney U test when the data are non-parametric. The interaction of respiratory minute volume, tidal volumes, RoR in the first 5 minutes after birth with time were analyzed with a linear mixed model, in which both study group (treatment) and time were taken in consideration. Other study parameters and basic characteristics were explored to test for differences between the study groups. If there were found to be significant differences with clinical relevance for the primary outcome, these parameters were incorporated in the linear mixed model.

RESULTS

A total of 109 eligible infants were born in the LUMC and AUMC during the study enrolment period between January 2018 and March 2019. 58 infants could not be included in this study because antenatal parental consent was refused, it was inappropriate to approach parents for considering enrolment in the study, the study was conflicting with other trials or due to practical reasons. Therefore, a total of 52 infants were randomized for initiation of resuscitation with either 100% or 30% O_2. 6 of these infants were excluded from analysis for outcomes on breathing effort and oxygenation due to failure to record physiological parameters with the RFM and pulse oximetry, 1 infant was excluded from complete analysis because he did not receive any respiratory support and 1 infant because deferred consent was not obtained (Figure 1).

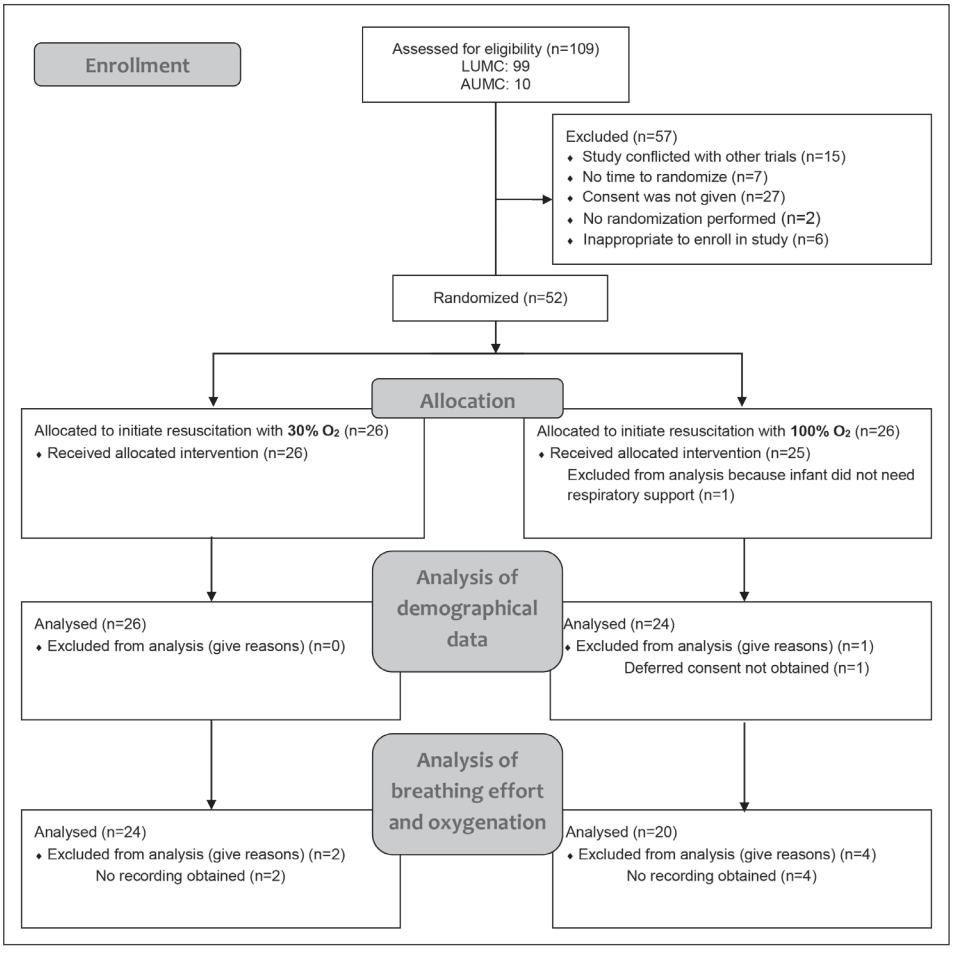

Figure 1 | CONSORT 2010 Flow chart

There are no significant differences between the 100% and the 30% O_2-group with regard to gestational age, gender, birth weight, percentage of infants that received a full course of antenatal steroids or mode of delivery in both the total group and the group where physiological parameters were recorded (Table 1, Table 2). Neither were there any differences regarding maternal medication use or complications during pregnancy that could have had an effect on respiration of the infant at birth (Table 1, Table 2).

The average FiO_2 received in the first 5 minutes after birth was significantly higher in the 100% O_2-group (100% O_2-group: FiO_2 0.91 (0.67 – 0.98) vs 30% O_2-group: FiO_2 0.48 (0.33 – 0.53), p<0.001), as was the average FiO_2 in the first 10 minutes after birth (100% O_2-group: FiO_2 0.69 (0.41 – 0.88) vs 30% O_2-group: FiO_2 0.45 (0.33 – 0.64), p=0.020).

Table 1 | Demographical data patients randomized

	100% O_2 n = 24	30% O_2 n = 26	*p*-value
Gestational age (weeks)[a]	27 $^{2/7}$ ± 1 $^{6/7}$	27 $^{1/7}$ ± 1 $^{4/7}$	0.746
Birth weight (grams)[a]	1000 ± 291	934 ± 258	0.405
Gender (% male)[c]	13/24 (54%)	9/26 (35%)	0.565
Mode of delivery (% caesarean section)[c]	14/24 (58%)	11/26 (42%)	0.396
Antenatal corticosteroids (% full course)[c]	11/24 (46%)	14/26 (54%)	0.606
Maternal medication use influencing respiration of the infant[c]	0/24 (0%)	0/26 (0%)	1.000
Complications during pregnancy[c]	7/24 (29%)	12/26 (46%)	0.255

Data are presented as mean ± SD for parametric data ([a]), median (IQR) for non-parametric data ([b]) and n (%) for categorical data ([c]).

Table 2 | Demographical data patients analyzed

	100% O_2 n = 20	30% O_2 n = 24	*p*-value
Gestational age (weeks)[b]	27 $^{6/7}$ (25 $^{2/7}$ – 28 $^{6/7}$)	27 $^{2/7}$ (25 $^{4/7}$ – 28 $^{3/7}$)	0.671
Birth weight (grams)[a]	992 ±309	931 ± 256	0.481
Gender (% male)[c]	11/20 (55%)	17/24 (71%)	0.352
Mode of delivery (% caesarean section)[c]	12/20 (60%)	10/24 (42%)	0.364
Antenatal corticosteroids (% full course)[c]	9/20 (45%)	14/24 (58%)	0.628
Maternal medication use influencing respiration of the infant[c]	0/20 (0%)	0/24 (0%)	1.000
Complications during pregnancy[c]	6/20 (30%)	12/24 (50%)	0.227

Data are presented as mean ± SD for parametric data ([a]), median (IQR) for non-parametric data ([b]) and n (%) for categorical data ([c]).

Breathing effort

The average minute volume/kg was significantly higher in the 100% O_2-group: (146.34 ± 112.68 mL/kg/min vs 74.43 ± 52.19 mL/kg/min, p=0.014). The minute volume in the first 5 minutes following birth was significantly higher in infants in whom resuscitation was initiated with 100% O_2 (area under the curve (AUC) 498.18 ± 390.10 vs 244.63 ± 168.42, p=0.014) (Figure 2), which represents a higher respiratory effort.

The average tidal volume and rate of rise to maximum tidal volumes in the first 5 minutes after birth were significantly higher in the 100% O_2-group (Table 3, Figure 3). There was a trend towards a higher breathing rate in the 100% O_2-group, although this difference did not reach statistical significance (Table 3, Figure 3). Although there was a trend towards a higher incidence of breaths with a tidal volume > 4 mL/kg (49 ± 25% vs 35 ± 24%, p=0.056), the incidence of recruitment breaths (> 8 mL/kg) did not differ between the study groups (Table 3).

Table 3 | Parameters of respiratory effort

	100% O_2 n = 20	30% O_2 n = 24	*p*-value
Average tidal volumes in first 5 minutes[a]	4.8 ± 3.8	3.8 ± 3.7	0.006
Average rate of rise to maximum tidal volume in first 5 minutes[a]	12.7 (5.7 – 18.0)	7.8 (3.3 – 13.3)	0.014
Breathing rate[b]	33 (14 – 52)	26 (11 – 36)	0.099
Time of mask ventilation in the first 10 minutes (s)[b]	23.6 (0.0 – 122.2)	108.3 (46.4 – 205.1)	0.021
Incidence of breaths with tidal volume > 4 mL/kg[a]	50 ± 24	35 ± 24	0.056
Incidence of recruitment breaths (> 8mL/kg)[b]	6 (1 – 24)	5 (0 – 18)	0.873

Data are presented as mean ± SD ([a]) and median (IQR) for non-parametric data ([b]).

The duration of mask ventilation was significantly shorter in the 100% O_2-group (23.6 (0.0 – 122.2) s vs 108.3 (46.4 – 205.1) s, p=0.021). While Apgar scores at 1 minute after birth were not significantly different between the study groups (6 (3 – 8) vs 5 (3 – 7), p=0.149), infants in the 100% O_2-group showed significantly higher Apgar score at 5 minutes after birth (8 (8 – 9) vs 8 (7 – 8), p=0.028).

Oxygenation

The average oxygen saturation in the first 5 minutes after birth was significantly higher in infants in the 100% O_2-group (85 (64 – 93) % vs 58 (46 – 67) %, p<0.001). In addition, there was a trend towards a higher heart rate in the 100% O_2-group (Table 4). Infants

in the 100% O_2-group had SpO_2 values between 90 - 95% for a significantly higher percentage of time in the first 5 minutes after birth, while at 5 - 10 minutes after birth this was not significantly different between the groups (Figure 4). A SpO_2 of 80 % was reached significantly earlier in the 100% O_2-group (57 (2 – 130) s after birth vs 133 (80 – 186) s after birth, p=0.013). In addition, the duration of hypoxia (SpO_2 < 25th percentile of reference ranges described by Dawson et al.(12)) was significantly shorter in the 100% O_2-group (Table 4). However, the duration of hyperoxia (SpO_2 > 95%) did not significantly differ between the study groups.

Table 4 | Parameters of oxygenation

	100% O_2 n = 20	30% O_2 n = 24	*p*-value
Average SpO_2 in first 5 minutes[b]	85 (64 – 93)	58 (46 – 67)	< 0.001
Average heart rate in first 5 minutes[a]	134 ± 24	120 ± 28	0.086
Duration of hypoxia (< 25th percentile of Dawson target ranges) in first 10 minutes (s)[b]	73 (0 – 189)	158 (116 – 184)	0.018
Duration of hyperoxia in first 10 minutes (s)[b]	99 (24 – 215)	79 (15 – 152)	0.394
Percentage of time of SpO_2 90-95% in first 5 minutes[b]	15 (2 – 38)	1 (0 – 9)	0.002
Percentage of time of SpO_2 90-95% in 5-10 minutes[b]	30 (21 – 41)	35 (19 – 58)	0.556
Incidence of intubation in delivery room[c]	0/20 (0%)	2/24 (8%)	0.493

Data are presented as mean ± SD for parametric data ([a]), median (IQR) for non-parametric data ([b]) and n (%) for categorical data ([c]).

Short-term clinical outcomes

Only 2 infants of the total study population needed intubation in the delivery room, both infants were randomized to the 30% O_2-group. There were no differences between the study groups with regard to intubation, incidence of IVH ≥ grade III or neonatal mortality (Table 5).

Table 5 | Short-term clinical outcomes

	100% O_2 n = 24	30% O_2 n = 26	*p*-value
Incidence of intubation in the delivery room	0/24 (0%)	2/26 (8%)	0.491
Incidence of intubation < 24 hours after birth	8/24 (33%)	7/26 (27%)	0.760
Incidence of IVH ≥ grade III	0/24 (0%)	4/26 (15%)	0.111
Incidence of neonatal mortality	2/24 (8%)	5/26 (19%)	0.420

Data are presented as n (%).

DISCUSSION

In this bi-center single blinded randomized controlled trial, the effect of initiating resuscitation with an FiO_2 of either 0.3 or 1.0 on breathing effort was evaluated. We demonstrated that breathing effort was significantly higher in preterm infants who started initially with 100% O_2 during stabilization as compared to infants initiating with 30% O_2, with minute volumes that were almost twice as high in the 100% O_2-group. Infants in the 100% O_2-group were significantly better oxygenated in the first 5 minutes after birth, with a shorter duration of hypoxia without an increase in hyperoxia. The better oxygenation and breathing effort are also reflected by a shorter duration in mask ventilation given in the 100% O_2-group. These results indicate that initiating with a high level of oxygen followed by careful titration to avoid hyperoxia, is a better option for stimulating breathing and decreasing the need for positive pressure ventilation.

The effectiveness of non-invasive ventilation is hampered in case of a closed glottis during apnea, preventing air from entering the lung. While non-invasive respiratory support is universally adopted as primary respiratory support for preterm infants at birth, the closure of the glottis between breaths underscores that stimulating spontaneous breathing is important for the success of this non-invasive approach. Evaluating the effect of interventions used in the delivery room on breathing effort of preterm infants is therefore important to guide clinicians in prioritizing interventions to increase the effectiveness of non-invasive respiratory support. Although there are multiple randomized trials performed on comparing the effect of a high versus low initial FiO_2 during stabilization of preterm infants at birth, none of these trials has evaluated breathing effort.(13, 18-22) The effect of oxygenation on breathing effort has so far only been demonstrated in an observational study where an increase in breathing effort was observed after switching FiO_2 from 0.21 to 1.0.(17) However, we recently demonstrated the effect of the use of 100% oxygen at birth on breathing effort in a spontaneously breathing preterm rabbit model (unpublished data). When compared to stabilization with room air, 100% oxygen led to a more stable breathing pattern with a lower variability in inter-breath interval, higher respiratory rate and less apnea. We now observed similar results in the current clinical study in preterm infants, even when oxygen was titrated instead of a continuous FiO_2 of 1.0 used in the preterm rabbit model.

The effect of other interventions used in the delivery room on breathing effort has been described previously.(23, 24) Applying a strict protocol of repetitive tactile stimulation to preterm infants at birth showed a non-significant but clinically relevant increase of breathing effort, as compared to stimulation on discretion of the caregiver.(23) In

addition, infants who have been administered caffeine in the delivery room showed a significantly higher breathing effort.(24) However, the magnitude of the difference in MV that is shown between the 100% O_2-group and the 30% O_2-group in the current study was not demonstrated before in any other trial on breathing effort of preterm infants at birth. This indicates that oxygen might be the major contributor to improving breathing effort at birth.

Oxygenation was significantly higher in infants in the 100% O_2-group, who showed significantly higher SpO_2 values, with a shorter duration of hypoxia, as compared with infants in the 30% O_2-group. Next to the initial FiO_2, all infants in this study were managed with the same respiratory support strategy. Additionally, the duration of mask ventilation needed by infants in the 100% O_2-group was significantly shorter compared to the 30% O_2-group. Therefore, the partial pressure gradient for oxygen is likely to be the major contributor to the improved oxygenation in the 100% O_2-group. Other trials on comparing high versus low initial oxygen concentrations for resuscitation of preterm infants at birth show conflicting results: SpO_2 values of infants initiating resuscitation with a low FiO_2 (<0.3) were significantly lower when compared to a high FiO_2 (>0.9),(13, 18, 20) whereas other trials detected no significant differences in oxygenation.(19, 21, 22) However, differences in titration protocols might have influenced these results, and there is heterogeneity in timepoints at which these outcomes have been assessed. Probably just as important is the prevention of hyperoxia, known to cause damage to tissues as well by an increased free oxygen radical production. However, there were no significant differences in the duration of hyperoxia between infants initiating resuscitation with either 30% or 100% O_2.

Improving oxygenation in the first 5 minutes after birth has gained increased attention, as it has become clear that persistent hypoxia after 5 minutes after birth is associated with an increased risk for IVH and mortality.(15) As hypoxia inhibits breathing both in the fetal situation as well as after birth, persistent hypoxia could reduce the effect of non-invasive ventilation resulting in an increased need for intubation and mechanical ventilation.(7) Our trial was not designed to detect significant differences in clinical outcomes. However, it should be noted that IVH and mortality occurred non-significantly more often in the 30% O_2-group.

The recent systematic review including trials on high versus low FiO_2 during resuscitation of preterm infants at birth showed no difference in clinical outcomes. However, the level of evidence of included studies was assessed to be very low. In our study we aimed to provide knowledge on one of the factors that could contribute to improve breathing

effort at birth. It was shown that oxygen is a major contributing factor, next to other factors that have previously been shown to increase breathing effort, such as tactile stimulation and caffeine administration in the delivery room.(23, 24) Incorporating these factors in the resuscitation guidelines on delivery room management might result in an increase in effectively supporting preterm infants non-invasively, leading to less intubation and mechanical ventilation and better clinical outcomes.

CONCLUSION

In this randomized controlled trial, we have demonstrated significantly higher breathing effort when resuscitation was initiated with 100% O_2 compared to 30% O_2. In addition, infants in the 100% O_2-group were significantly better oxygenated with a shorter duration of hypoxia without increasing the duration of hyperoxia. These results suggest that oxygenation might be a primary determinant in stimulating breathing and decreasing the need for positive pressure ventilation in preterm infants at birth.

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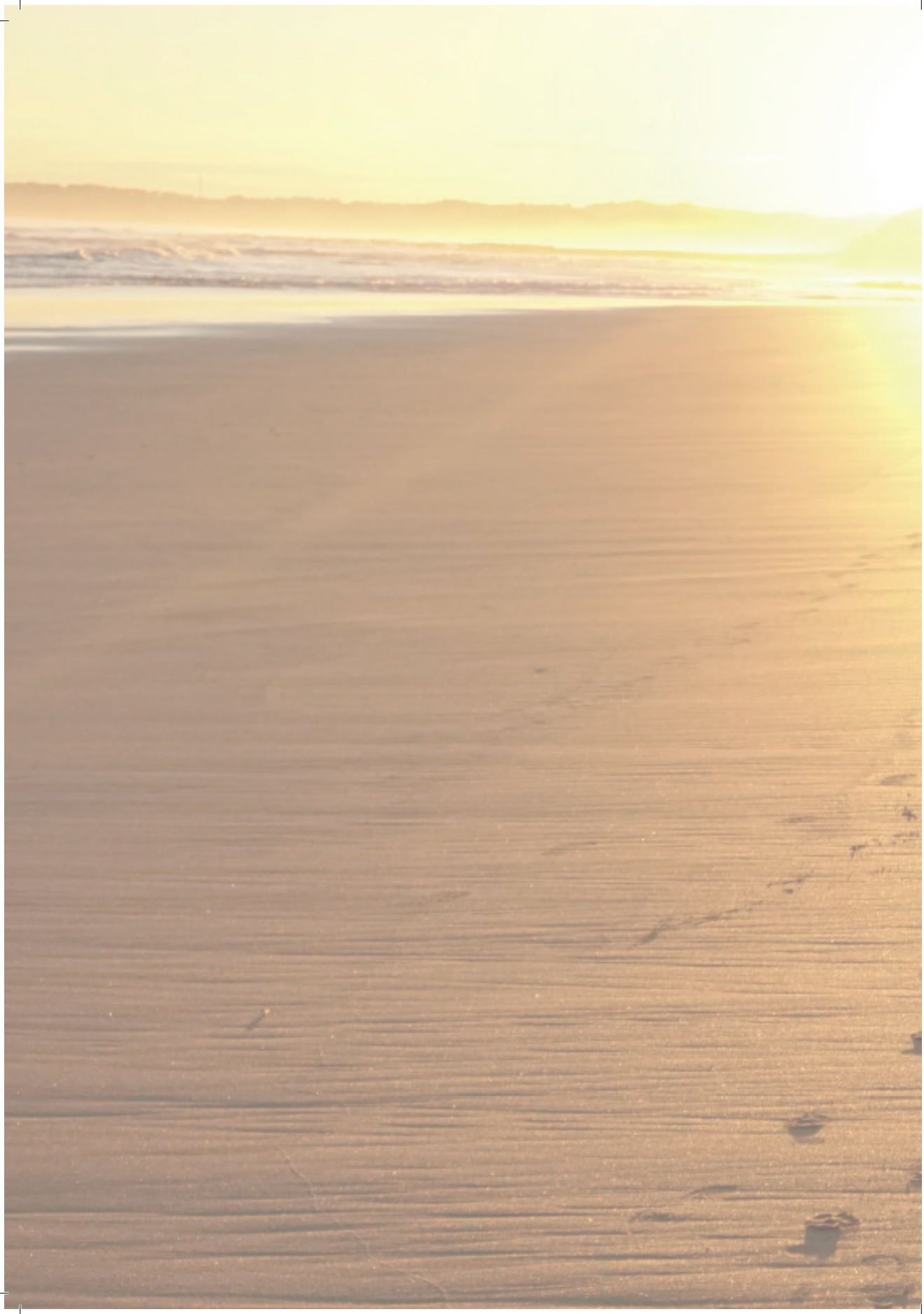

CHAPTER 7

Caffeine to improve breathing effort of preterm infants at birth: a randomized controlled trial

J Dekker
SB Hooper
JJ van Vonderen
RSGM Witlox
E Lopriore
AB te Pas

Pediatric Research 2017;82(2):290-296

ABSTRACT

BACKGROUND

Caffeine promotes spontaneous breathing by antagonizing adenosine. We assessed the direct effect of caffeine on respiratory effort in preterm infants at birth.

METHODS

30 infants of 24 – 30 weeks of gestation were randomized for receiving caffeine directly after birth in the delivery room (caffeine DR-group) or later in the neonatal intensive care unit (control-group). Primary outcome was respiratory effort, expressed as minute volume, tidal volumes, respiratory rate, rate of rise to maximum tidal volume (RoR) and recruitment breaths at 7 - 9 minutes after birth.

RESULTS

After correction for gestational age, minute volumes ((mean ± SD) 189 ± 74 mL/kg/min vs 162 ± 70 mL/kg/min, $p<0.05$) and tidal volumes ((median (IQR) 5.2 (3.9 – 6.4) vs 4.4 (3.0 – 5.6) mL/kg, $p<0.001$) were significantly greater in caffeine DR-group. Although respiratory rates were similar ((mean ± SD) 35 ± 10 breaths/minute vs 33 ± 10 breaths/minute), RoR increased significantly ((median (IQR) 14.3 (11.2 - 19.8) mL/kg/s vs 11.2 (7.9 – 15.2) mL/kg/s, $p<0.001$) and more recruitment breaths were observed (13% vs 9%, $p=0.001$).

CONCLUSION

Caffeine increases respiratory effort in preterm infants at birth, but the effect on clinical outcomes needs further investigation.

INTRODUCTION AND RATIONALE

During the transition after birth, lung aeration is pivotal for the changes in respiratory and cardiovascular function that are required for survival.(1, 2) Although most preterm infants breathe at birth, their respiratory drive is weak and often insufficient to aerate their lungs and establish gas exchange.(3-7) To minimize injury, intubation and mechanical ventilation is now avoided, and the focus of respiratory care has shifted to non-invasive ventilation, involving positive pressure support of breathing and/or ventilation via face mask.(6, 8)

Recent studies have demonstrated that intermittent positive pressure ventilation (IPPV) applied via mask in infants with insufficient respiratory effort, was often ineffective unless the infant took breaths during IPPV.(3) Mask ventilation is often hampered not only by leak, but also by obstruction caused by active larynx adduction.(3, 4, 9) Antenatally, active larynx adduction during apnea is needed for lung expansion and lung growth(9), and this pattern of activity might temporarily persist at birth. Logically, the best way to open the larynx is to stimulate spontaneous breathing, which is also likely to be the most gentle and effective way of providing respiratory care without causing injury**.** Currently, only tactile stimulation is recommended to stimulate breathing.(10-12)

Caffeine promotes breathing in infants by antagonizing the suppressive effects of adenosine and is a safe and effective treatment for apnea of prematurity.(13, 14) When caffeine is given in the first hours after birth, diaphragmatic activity increased at 5 minutes after caffeine administration, which correlates with an increased tidal volume (Vt).(15) Also, the need for vasopressors and the incidence of intubation are lower when caffeine is given in the first few hours after birth, compared with that given after 12 hours.(16) In addition, the incidence of bronchopulmonary dysplasia was lower when caffeine was given in the first 2 days of life.(17, 18) Although these studies demonstrated the effects of caffeine in the first days after birth, there is little data on the direct effect of caffeine on respiratory effort or whether gestational age (GA) influences the effect of caffeine. Bairam et al.(19) showed in 2 – 5 day-old term lambs that Vt_i increased after a loading dose of caffeine. Kraaijenga et al. confirmed this in 1 – 4 day-old preterm infants by measuring diaphragmatic activity after caffeine administration. (15, 19) Within 5 minutes after administration, diaphragmatic activity increases, resulting in a 30% increase in tidal volumes.(15) Higher tidal volumes could potentially lead to greater lung recruitment and improved oxygenation, which can result in an increased respiratory drive.(20) We hypothesized that this effect of caffeine can be duplicated when caffeine is administered directly after birth.

We aimed to evaluate the respiratory effort of preterm infants at birth when caffeine was either administered directly after birth in the delivery room or after admittance to the neonatal intensive care unit (NICU).

METHODS

An unblinded randomized controlled trial was conducted at the Leiden University Medical Center. Preterm infants born between 24^{+0} - 29^{+6} weeks of gestation were included. We excluded infants with congenital abnormalities or conditions that might have an adverse effect on breathing or ventilation, including congenital diaphragmatic hernia, tracheoesophageal fistula, or cyanotic heart disease. Infants participating in other trials on respiratory effort were also excluded. Infants were randomized using sequentially numbered sealed envelopes to either receive a loading dose of caffeine base (10 mg/kg, based on estimated or measured birth weight) at birth in the delivery room (caffeine DR-group) or later after arrival in the NICU (caffeine NICU-group). Consecutive caffeine base therapy was given in both groups according to the local protocol (5 mg/kg, 24 h after loading dose). Allocation was stratified by GA (24^{+0} – 26^{+6} or 27^{+0} – 29^{+6} weeks) using blocks of 4. When randomized for caffeine administration in the delivery room, a butterfly needle (21G) that was prefilled with saline and attached to a saline-filled 5 mL syringe was inserted in the umbilical vein just above the umbilicus after a chlorhexidine swap. Endovascular location of the needle tip was confirmed by flushing in saline and withdrawing blood. A bolus of caffeine was administered within the first 7 minutes after birth. After the administration of caffeine, a further 2 mL flush of saline was given.

None of the mothers received general anesthesia or iv narcotics close to delivery. In both groups, standard care was provided in both the delivery room and after arrival in the NICU. A Neopuff™ infant T-piece resuscitator (Fisher & Paykel Healthcare LTD, Auckland, New Zealand) was used for respiratory resuscitation. For stabilization at birth, local resuscitation guidelines were followed, including delayed cord clamping and early start of continuous positive airway pressure (CPAP) for all infants with a GA < 32 weeks. In addition, a sustained inflation of 15 s with a pressure of 20 cm H_2O was given to apneic and bradycardic infants. A second sustained inflation of 15 s with a pressure of 25 cm H_2O was given when infants remained apneic and bradycardic, after which IPPV was started. Intubation was considered when effectiveness of mask ventilation could not be guaranteed or prolonged mask ventilation was needed. Respiratory function monitoring (RFM) was recorded at birth, which is also standard clinical care in our

centre to have a direct feedback device for respiratory support. This monitor was used to measure inflation pressures, gas flow, Vt, average minute volume (MV), and average rate of rise to maximum tidal volume (RoR). The monitor utilizes a small (dead space 1 mL) variable orifice anemometer to measure gas flow in and out of a face mask. This signal was automatically integrated to provide inspired and expired tidal volume. RFM measurements were collected during the first 10 minutes after birth. All signals measured were digitized and recorded at 200 Hz using the New Life Box physiological recording system with Polybench software (Advanced Life Diagnostics, Weener, Germany). Pulmochart software (Advanced Life Diagnositcs, Weener, Germany) was used for analyzing recorded data. Oxygenation and heart rates were measured with a Masimo SET pulse oximeter (Masimo corporation, Irvine, CA, USA). The pulse oximetry probe was placed around the ulnar aspect of the infant's right wrist. Fraction of inspired oxygen (FiO_2) was measured with a Teledyne oxygen analyzer (Teledyne Analytical Instruments, City of Industry, CA, USA) inserted into the inspiratory limb of the Neopuff circuit.

The primary outcome was respiratory effort, expressed as MV of spontaneous breathing at 7 - 9 minutes after birth. Also, the Vt, respiratory rate, the RoR and recruitment breaths (% of Vt > 8 mL/kg) were used as parameters of respiratory effort. Maximum FiO_2, oxygen saturation (SpO_2), and heart rate at 7 - 9 minutes after birth were also measured. Analyses were performed on these outcomes at 7 - 9 minutes after birth; as we assumed that caffeine could be administered within the first 7 minutes after birth, most infants would be respiratory stable at 7 minutes, and were transported to the NICU 10 minutes after birth.

To observe the direct effect of caffeine we compared the MV in the minute before (period 0) with the minute after (period 1) caffeine administration in the caffeine DR-group. The frequency of expiratory holds and panting were also compared, using the definitions previously described by te Pas et al.(21) Basic characteristics of all included infants were collected: GA, birth weight, gender, Apgar score, antenatal use of corticosteroids, mode of delivery, the need for a sustained inflation and cord pH and BE. Data were also collected on short-term clinical outcomes as intraventricular hemorrhage, intubation, and need for surfactant administration during the admission on the NICU.

Based on the previous studies of Bairam et al. and Kraaijenga et al.(15, 19) that examined the direct effect of caffeine, we calculated that with an α of 0.05 and β of 0.8, 26 infants were needed to detect a 150 mL/kg/min increase in MV with a SEM of 60.
The ethical committee of the Leiden University Medical Center approved the study

protocol. Parental informed consent was obtained prior to randomization, and in emergency situations with immediate delivery required or when approaching parents for antenatal consent was not considered appropriate, consent was obtained retrospectively as soon as possible after birth.

This study was registered in www.trialregister.nl, with registration number NTR4896.

Statistical analysis was performed with SPSS software version 23.0 (SPSS, Chicago, Illinois). The parameters of both groups were tested for normality using the Kolmogorov-Smirnov and Shapiro-Wilkinson test. The caffeine DR-group and caffeine NICU-group were compared using Student's t-test for parametric and the Mann-Whitney U test for non-parametric comparisons for continuous variables, and the χ^2 test for categorical variables. Results are presented as mean ± SD for normally distributed values or median (IQR) for non-normally distributed values. P-values <0.05 were considered statistically significant. Reported p-values are two-sided. MV was calculated using only tidal volumes recorded without mask leakage, and dividing it by the number of seconds for which data were present. Of all other study parameters, calculations were made based on available data. Despite randomization of infants, a significant and clinically important baseline imbalance in GA between the two groups was discovered. As a result, we performed a post-hoc analysis to adjust the outcome parameters for the GA and examined the relationship between GA and MV together and separately in both groups using PRISM software (Irvine, CA, USA). Reported results are corrected for GA.

RESULTS

Between November 2014 and August 2015, 76 eligible infants were born in the Leiden University Medical Center with a GA of 24 - 30 weeks. In total, 23 infants were not included because the study was conflicting with other trials, the monitor was not available or consent was not given. Also, 23 infants of 27-30 weeks' gestation were not included as we reached full recruitment for that stratification group.

Therefore, 30 infants were randomized. Of these, 4 infants were excluded because of failure to obtain recordings (1 infant in caffeine DR-group, 3 infants in caffeine NICU-group), and 1 infant was excluded because the timing of administration of caffeine exceeded the time stated in the protocol. Two infants (one infant in each group) remained apneic at 7 - 9 minutes after birth, and outcome parameters could not be obtained from these infants. Therefore, 23 infants were analyzed - 13 in the caffeine

DR-group and 10 in the caffeine NICU-group (Figure 1). A total of 1840 spontaneous breaths were analyzed - 1061 in the caffeine DR-group and 779 in the caffeine NICU-group. Of 23 infants included in analysis, 16/23 (70%) of parents gave informed consent retrospectively.

Infants included in the caffeine DR-group had a significantly lower GA, despite randomization (Table 1). There were no differences in birth weight, gender, Apgar score at 5 minutes, use of antenatal corticosteroids, mode of delivery, need for sustained inflation, cord pH or Base Excess at birth. Caffeine was given at a median (IQR) time of 4.4 (3.6 – 5.5) minutes after birth in the caffeine DR-group, and at 48 (32.8 – 107.3) minutes after birth in the caffeine NICU-group.

Respiratory effort at 7 – 9 minutes after birth

At 7 - 9 minutes after birth, MV was significantly (p=0.006) related to GA (Figure 2). When corrected for GA, MV was significantly greater in the caffeine DR-group than in the caffeine NICU-group (189 ± 74 mL/kg/min vs 162 ± 70 mL/kg/min, p<0.05, OR (95%

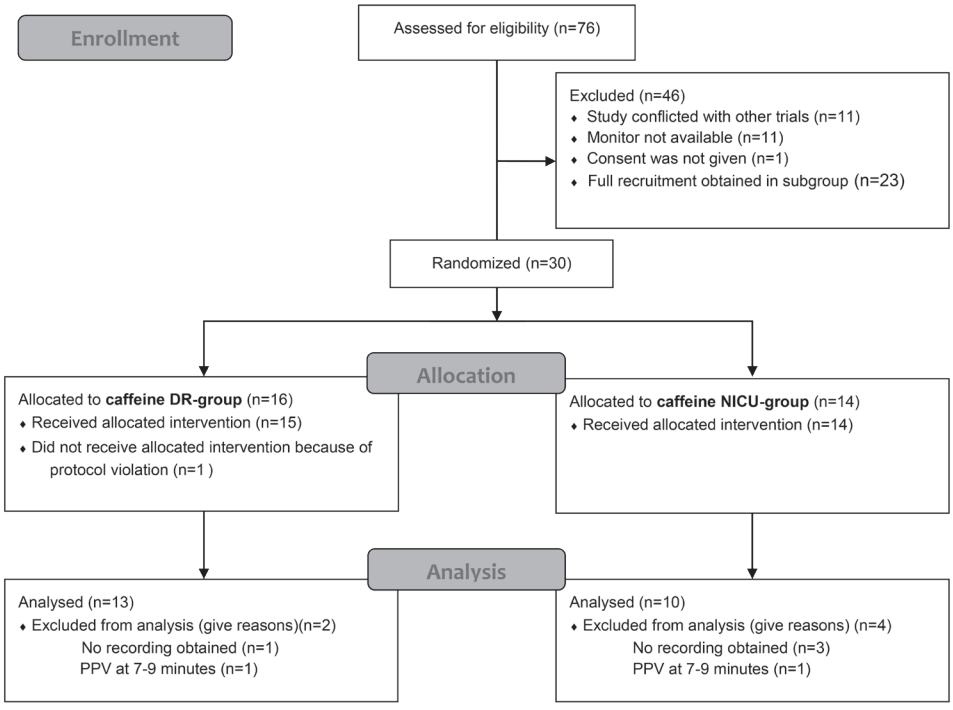

Figure 1 | CONSORT flow diagram

CI) 53.1 (18.0 – 88.3)). When analyzed within each group (Figure 2), the relationship between MV and GA was significant in the caffeine DR-group (p=0.0008; slope = 4.1 mL/min/kg/day), but not in the caffeine NICU-group (p=0.09; slope = 2.3 mL/min/kg/day).

Vt, RoR, percentage of recruitment breaths and heart rate were significantly higher (all p<0.001) in the caffeine DR-group as compared to the caffeine NICU-group (Table 2). There were no differences in respiratory rate, maximum FiO_2 and SpO_2 (Table 2).

Table 1 | Demographical data

	Caffeine DR-group	Caffeine NICU-group	*p*-value
Gestational age (weeks)b	27 (26 – 28)	28.5 (27 – 29)	0.049
Birth weight (grams)[b]	870 (767 – 1198)	960 (731 – 1450)	ns
Gender (% male)[c]	7/13 (54%)	6/10 (60%)	ns
Apgar score at 5 minutes[b]	9 (7 – 9)	8 (7 – 9)	ns
Antenatal use of corticosteroids[c]	13/13 (100%)	10/10 (100%)	ns
Mode of delivery (% caesarean section)[c]	5/12 (42%)	6/10 (60%)	ns
Sustained inflation[c]	9/13 (69%)	9/10 (90%)	ns
Cord pH after birth[b]	7.33 (7.22 – 7.36)	7.26 (7.20 – 7.29)	ns
Cord BE after birth[a]	-4.6 ± 3.1	-5.2 ± 2.6	ns
Intubation during NICU admission[c]	7/13 (54%)	4/10 (40%)	ns
Surfactant administration[c]	9/13 (69%)	5/10 (50%)	ns
Endotracheally	5/13 (39%)	2/10 (20%)	ns
Minimal invasively	4/13 (31%)	3/10 (30%)	ns
IVH (all grades)[c]	3/13 (23%)	3/10 (30%)	ns

Data are presented as mean ± SD for parametric data ([a]), median (IQR) for non-parametric data ([b]) and n (%) for categorical data ([c]).

Direct effect of caffeine

In the caffeine DR-group, both the spontaneous breathing MV ((mean ± SD) 236.8 ± 106.6 mL/kg vs 134.8 ± 103.9 mL/kg, p<0.05, OR (95% CI) 39.2 (-1.5 – 79.9)) and inspired tidal volumes ((median (IQR)) 5.3 (3.8 – 8.2) mL/kg vs 4.1 (2.4 – 7.2) mL/kg, p<0.001, OR (95% CI) 1.4 (0.9 – 1.9)) were significantly greater in the minute after caffeine administration compared with that in the minute before caffeine administration. There were no significant differences in breathing pattern (percentage of time panting, percentage of expiratory holds) between the minutes before and after caffeine administration for infants receiving caffeine directly after birth (Table 3).

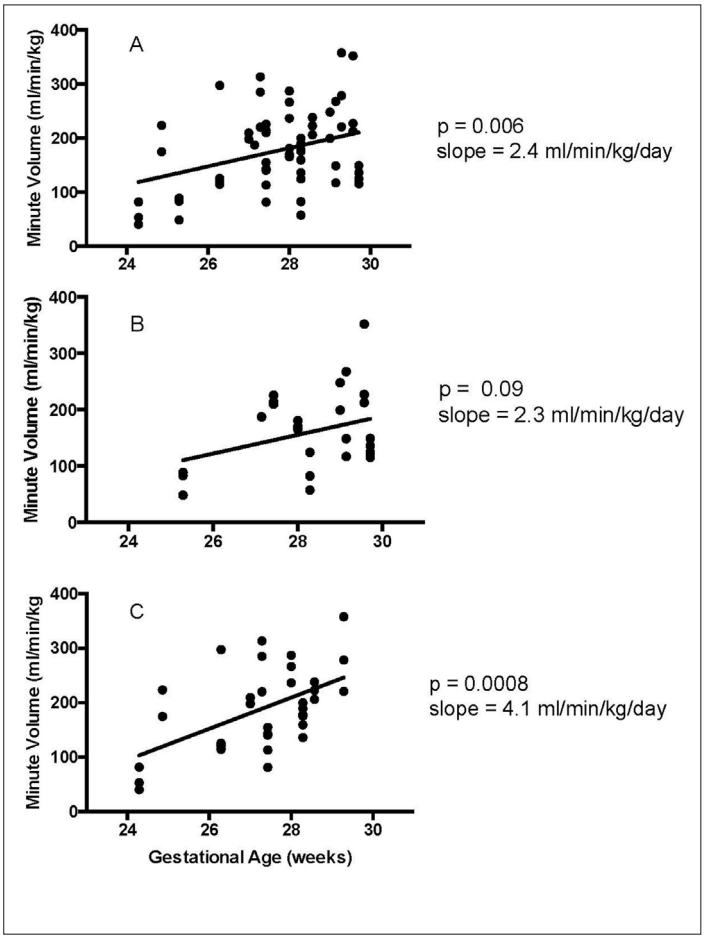

Figure 2 | Minute volume in relation to gestational age

Short-term clinical outcomes

There were no differences between the groups in short-term clinical outcomes. Intubation rates following NICU admission, surfactant administration, either by endotracheal tube or by minimal invasive surfactant therapy, and all grades of intraventricular hemorrhage were similar in the caffeine DR-group and caffeine NICU-group, respectively (Table 1).

Table 2 | Respiratory parameters at 7 - 9 minutes

	Caffeine DR-group	Caffeine NICU-group	*p*-value	OR (95% confidence interval)
Minute volume (mL/kg)[a]	189 ± 74	162 ± 70	< 0.05	53.1 (18.0 – 88.3)
Average rate of rise to maximum tidal volumes (mL/kg/s)[b]	14.3 (11.2 – 19.8)	11.2 (7.9 – 15.2)	< 0.001	5.5 (4.8 – 6.3)
Average inspired tidal volume (mL/kg)[b]	5.2 (3.9 – 6.4)	4.4 (3.0 – 5.6)	< 0.001	0.8 (0.5 – 1.0)
Recruitment (number of breaths with inspired tidal volume/kg > 8 mL)[c]	142/1061 (13%)	67/779 (9%)	0.001	1.6 (1.2 – 2.2)
Respiratory rate/minute[a]	35 ± 10	33 ± 10	ns	4.7 (-0.5 – 9.9)
Heart rate (bpm)[b]	157 (141 – 170)	146 (135 – 160)	< 0.001	9.7 (8.2 – 11.2)
Maximum FiO_2[a]	0.55 ± 0.21	0.63 ± 0.27	ns	10.0 (-15.7 – 35.7)
FiO_2 when leaving the delivery room[b]	0.35 (0.31 – 0.40)	0.39 (0.32 – 0.63)	ns	7.8 (-12.2 – 27.7)
SpO_2 (%)[b]	91 (87 – 94)	91 (88 – 94)	ns	-0.2 (-0.7 – 0.4)

Data are presented as mean ± SD for parametric data ([a]), median (IQR) for non-parametric data ([b]) and n (%) for categorical data ([c]).

Table 3 | Difference in breathing pattern

	Minute before caffeine	Minute after caffeine	*p*-value	OR (95% confidence interval)
Percentage of expiratory holds[a]	43.5 ± 16.8	43.1 ± 28.7	ns	8.0 (-9.5 – 25.5)
Percentage of time panting[b]	0 (0 – 18)	0 (0 – 6)	ns	-0.8 (-7.8 – 6.1)

Data are presented as mean ± SD for parametric data ([a]) and median (IQR) for non-parametric data ([b]).

DISCUSSION

In this randomized controlled trial, we found that MV at 7 – 9 minutes after birth was significantly greater in the caffeine DR-group than in the caffeine NICU-group, after correction for GA. Although respiratory rate did not increase, all other parameters assessing respiratory effort increased significantly by caffeine administration, including Vt, RoR and the percentage of recruitment breaths. We also observed an immediate increase in respiratory effort in the minute after caffeine administration in caffeine-treated infants. There was a moderate but significant increase in heart rate, but oxygen need and SpO_2 did not differ between the groups. These results indicate that caffeine at birth could play a role in stimulating breathing during transition at birth.

Studies have reported the effect of caffeine in preterm infants with apnea of prematurity(13, 14), whereas the focus of more recent studies has shifted towards the timing of the caffeine administration and its effect on breathing and ventilation.(15) Our study confirmed the increase in Vt_i observed in the study of Kraaijenga et al.(15), although they did not report the immediate effect of caffeine and the infants were 1 - 4 days old. They observed increased diaphragmatic activity at 5 minutes after caffeine administration and increased Vt_i, measured using inductive plethysmography, in the first hour after administration.

Bairam et al.(19) observed a large increase in MV when caffeine was administered to 2 - 5 day-old term lambs with well-established respiratory rhythms. Based on this stimulatory effect in lambs, we expected caffeine to stimulate MV and other respiratory parameters directly after birth in preterm infants. In our study, the stimulatory effect of caffeine was clear with regard to increasing RoR, Vt_i and frequency of recruitment breaths. We also found a significant (p=0.006) relationship between MV and GA in all infants at 7 - 9 minutes after birth (Figure 2). As the GA was different between the groups, despite randomization, we adjusted MV for GA. Adjusted for GA, MVs were significantly greater in caffeine treated infants than control infants. Furthermore, in caffeine treated infants, we found that the relationship between MV and GA was highly significant (p=0.0008), with MV increasing at a rate of 4.1 mL/min/kg/day. In contrast, in control infants the relationship between MV and GA just failed to reach statistical significance (p=0.09), because of the small number of observations, with MV tending to increase at only 2.3 mL/min/kg/day. These findings indicate that caffeine enhances the GA-related increase in MV and that the stimulatory effect of caffeine on MV increases with GA.

Te Pas et al.(21) described that braked expirations occur often in preterm and term infants, but expiratory holds occurred significantly more often in preterm infants. In this situation, the glottis closure combined with abdominal muscle contraction and increase in intrathoracic pressure helps to maintain functional residual capacity. In our study, the percentage of expiratory holds in the minute after caffeine administration did not differ from the minute before caffeine, but at 7 – 9 minutes after birth the percentage of expiratory holds was significantly greater in the caffeine DR-group. The increase in respiratory effort observed at 7 – 9 minutes after birth might be explained by a change in breathing pattern.

Caffeine antagonizes adenosine at its receptor, and thereby stimulates the respiratory center in the medulla, and increases skeletal muscle tone, diaphragmatic contractility

and the sensitivity to carbon dioxide.(22, 23) The levels of adenosine, however, may vary considerably in infants under different conditions, such as the mode of delivery and the presence of hypoxemia.(24) Irestedt et al.(24) showed higher plasma adenosine concentrations in infants born vaginally compared to infants born with caesarean section, possibly due to hypoxemia caused by uterine contractions. Indeed, in piglets in the first 3 days after birth, hypoxemia can increase adenosine levels and reduce MVs.(25) However, administration of aminophylline, a xanthine-derivative, counteracted the effect of adenosine and increased MVs by 43.5 %, an effect that persisted for at least 6 minutes despite persisting hypoxemia.(25) Therefore, the effect of caffeine may also differ at birth because of different levels of hypoxemia. It is possible that different dosages of caffeine are needed to compete with the level of adenosine present at birth or to overcome other variables.

In this study, a bolus of 1 mL/kg of caffeine, followed by a 2 mL flush of saline, was administered in the caffeine DR-group. The effect of the administration of a volume bolus on cerebral perfusion is a well-discussed subject. In the study by Kooi et al.(26), cerebral perfusion did not improve after the volume expansion (15 mL/kg) in the first day of life in infants with a GA of 25 - 28 weeks, although this might be caused by the presence of an adequate cerebral autoregulation. In the present study, it is not clear how the administration of the small bolus of caffeine and saline might have influenced cerebral outcomes. There are additional factors influencing these outcomes, as mentioned by Rajani et al.(27) in their review on delivery room management. The use of positive pressure ventilation and rapid $PaCO_2$ changes influence cerebral blood flow, thereby increasing the risk of intraventricular hemorrhage. However, in our study there were no differences between the caffeine DR-group and caffeine NICU-group by means of any grade of intraventricular hemorrhage or periventricular leucomalacia.
This study is limited by the lack of a placebo-controlled group. It is theoretically possible that the flush of saline given, before caffeine was administered, could have a positive effect on the breathing effort. When reviewing the monitor recordings, we did not observe any effect when a flush of saline was given before the administration of caffeine; however, the caregivers were not blinded for the allocated intervention when reviewing the monitor recordings.

This study is also methodologically limited by excluding study participants and thereby not reaching the proposed sample size. Infants were excluded because of failure of the recordings, which was only visible during analysis. Therefore, no additional infants could be included. Another methodologic limitation is the sample size calculation, which was based on experimental data from 2 – 5 day-old lambs, and data from preterm infants

multiple days after birth. The proposed change in MV of 150 mL/kg appears to be too optimistic, as the overall mean ± SD MV in this study was 152 ± 74 mL/kg in preterm infants during the first minutes after birth.

In addition, infants in the caffeine DR-group had a significantly lower GA. Our finding of a significant relationship between MV and GA indicates that older infants have a more developed respiratory control system. As this could have influenced the outcomes, resulting in an underestimation of the effect size, we corrected analyses for GA using multiple regression.

CONCLUSION

In this small randomized trial, we observed a direct positive effect of administering caffeine on the respiratory effort in preterm infants at birth. The results of this study provide evidence, indicating that caffeine could play a role in stimulating breathing in preterm infants during the transition. Further studies are needed to explore whether the use of caffeine in the delivery room might contribute to a decrease in delivery room intubations or additional long-term outcomes as bronchopulmonary dysplasia.

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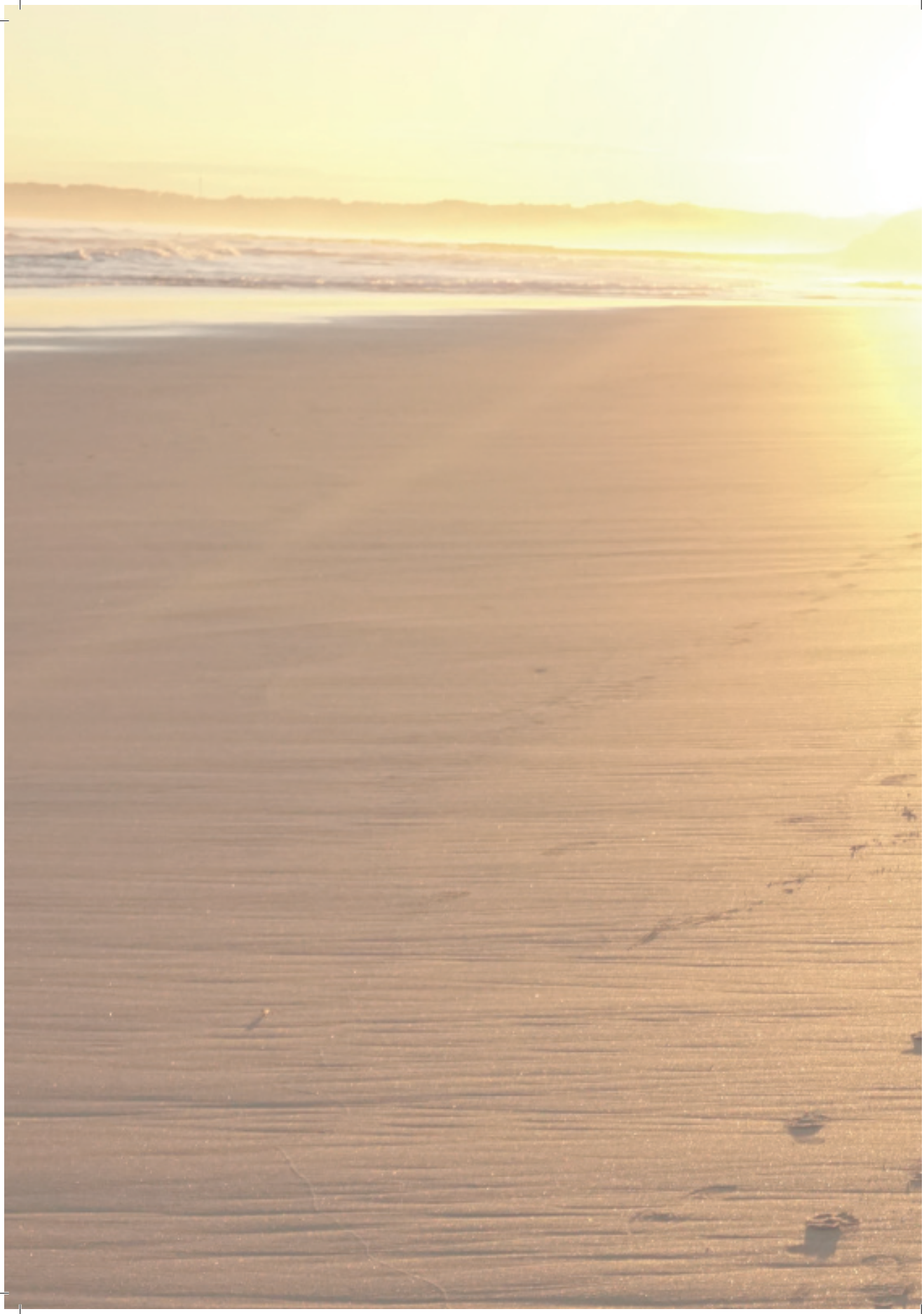
PART THREE

MAINTAINING SPONTANEOUS BREATHING AFTER BIRTH



CHAPTER 8

Sedation during minimal invasive surfactant therapy in preterm infants

J Dekker
E Lopriore
M Rijken
EGJ Rijntjes-Jacobs
VEHJ Wintjens
AB te Pas

Neonatology 2016;109:308-313

ABSTRACT

BACKGROUND

There is no data available whether sedation should be given during minimally invasive surfactant therapy (MIST). The aim of this study was to compare the level of comfort of preterm infants receiving sedation vs no sedation for MIST.

METHODS

A retrospective study of preterm infants receiving MIST was performed in the Leiden University Medical Center in 2014. Sedation (propofol 1 mg/kg) was optional and left to the discretion of the caregiver. Standardized COMFORTneo scores were compared and COMFORTneo < 14 was considered comfortable. Basic characteristics and complications were noted.

RESULTS

In 38 infants receiving MIST, 23 received propofol and 15 were not sedated. Mean ± SD gestational age (29 ± 2 weeks vs 29 ± 3 weeks) and birth weight (1312 ± 483 grams vs 1469 ± 588 grams) were not different. Median (IQR) COMFORTneo was not different between the groups before (11 (9 - 15) vs 10 (8 - 12)) and after MIST (10 (8 - 12) vs 9 (8 - 10)), but lower in the sedated group during MIST (12 (9 - 17) vs 20 (15 - 23), p=0.002) with more often COMFORTneo < 14 (56% vs 11%, p=0.04). Duration of MIST (2 (2 - 4) minutes vs 3 (2 - 7) minutes) and occurrence of bradycardia (13% vs 33%) and hypotension (21% vs 18%) were not different. Although not significant, intubation occurred more often in the sedated group (during MIST: 9% vs 0%, < 24 hours after MIST: 26% vs 13%). During MIST, oxygen saturation < 80% lasted longer in the sedated group (3 (2 - 4) minutes vs 1 (0 - 2) minutes, p=0.001) and nasal intermittent positive pressure ventilation (nIPPV) was applied more (100% vs 33%, p<0.001).

CONCLUSION

Preterm infants receiving MIST were more comfortable when sedation was given, but needed ventilation more often. A randomized controlled trial is warranted to test whether the benefit of sedation outweighs the risks of complications.

INTRODUCTION AND RATIONALE

Many preterm infants need surfactant therapy to diminish alveolar surface tension and work of breathing caused by respiratory distress syndrome (RDS), so as to avoid the occurrence of atelectasis.(1) While intubation and mechanical ventilation is increasingly being avoided(2, 3), infants are still being intubated and mechanically ventilated for surfactant therapy.(4) Mechanical ventilation can, however, lead to lung injury and ultimately to bronchopulmonary dysplasia (BPD).(5) The incidence of BPD decreases when non-invasive ventilation increases.(6) In this context, minimal invasive surfactant therapy (MIST) techniques are promising, in which surfactant is administered to a spontaneously breathing infant who then remains on continuous positive airway pressure (CPAP).(7, 8) During MIST, the vocal cords are visualized by the use of a laryngoscope and the trachea is catheterized using a semirigid catheter, and then the surfactant is instilled.(4) To date, most Neonatal Intensive Care Unit (NICU) centers in the Netherlands have adopted this procedure.

There is consensus that an endotracheal intubation procedure should be performed while the infant is adequately sedated.(9, 10) However, there is an ongoing debate whether or not sedation should be used during MIST as the presence of spontaneous breathing is a pre-requisite for the procedure. So far, there are no studies concerning sedation during this procedure. In the recent published studies and current trials on MIST, no sedation is given before MIST.(4)

By using a laryngoscope during MIST, pharyngeal stretching triggers sympathic and parasympathetic reflexes, which could lead to cardiovascular responses.(10) Also, when the infant is not sedated, efforts to resist the laryngoscope and attempts to cry can cause an increase in intracranial pressure, which could result in impairment of the venous return of the brain and intracranial venous hypertension.(9, 11, 12) This can then contribute to the risk of intraventricular hemorrhage.(9) In addition, laryngoscopy is associated with apnea, increased blood pressure, decreased heart rate and decreased transcutaneous PO_2.(13-15) These differences in vital signs were greater in infants who did not receive any premedication.(15) However, many analgesic agents used for neonatal intubation have been studied, and side effects such as respiratory depression, hypotension, muscle rigidity, increased intracranial blood pressure and decreased cerebral blood flow have been reported.(16, 17)

Propofol is routinely used as premedication for endotracheal intubation at our center. Although the maintenance of spontaneous breathing is described as an advantage of propofol, hypotension and respiratory depression have been reported.(17)

The use of propofol during MIST is currently left to the discretion of the attending neonatologist when discomfort is anticipated. Propofol may then contribute to more comfort and less resistance from the infant during the procedure, which can increase the success of the procedure. However, administering propofol could also cause respiratory depression and increase the chance for non-invasive intermittent positive pressure ventilation or, when respiratory depression persists, intubation and mechanical ventilation. There is no data available whether the use of propofol for more comfort during MIST outweighs the risk for failure of the procedure and the occurrence of complications.

We performed a cohort study to compare the level of comfort and the occurrence of complications of infants receiving sedation with infants receiving no premedication during MIST.

METHODS

A retrospective cohort study was conducted at the neonatal department of the Leiden University Medical Center. All admitted infants receiving surfactant by MIST in the year 2014 were included in this study. We only included the first MIST, repeated surfactant doses by MIST were excluded. The criteria receiving surfactant by MIST were: GA 25^{+6} – 36^{+6} weeks of gestation, no need for imminent intubation, adequate respiratory drive, CPAP level ≥ 8 cm H_2O and FiO_2 > 0.3.

According to the local protocol the decision whether to give sedation for MIST was left to the discretion of the attending neonatologist. For endotracheal intubation, intravenous propofol 2.5 mg/kg is standardly used for sedation in our unit. However, to maintain spontaneous breathing and reduce the risk of side effects, a reduced dose of intravenous propofol (1 mg/kg) was administered before MIST. In each infant, non-pharmacological techniques for comfort were also performed, which consisted of the administration of oral sucrose 24% in the cheek pouch of the infant along with a pacifier at least 2 minutes before the procedure, and swaddling the infant in a swaddling cloth to keep the infant contained.

MIST was performed using the method as described earlier by Dargaville et al.(8), in which the vocal cords are visualized using a laryngoscope, where after a semi-rigid angiocatheter is orally introduced to catheterize the trachea.

The infants receiving propofol (sedated group) were compared with the infants without premedication (non-sedated group). Nurses in our unit routinely score the comfort of the infants using the COMFORTneo score, which is validated for measuring objectively the (dis)comfort of a preterm infant.(18) Interrater reliability was assessed by Caljouw et al.(18), who found that the COMFORTneo score is reliable to measure distress in preterm infants. With a score < 14, the infant's comfort is considered to be acceptable. The COMFORTneo score before, during and after MIST of both groups were retrieved and noted. In our unit, interrater reliability between nurses of the COMFORTneo research group (Caljouw et al.(18)) and other NICU nurses was assessed in ten clinical situations, and nurses could measure comfort using the COMFORTneo score if they had achieved a Cohen's $\kappa > 0.6$. Cohen's κ measures the interrater agreement, where 0 means no agreement and 1 means total agreement.(19)

Both COMFORTneo scores and basic characteristics (gestational age, gender and birth weight) were gathered, as well as complications of MIST and the administration of propofol. These complications included the need for nIPPV, intubation, the occurrence of desaturation (oxygen saturation < 80 %), hypotension (mean mmHg < gestational age) or bradycardia (heart rate < 80 bpm). The differences in heart rate between the interval before the MIST and during the MIST were also compared for the sedated and the non-sedated groups.

All study data were retrieved from the digital medical charts (PDMS, MetaVision iMDsoft, Leiden, The Netherlands), a clinical information system designed especially for use in NICUs. In this system, each parameter is noted every minute.

This was a retrospective study and did not need to comply with the Dutch law on Medical Research in Humans; the Research Ethics Committee issued a statement of no objection.

Statistical analysis

Because of the retrospective nature of this study, a convenience sample was used. No power calculation was performed because there was no data regarding sedation during MIST. Statistical analysis was performed using SPSS 22 (IBM SPSS Statistics). The parameters of both groups were tested for normality using Kolmogorov-Smirnov and Shapiro-Wilkinson. The groups were compared using Student's t-test for parametric variables and the Mann-Whitney U test for non-parametric comparisons for continuous variables, and the X^2 test for categorical variables. Results are presented as mean ± SD for normally distributed values or median (IQR) for non-normally distributed values. $P < 0.05$ was considered statistically significant. Reported p-values are two-sided.

RESULTS

During the one-year period, 310 infants with a gestational age between 26^{+0} and 36^{+6} weeks were admitted to the NICU. In 38 infants, surfactant was given by MIST, of which 23 infants received propofol (supplemental video 1; www.karger.com/doi/10.1159/000443823) and 15 infants were not sedated (supplemental video 2) based on the discretion of the attending neonatologist. Reasons given for prescribing propofol were expected discomfort. There were no significant differences in mean ± SD gestational age (sedation vs no sedation: 29 ± 2 weeks vs 29 ± 3 weeks), birth weight (1312 ± 483 grams vs 1469 ± 588 grams) and the percentage of males (61% (14/23) vs 73% (11/15)). The median (IQR) duration of the procedure did not significantly differ between the groups (2 (2 - 4) minutes vs 3 (2 - 7) minutes).

Comfort

Surfactant was administered by MIST 38 times, in which comfort was scored before, during and after the procedure in 76% (29/38), 71% (27/38) and 71% (27/38) of the procedures, respectively. Both before and after MIST the median (IQR) COMFORTneo score did not differ between the study groups, but the COMFORTneo score during the procedure was significantly lower in the sedated group when compared to the non-sedated group (Table 1). The COMFORTneo score was significantly more often < 14 in the sedated group during MIST (56% vs 11%, $p<0.05$). There was a significant positive correlation between both gestational age ($r=0.419$, $p<0.05$) as postnatal age ($r=0.435$, $p<0.05$) and COMFORTneo score during the procedure. However, as previously mentioned the gestational age did not differ between the sedated and the not sedated group.

Table 1 | Results: comfort

	Sedated group n = 23	Non-sedated group n = 15	*p*-value
COMFORTneo score before MIST	11 (9 – 15) *n = 18*	10 (8 – 12) *n = 11*	ns
COMFORTneo score during MIST	12 (9 – 17) *n = 17*	20 (15 – 23) n = 10	0.002
% COMFORTneo score < 14 during MIST	9/16 (56%)	1/9 (11%)	0.04
COMFORTneo score after MIST	10 (8 – 12) *n = 23*	9 (8 – 10) *n = 9*	ns

Data is presented as median (IQR) for non-parametric data, and n (%) for categorical data.

Complications

The occurrence of complications could be retrieved from the digital medical chart for all patients. Bradycardia and hypotension occurred in a few infants, but these were not significantly different between the groups (Table 2). There was no significant increase or decrease in heart rate during the procedure in both groups. The median (IQR) duration of oxygen desaturation of all infants (< 80 %) was longer in the sedated group (3 (2 - 4) minutes vs 1 (0 - 2) minutes, p <0.01). All patients in the sedated group needed nIPPV temporarily due to apnea and saturation < 80 % during MIST as compared to 33 % in the non-sedated group (p<0.001) (Table 2). 2 infants (8%) in the sedated group were intubated during MIST due to failure of the procedure because the trachea could not be catheterized, while this did not occur in the non-sedated group (ns). There was no significant difference in occurrence of intubations in the first 24 hours after MIST between the two groups (Table 2). The intubated infants had a mean ± SD gestational age of 28 ± 2 weeks and a birth weight of 1109 ± 454 grams. Reasons to intubate in the first 24 hours after MIST were apneas in 3 cases, no improvement or an increase in FiO_2 need in 3 cases, and no adequate respiratory drive before the procedure with persistent apnea after the procedure in 2 cases (Table 3).

Table 2 | Results: complications

	Sedated group n = 23	Non-sedated group n = 15	*p*-value
nIPPV during MIST[a]	23/23 (100%)	5/15 (33%)	< 0.001
Intubation in the first 24 hours after MIST[a]	6/23 (26%)	2/15 (13%)	ns
Intubation during MIST[a]	2/23 (9%)	0/15 (0%)	ns
Duration of SpO_2 < 80% during MIST (minutes)[b]	3 (2 – 4)	1 (0 – 2)	0.001
Hypotension during MIST[a]	3/14 (21%)	2/11 (18%)	ns
Bradycardia during MIST[a]	3/23 (13%)	5/15 (33%)	ns
Heartrate before – during MIST (bpm)[b]	-4 (-10 - -1)	-2 (-7 – 3)	ns

Data is presented as n (%) for categorical data (a) and median (IQR) for non-parametric data (b).

Table 3 | Reasons for intubation

	Sedated group n = 8	Non-sedated group n = 2	*p*-value
Trachea could not be catheterized	2/8 (25%)	0/2 (0%)	ns
Infant did not meet MIST criteria before procedure	2/8 (25%)	0/2 (0%)	ns
Apnea	2/8 (25%)	1/2 (50%)	ns
No improvement/increase FiO_2 needed	2/8 (25%)	1/2 (50%)	ns

Data is presented as n (%) for categorical data.

DISCUSSION

In this retrospective cohort study, we observed that infants receiving propofol had a higher level of comfort compared to the infants receiving no sedation before MIST. However, infants receiving propofol desaturated for a longer period, needed temporarily nIPPV more frequently during the procedure and, although this did not reach significance, more often infants were intubated during or within 24 hours after MIST. All other complications (hypotension and bradycardia) were not different between the groups.

Administration of surfactant without intubation and mechanical ventilation, using procedures such as MIST, has recently received increased attention and many neonatal units have adopted the method.(4, 7, 20, 21) There is however no consensus on whether the procedure should be performed with or without the use of premedication, which has been a hot topic for debate. This is the first study describing the comfort of preterm infants receiving MIST using objective measurements and the effect of sedation vs no sedation. It is argued by Dargaville et al(4) that MIST can generally be performed without creating discomfort in the infants, however, this was not objectively evaluated. There is consensus that an intubation procedure should be performed while the infant is adequately sedated.(9, 10) Laryngoscopy can be painful and is associated with crying and increased changes in intracranial pressure when infants are awake.(9, 11, 12) It could be reasoned that this also accounts for MIST as laryngoscopy is also performed.

Although propofol has been used for the intubation-surfactant-extubation (INSURE) method, its usage for MIST has not been described before.(17) Propofol is used because of its short acting character and it is also used in our unit as sedation for endotracheal intubation. We have chosen to use a low dose of propofol for MIST to minimize the risk of apnea and hypotension. Propofol has an anxiolytic effect in low dosages, but it is not known whether propofol has analgesic effects or not.(22) Propofol is effective for obtaining hypnosis and muscle relaxation in endotracheal intubation.(16, 17) One of the complications of propofol is respiratory depression.(17) This study has shown that all infants receiving propofol needed nIPPV temporarily, compared to 33 % of infants receiving no propofol. However, respiratory drive might be depressed by the use of propofol; of all sedated infants, 2/23 infants (9 %) needed to be intubated during the procedure due to other reasons than respiratory depression, as the trachea could not be catheterized. To make solid conclusions about respiratory depression caused by propofol for MIST, a randomized controlled trial is warranted.

When high doses of propofol were used (3 and 6 mg/kg), hypotension was considered a significant complication during and within 1 hour after intubation.(23, 24) In contrast, other studies reported that hypotension did not occur when propofol 2.5 mg/kg was used.(16, 25) Interestingly, Welzing et al.(17) also noted a high incidence of hypotension even when a dosage of 1 mg/kg was used. However, our study does not confirm this as we did not observe more hypotension when we used this low dose compared to no sedation.

The MIST procedure has been recently introduced in our unit and it is possible that infants would be more comfortable in a center with extensive experience in the procedure. However, the MIST procedures in our study were performed by neonatologists who were well trained in endotracheal intubation and laryngoscopy. Also, there were no significant differences in duration of the procedure and the frequency of complications in the non-sedated group appeared to be low.

The decision for the use of propofol as premedication for MIST was left to the discretion of the attending neonatologist, and a selection bias could have occurred. The reason given by the caregivers for the decision to use propofol was expected discomfort. However, gestational age, birth weight and COMFORT score before the procedure were not different between the groups, which makes it less likely that these items influenced the decision of the caregiver to give sedation or not.

This was a small retrospective cohort study with a convenience sample. We already reached a large difference in COMFORTneo score in this small group, but it is possible that differences in complications would have reached significance in a larger group. Although we could not retrieve the COMFORTneo score in all infants, this was equally distributed among the study groups. In addition, comfort was scored by nurses who were not blinded to the treatment given, which could influence the COMFORTneo scores and cause observer bias.

CONCLUSION

In summary, we observed more comfort in preterm infants when they received a low dose of propofol (1 mg/kg) as premedication for MIST, but an increase in respiratory complications and non-significantly more intubations. However, we do not consider this study to be conclusive and a randomized controlled trial is warranted to determine whether the benefit of sedation in comfort outweighs the risks for complications.

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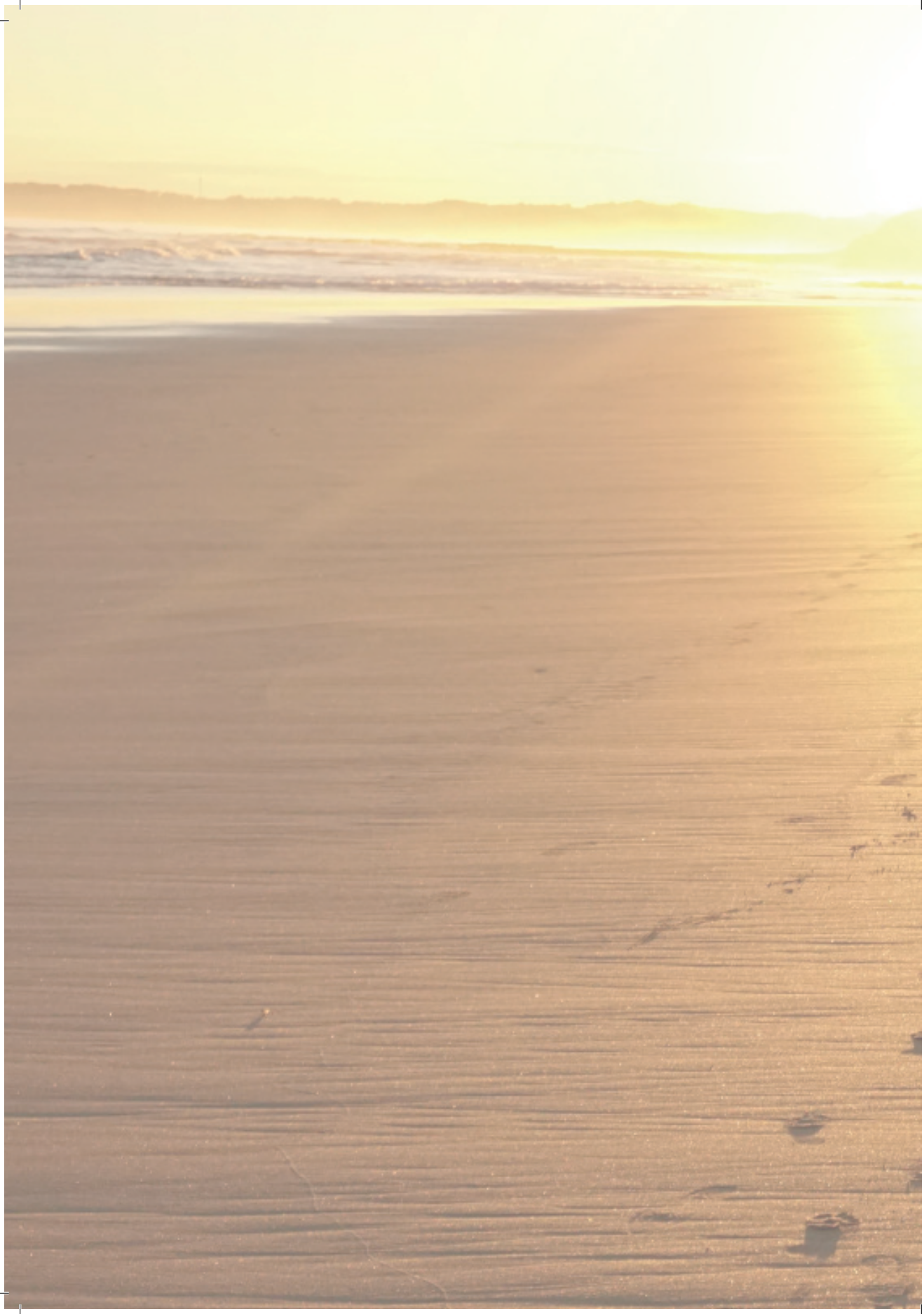

CHAPTER 9

Sedation during minimal invasive surfactant therapy: a randomized controlled trial

J Dekker
E Lopriore
HA van Zanten
RNGB Tan
SB Hooper
AB te Pas

Arch Dis Child Fetal Neonatal Ed 2018;0:F1-F6

ABSTRACT

AIM

Although sedation for endotracheal intubation of infants is widely adopted, there is no consensus whether sedation should be used for minimal invasive surfactant therapy (MIST). We compared, in a randomized controlled setting, the level of stress and comfort of preterm infants during MIST with and without receiving low dose sedation.

METHODS

Infants between 26 - 36 weeks gestational age were randomized to receive either low dose sedation (1 mg/kg propofol iv) or no premedication during MIST procedure. Standard comfort care was given in both groups, which consisted of administering sucrose in the cheek pouch of the infant and containment. Primary endpoint was the percentage of infants assessed to be comfortable during the procedure (COMFORTneo score < 14). Secondary parameters included complications of both the MIST procedure and low dose sedation administration.

RESULTS

In total 78 infants were randomized and analyzed, with a median (IQR) gestational age of 29^{+0} (28^{+0} – 32^{+0}) weeks. The percentage of infants with a COMFORTneo score < 14 during MIST was significantly higher in the sedated group (32/42 (76 %) vs 8/36 (22 %), p<0.001). The incidence of desaturation (SpO_2 < 85 %) during the procedure was significantly higher in the sedated group (38/42 (91 %) vs 25/36 (69 %), p=0.023), and infants needed more often nasal intermittent mandatory ventilation during the procedure (39/42 (93 %) vs 17/36 (47 %), p<0.001). There were no differences in incidence of hypotension, bradycardia, intubation or pneumothoraxes.

CONCLUSION

Low dose sedation increased comfort during MIST procedure in preterm infants, but the need for transient non-invasive ventilation was increased.

INTRODUCTION AND RATIONALE

Many preterm infants have respiratory distress syndrome due to surfactant deficiency.(1) Although nasal continuous positive airway pressure (CPAP) is effective as the initial means of respiratory support in most premature infants, a proportion of infants require surfactant therapy in order to succeed on CPAP.(2, 3) However, this has traditionally involved intubation followed by mechanical ventilation. Avoiding mechanical ventilation has the potential to decrease the risk for intraventricular hemorrhage (IVH), bronchopulmonary dysplasia (BPD) and death.(4, 5) This can be achieved with MIST, where surfactant is administered to a spontaneously breathing infant who then remains on CPAP.(6, 7) The most common method for this approach involves visualising the vocal cords with a laryngoscope and catheterising the trachea using a semi-rigid catheter, after which the surfactant is instilled.(8) Currently, many Neonatal Intensive Care Unit (NICU) centers, including in the Netherlands, have adopted this procedure.

There is general consensus that an infant should be adequately sedated when an endotracheal intubation procedure is performed.(9-12) Although it is known that visualising the vocal cords with a laryngoscope is highly uncomfortable(13), there is still no consensus on whether or not to use sedation during MIST, particularly when a laryngoscope is used. The use of a laryngoscope can lead to cardiovascular responses and attempts to resist the laryngoscope might result in an increased risk of intraventricular hemorrhage by impairing cerebral venous return.(14-17) While sedation might also increase the chance for an uneventful, smooth and successful procedure, sedation might compromise the infant's respiratory drive which is a prerequisite for MIST. Reported experience from feasibility studies suggests that the MIST procedure is generally well tolerated without any premedication.(1, 6, 8) However, Klotz et al.(18) reported in a European survey that almost half of the neonatologists use premedication during MIST procedures, including sedatives.

We have performed a randomized controlled trial where we hypothesized that low dose sedation would increase the comfort of preterm infants during the MIST procedure with minimal side-effects. The aim of this study was to compare the level of comfort of preterm infants receiving low dose sedation versus no sedation during MIST procedure.

METHODS

A randomized controlled trial was conducted at the Leiden University Medical Center, including all preterm infants with a gestational age between 26 - 37 weeks needing surfactant therapy for respiratory distress syndrome according to the local criteria (FiO_2 > 0.3 and PEEP ≥ 8 cm H_2O). Opaque sealed envelopes were used to determine randomization: low dose sedation or no premedication. We excluded infants who had an imminent need for intubation because of respiratory insufficiency, expressed by apnea and/or persistent acidosis. Infants with a pneumothorax or pulmonary hemorrhage were also excluded. All infants < 30 weeks gestational age (GA) received caffeine in the delivery room or at admission to the NICU. Infants > 30 weeks GA received caffeine in case of recurrent apnea. Allocation was stratified by GA (26^{+0} – 31^{+6} and 32^{+0} – 36^{+6} weeks) using variable block sizes (4-8).

The intervention consisted of the use of administering intravenous propofol (1 mg/kg) for sedation during the MIST procedure. This is a reduced dose compared to the standard dose of 2.5 mg/kg used for neonatal intubation, to prevent side effects as respiratory depression. Propofol was administered intravenously, either by peripheral or central vein dependent on the iv access point of the infant. The administration was performed slowly directly before MIST, to refrain from thoracic rigidity and pain at administration point. Both groups received standard comfort care, which consisted of administering sucrose 24% in the cheek pouch of the infant and containing the infant during the MIST by swaddling the infant or gently placing the hands of a caregiver on the infant's body.

The main study parameter was the COMFORTneo score; the primary endpoint was the percentage of infants with a COMFORTneo score < 14 during the procedure. The COMFORTneo score is a validated instrument for measuring objectively the (dis) comfort of a preterm infant,(19) and is used by trained NICU nurses as standard of care to assess the comfort of a preterm infant every shift at the NICU of the LUMC. The procedure was video recorded, where both the face of the infant and the motions of one of the extremities could be observed, while the other extremities were contained. The recordings were coded and edited so that the administration of sedation was not visible, and two independent NICU nurses, blinded for the allocation, reviewed the recordings and measured the level of comfort using the COMFORTneo scale. Both nurses assessed comfort in 10 MIST procedures, after which interrater reliability between these nurses was assessed. Cohen's κ was used to assess interrater reliability, where 0 means no agreement and 1 means total agreement. A Cohen's κ > 0.4 was considered reasonable. When interrater reliability appeared to be < 0.4, an additional NICU nurse

assessed the same 10 MIST procedures, to find reasonable reliability between two NICU nurses. When reasonable Cohen's κ was achieved within two NICU nurses, the remaining MIST procedures were subdivided into those two nurses for assessment.

Secondary study parameters were: the occurrence of nasal intermittent mandatory ventilation (nIMV) during and immediately after the procedure, intubation need during the procedure and within 24 hours, number of attempts of insertion of the angio-catheter, duration of the procedure, complications occurring during the procedure (desaturation < 85 %, hypotension with mean < GA, bradycardia < 80 bpm, nasal hemorrhage), other complications (pneumothorax, pulmonary hemorrhage, resuscitation, intraventricular hemorrhage ≥ grade 3, death), and heart rate and blood pressure before, during and after the procedure. Oxygenation and heart rate were measured with the Masimo SET pulse oximeter. Arterial blood pressure was measured using an IntelliVue MP30 Philips Monitor. If an arterial line was not present, blood pressure was measured non-invasively using an appropriately sized neonatal cuff (Philips). All clinical parameters were stored every minute in the local Patient Data Management System (Metavision, IMDSoft, Tel Aviv, Israel).

As there was no data available on comfort during MIST to base the sample size on, we based our sample size calculation on a study that compared intubation during sevoflurane anaesthesia with awake intubation in preterm infants.(20) In this trial, the incidence of hypertension, which is a sign of discomfort, was 25% in the anaesthetized infants as compared to 56% in awake infants. To detect a comparable decrease in the incidence of COMFORTneo score > 14 when using propofol, with a power of 80% and an α error of 5% (two-tailed test), we required 39 infants for each arm.

The ethical committee of the LUMC approved the study protocol. Informed parental consent was obtained if time permitted to do so before MIST. However, when MIST was imminent and there was no time to approach parents for consent or this was considered inappropriate, deferred consent was asked at a more appropriate time after the procedure. This study was registered in www.trialregister.nl, with registration number NTR5010.

Statistical analysis was performed with SPSS software version 23.0 (SPSS, Chicago, Illinois). Demographics of the sedated and the non-sedated group were compared by α^2 test, Student's t-test and Mann-Whitney U test based on normality of the data. Linear study parameters that were assessed once per infant were compared by a two-way factorial ANOVA or a linear mixed-effect regression model in multiple assessments,

including both the randomization and stratification group. Categorical outcomes were assessed by Fisher's exact test. Two-sided p-values <0.05 were considered statistically significant.

RESULTS

A total of 110 eligible infants were admitted to the NICU between January 2015 and July 2017, of which 22 infants were not randomized (Figure 1). However, 4 infants in the sedated group and 6 infants in the non-sedated group were excluded from analysis. A total of 78 infants were analyzed, of which 42 in the sedated group and 36 in the non-sedated group. There were no differences between the groups in GA, birth weight or gender (Table 1).

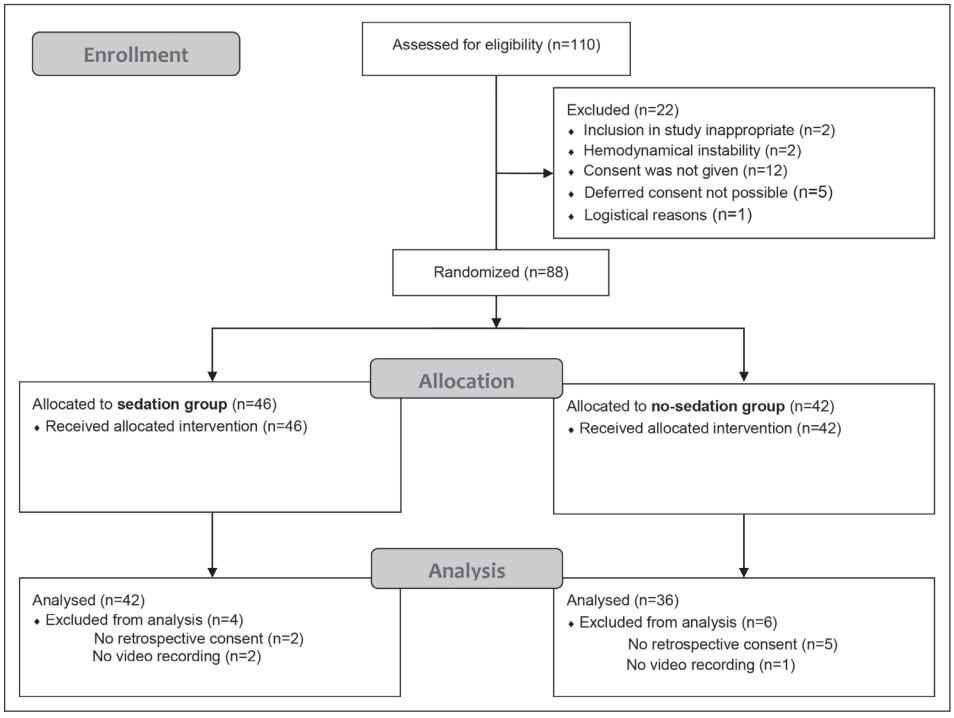

Figure 1 | CONSORT flow diagram

Table 1 | Demographical data

	Sedation group n = 42	No sedation group n = 36	*p*-value
Gestational age[a]	29^{+0} (27^{+5} – 32^{+0})	29^{+0} (28^{+0} – 31^{+0})	0.731
Birth weight (grams)[b]	1475 ± 575	1502 ± 606	0.837
Gender (% males)[c]	26/42 (62%)	20/36 (56%)	0.647

Data are presented as median (IQR) for non-parametric data ([a]), mean ± SD for parametric data ([b]) and n (%) for categorical data ([c]).

MIST procedure

Time point after birth when MIST procedure was performed was not different (sedated vs non-sedated group: median (IQR) 5.5 (3 – 15) hours vs 6.5 (3 – 14) hours, p=0.7). The number of attempts needed to insert the angio-catheter in the trachea was not different (1 (1 – 2) attempts vs 1 (1 – 2) attempts, p=0.982). There was no difference in total duration of the MIST procedure (246.1 ± 174.8 s vs 246.1 ± 178.4 s, p=0.641).

COMFORTneo score

The percentage of infants with a COMFORTneo score < 14 during MIST was significantly higher in the sedated group when compared to the non-sedated group (32/42 (76%) vs 8/36 (22%), p<0.001).

The mean ± SD COMFORTneo score during MIST was significantly lower in the sedated group compared with the non-sedated group (12 ± 3 vs 17 ± 4, p<0.001).

Complications

The incidence of desaturation was significantly higher in the sedated group (38/42 (91%) vs 25/36 (69%), p=0.023). There was no difference in mean blood pressure in the 30 minutes before MIST, during MIST and 30 minutes after MIST (Table 2). The incidence of hypotension was not significantly different between the groups (p=0.282), as was volume expansion as treatment of hypotension (p=1.000) (Table 2). In both groups, heart rate was significantly lower during and after the procedure when compared to before the MIST procedure. In the sedated group, the difference in heart rate was significantly greater between the periods before, during and after MIST when compared to the non-sedated group (p=0.002). There was no difference in the occurrence of bradycardia (heart rate < 100 bpm, p=0.556) (Table 2).

More infants in the sedated group needed nIMV during MIST (39/42 (93%) vs 17/36 (47%), p<0.001), but there was no difference in duration of nIMV given (median (IQR) time 7 (3 – 21) minutes vs 6 (3 – 12) minutes, p=0.274). Applying nIMV was not influenced by the different GA strata (Figure 2).

Table 2 | Demographical data

	Sedation group n = 42	No sedation group n = 36	*p*-value
Incidence of desaturation[a]	38/42 (91%)	25/36 (69%)	0.023
Incidence of hypotension[a]	9/30 (30%)	2/17 (12%)	0.282
Treatment for hypotension[a]	3/9 (33%)	0/2 (0%)	1.000
Blood pressure (mean mmHg)[b]			0.145
Before MIST	37 ± 8	38 ± 7	
During MIST	35 ± 7	39 ± 5	
After MIST	35 ± 7	38 ± 4	
Incidence of bradycardia[a]	9/42 (21%)	5/35 (14%)	0.556
Heart rate (beats per minute)[b]			0.002
Before MIST	151 ± 16	148 ± 13	
During MIST	143 ± 14	147 ± 13	
After MIST	141 ± 13	146 ± 14	
Need for nIMV[a]	39/42 (93%)	17/36 (47%)	< 0.001
Duration of needed nIMV (minutes)[c]	7 (3 – 21)	6 (3 – 12)	0.274
Intubation during MIST[a]	1/42 (2%)	4/36 (11%)	0.175
Intubation < 24 h after MIST[a]	10/42 (24%)	6/36 (17%)	0.576
Incidence of pneumothorax[a]	3/42 (7%)	1/36 (3%)	0.620
Incidence of pulmonary hemorrhage[a]	1/42 (2%)	0/36 (0%)	1.000
Intraventricular hemorrhage ≥ grade III[a]	2/42 (5%)	0/36 (0%)	0.497
Death[a]	1/42 (2%)	1/36 (3%)	1.000

Data are presented as n (%) for categorical data ([a]), mean ± SD for parametric data ([b]) and median (IQR) for non-parametric data ([c]).

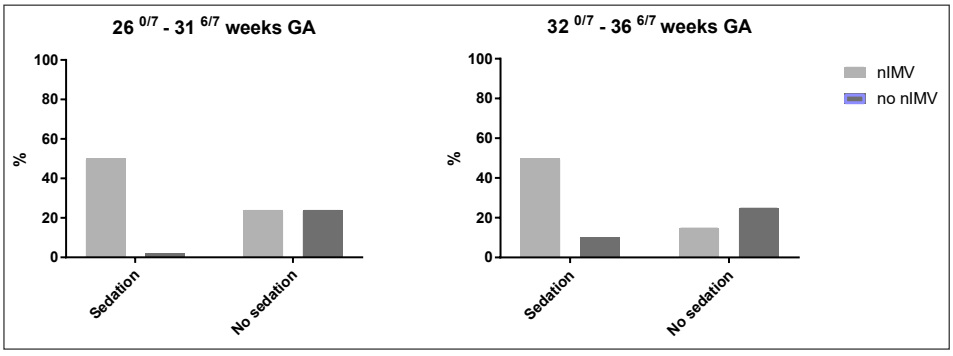

Figure 2 | Need for nasal intermittent mandatory ventilation (nIMV) during minimal invasive surfactant therapy

Only 1 infant in the sedated group was intubated during MIST, while 4 infants were intubated in the non-sedated group (1/42 (2%) vs 4/36 (11%), p=0.175). Overall, the rate of intubation in the first 24 hours after MIST was similar in the two groups (10/42 (24%) vs 6/36 (17%), p=0.576). GA stratum did not influence intubation rate.

The incidence of pneumothorax (3/42 (7%) vs 1/36 (3%); p=0.620) and pulmonary hemorrhage (1/42 (2%) vs 0/36 (0%), p=1.000) was not different. There were no differences in rates of intraventricular hemorrhage ≥ grade 3 or mortality. None of the included infants needed resuscitation during the MIST procedure.

DISCUSSION

In this randomized study we observed that during the MIST procedure more infants were comfortable and the average COMFORTneo score was lower when low-dose sedation was given. However, infants that received sedation were more likely to desaturate and receive nIMV. There were no differences in other complications of the procedure or the use of sedation. This implies that when accepting a temporary need for nIMV, low-dose sedation could be used to increase the comfort of preterm infants during the MIST procedure, without major clinical implications.

Resistance of the infant during laryngoscopy can lead to an increased risk of intraventricular hemorrhage by impairing cerebral venous return.(14-17) Therefore, more comfort during the procedure might be beneficial. The results of the meta-analyses of studies where no sedation is used, are conflicting.(21,22) While Kribs et al.(2007) and Klebermass-Schrehof et al.(2013) described a decrease in IVH, Aldana-Aguirre et al.(2016) reported no reduction in IVH. We have reported no differences in rate of IVH between the randomization groups, yet this trial was not powered on this outcome.

Cruz et al.(21) reported that infants in the NICU experience many invasive procedures each day, with the highest frequency in the vulnerable first week of life. Thereby, infants with the lowest GA and at the highest risk for neurological impairment received the lowest amount of analgesic interventions.(21) Procedural pain can affect neurodevelopment, as the exposure to multiple painful procedures can lead to on-going stress.(22) There is evidence that these adverse events can be prevented or minimized by using both analgesic and non-pharmacological interventions during painful procedures.(23, 24) On the other hand, the use of analgesia can lead to adverse effects in the brain as well.(25) A high level of analgesia correlates strongly with reduced cerebellar volume and poorer cognitive and motor outcomes in infancy.(26-28) However, in our study we used a single low dose of propofol, which reduced the stress of a painful stimulus in this vulnerable population and thereby might influenced neurodevelopment rather positively than negatively.

While this is the first trial focussing on sedation during MIST, the use of sedation for administrating surfactant by intubation-surfactant-extubation (INSURE) has previously been studied.(29) In both the INSURE and the MIST procedure, the sedative agent should have a rapid distribution and redistribution.(29, 30) Remifentanil is described to have these characteristics, and is therefore used in many clinical applications in neonates.(31) However, remifentanil was found to be unsuitable as premedication for INSURE, as it did not provide adequate sedation.(30) In addition, remifentanil is a potent respiratory depressant, which makes it unsuitable as a sedative for MIST.(31)

Propofol however, has been widely used as well during intubation and other procedures for sedation and anaesthesia.(32-34) Thereby, the level of sedation achieved by the use of propofol was considered satisfactory during surgery in the study of Piersigilli et al.(33), but this was not evaluated objectively.

One of the most noted side effects of propofol is the occurrence of hypotension, although the incidence varies in studies. Hypotension was not reported in the study which used a dose of 2 mg/kg propofol iv(35), while hypotension was reported in studies with an even lower dose of propofol.(36, 37) In our study, the observed rate of hypotension is similar to our previous observational report.(38) In most infants, hypotension was mild and transient and only 3/9 infants (33 %) needed a fluid bolus as treatment.

Only 44 % of infants receiving MIST in the study of Dargaville et al.(8) needed positive pressure ventilation, which we confirm in our non-sedated group (47 % needed nIMV). However, we report a significantly higher incidence of need for nIMV in the sedation group (93 %). This is due to a higher incidence of desaturation, which was comparable to the study of Descamps et al.(39) However, the incidence of need for intubation during or within 24 hours after the procedure, did not differ between the randomization groups, thereby indicating that the need for nIMV was transient.

Intermittent hypoxemia is associated with an impaired neurodevelopmental outcome.(40) However, infants born preterm are intrinsically at risk for intermittent hypoxic episodes during the first 6 - 8 weeks after birth due to immaturity and many other factors, with a mean of 100 - 800 hypoxic episodes occurring each week.(40) It is therefore difficult or even impossible to differentiate the effect of the single hypoxic episode during MIST procedure on outcome.

The neonatologists performing MIST in this trial were not blinded for the allocated treatment. Although there was clinical equipoise, awareness of the treatment allocation

could have influenced the use of nIMV. In contrast, the NICU nurses who analyzed the primary outcome were blinded, therefore this parameter was not influenced by the study design.

Another limitation of this study is the insufficient sample size to perform sub-analyses based on different GA strata. However, as we have included GA strata in the models for statistical analyses, our results are corrected for GA.

CONCLUSION

In this randomized controlled trial, we observed that preterm infants were more often comfortable during the MIST procedure while more often desaturation occurred and nIMV was given. The low dose sedation could help increase comfort during MIST and might reduce the risk for neurodevelopmental complications due to stress of a painful stimulus. However, only a large and adequately powered randomized trial on this significant clinical outcome can be conclusive on this statement.

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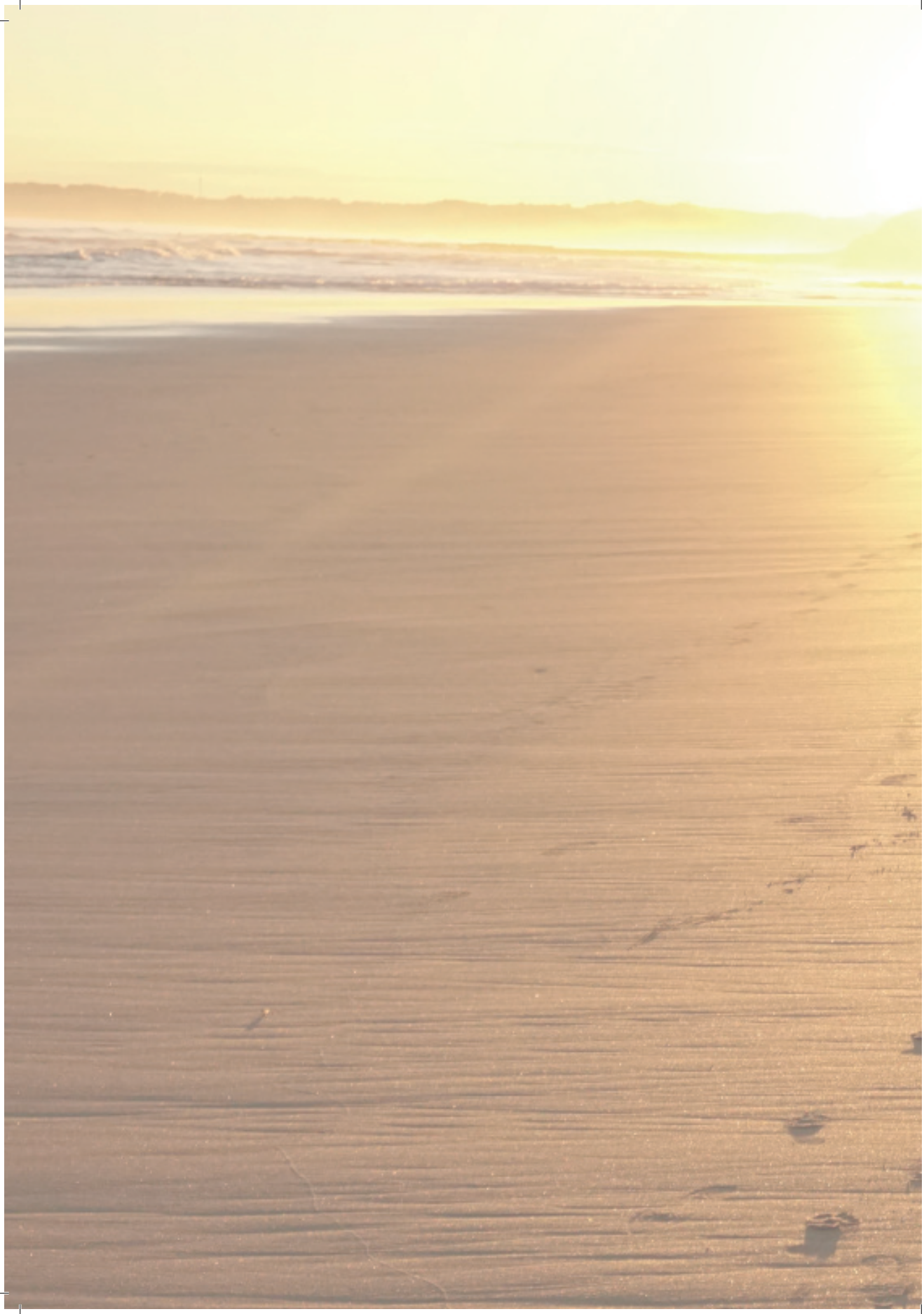
PART FOUR

DISCUSSION AND SUMMARY



GENERAL DISCUSSION

Based on two reviews:

Dekker J, van Kaam AH, Roehr CC, Flemmer AW, Foglia EE, Hooper SB, te Pas AB. *Stimulating and maintaining spontaneous breathing during transition of preterm infants.* Pediatric Research 2019 June.

Te Pas AB, Hooper SB, Dekker J. *The changing landscape in supporting preterm infants at birth.* Neonatology 2019;115:392-397.

INTRODUCTION

Compared to term infants, the respiratory system of preterm infants is structurally and biochemically immature, with a highly compliant chest wall, a large gas diffusion barrier and stiff lungs due to structural immaturity and surfactant deficiency.(1) Although most preterm infants breathe at birth, respiratory support is often needed to ensure adequate gas exchange.(2, 3) While traditionally infants were intubated and mechanically ventilated, there is considerable evidence that this approach increases the risk of lung and brain injury with subsequent long-term impairment of lung function and neurodevelopment.(4, 5) To avoid injury, the focus of respiratory support has therefore shifted towards more non-invasive approaches, such as applying positive pressure support or ventilation via face mask.(6-10) In addition, immediate cord clamping is necessary when stabilizing preterm infants in order to transfer the baby to the resuscitation table. However, the interventions that are currently recommended for stabilization/resuscitation of the preterm infant are largely based on long held beliefs or scientific knowledge coming from fetal or fully transitioned animal models. We know very little about how mask ventilation strategies interact and integrate with the infant's physiology as it transitions a from a fetal into a neonatal phenotype.

The effectiveness of non-invasive ventilation might be hampered by a number of reasons. For instance, mask leak is often not recognized by the caregiver and represents one of the major causes of ineffective ventilation, as it reduces the administered tidal volumes. (11-13). Ventilating non-invasively by face mask without leakage requires training and experience.(12-15) In addition, commercially available face masks are commonly not of an appropriate size for the infant's face, particularly in preterm infants, making it difficult to avoid placing the rim over the chin or eyes.(16-18) As a result, efforts have been made to implementing extra training(19), improving ventilation devices(20) and designing different masks(21). Another complication is that, in an effort to minimize mask leak during ventilation, caregivers may also inadvertently further reduce the effectiveness of ventilation by pressing too hard and obstructing the upper airways. (13, 22, 23)

The adducted larynx at birth has so far been overlooked as a possible cause for obstruction. Lung aeration can only take place in case of an open airway – including the larynx.(2, 24, 25) However, during fetal life the larynx is chronically adducted to promote lung expansion and thereby lung growth and it is unknown when and how the larynx adapts to the new function after birth.(25, 26) There is now evidence that immediately after birth the larynx continues to function as it does in fetal life and remains mostly

closed, making ventilation strategies inadequate when applied non-invasively.(24) This was recently demonstrated in a preterm rabbit model showing that at birth, the larynx is predominantly closed during apnea, and opens only briefly when a breath is taken. This pattern changes and the larynx remains mainly open once a stable breathing pattern has been established.(24) This explains the distention of the upper airway that can occur during mask ventilation, as has been demonstrated in preterm lambs and infants by van Vonderen et al.(27) This should be taken into account when targeting ventilation, because tidal volumes could be interpreted as 'appropriate' during mask ventilation, while the closed larynx prevents lung aeration and gas exchange which results in inadequate ventilation. This finding could completely change the perspective of applying non-invasive ventilation at birth. The mechanisms controlling how the larynx functions is still unclear, but preliminary results suggest that it is closely linked to the stimulation/suppression of breathing; when breathing is stimulated the larynx is open, but when breathing is inhibited the larynx closes. As such oxygenation and aeration of the lung play an important role in this. This almost sounds like a catch 22: for a patent larynx you need lung aeration and oxygenation, but for lung aeration and oxygenation you need a patent larynx. However, spontaneous breathing seems to be able to break through this impasse. The larynx will open during a spontaneous breath, allowing air to enter the lung. Stimulating spontaneous breathing of preterm infants at birth could therefore enhance the success of non-invasive ventilation at birth.

Maintaining spontaneous breathing is also an important goal in the first hours after admission to the Neonatal Intensive Care Unit (NICU). While the majority of preterm infants leave the delivery room supported by non-invasive ventilation, a proportion of them will suffer from severe respiratory distress syndrome, for which administration of exogenous surfactant might be needed.(28) Traditionally, intubation and subsequently mechanical ventilation was required to administer surfactant, increasing the risk for ventilation-induced-lung-injury.(29) However, recent trials have demonstrated the feasibility and efficacy of surfactant administration in a minimal invasive way, thereby omitting intubation.(30, 31) A stable respiratory drive is a prerequisite to make this procedure successful in avoiding intubation and mechanical ventilation.

We will discuss the interventions to stimulate and maintain spontaneous breathing of preterm infants at birth and in the first hours after admission in the NICU, which was the aim of this thesis.

STIMULATING SPONTANEOUS BREATHING AT BIRTH

Tactile stimulation

The incidence, methods and effect of tactile stimulation in preterm infants at birth is described in **Chapter 1 & 2**.

Chapter 1 describes a large variability in practice with regard to tactile stimulation. The primary method of stimulation varied from rubbing the soles of the feet to rubbing the chest and/or back. A similar variety in stimulation methods was described by Baik-Schneditz et al.(32-34) This wide variety of practices might be due to the international resuscitation guidelines not being very specific on timing and methods of recommended tactile stimulation.(35, 36)

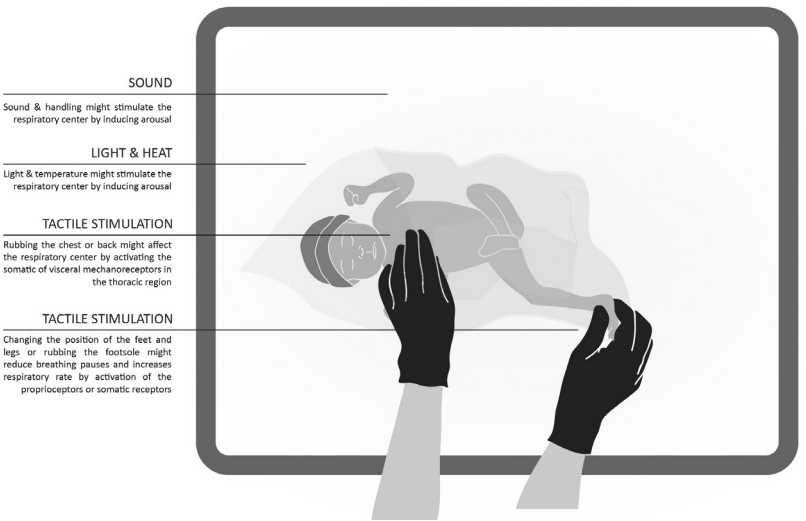


Figure 1 | Pathways involved by different methods of stimulation.

By using different methods of tactile stimulation, activation of different sensory pathways could be involved (Figure 1). Stimulation of proprioceptors activated by changing the position of the feet and legs by rubbing the soles of the feet has been shown to reduce breathing pauses and increase respiratory rate.(37, 38) Rubbing the chest or back, thereby activating the somatic or visceral mechanoreceptors in the thoracic region might affect the respiratory center as well.(39, 40) Activating a larger number of receptors by applying stimulation to a larger cutaneous surface area might increase the effect. However, the most effective method of stimulation remains unclear.

The true effect of tactile stimulation on respiratory effort is difficult to determine in human preterm infants. Clinical equipoise for omitting stimulation is not possible as tactile stimulation has been common practice for many years and has become a fundamental intervention of resuscitation, even though recent studies described that one third to even two thirds of infants do not receive any stimulation.(33, 34) Therefore, the effect of standard stimulation (stimulation at the discretion of the caregiver) was compared to a strict protocol of repetitive stimulation in our randomized controlled trial in preterm infants at birth (**chapter 2**). It was hypothesized that repetitive stimulation, consisting of stimulation episodes of 10 seconds alternated with pauses of 10 seconds, would improve breathing effort. Pauses in stimulation were included in an attempt to avoid habituation of the reflex.(41) Respiratory effort was shown to be higher in the repetitive stimulation group, but these differences did not reach statistical significance. However, while infants in the repetitive stimulation group had significantly better oxygenation, despite requiring a significantly lower fraction of inspired oxygen (FiO_2), the findings of an increased respiratory effort were clinically relevant and indicate that applying repetitive stimulation may facilitate the respiratory transition of preterm infants at birth. (42)

The incidence of stimulation has been studied retrospectively in many studies, and was shown to vary between centers: from 35 % in the study of Baik-Schneditz et al. to 90 % in the study of van Henten et al.(32-34, 43) Lower percentages of stimulation were reported in infants < 30 weeks of gestation, which are wrapped in a polyethylene bag. (32, 34) The polyethylene bag might form a physical barrier and thereby contributes to the omission of stimulation.(44, 45) However, these infants usually need more (respiratory) support and could benefit from receiving tactile stimulation.

It is important to note that, while we have described an incidence of stimulation of 67 % during our retrospective study (**Chapter 1**), the incidence increased when a clinical trial was performed, as described in **Chapter 2**. Despite the fact that infants in the standard stimulation group were supposedly stimulated based on clinical indication, the incidence of stimulation appeared to be 96 %.(32-34, 42) It is likely that performing a study on a maneuver like tactile stimulation produced a Hawthorne effect, leading to an increase in applying stimulation in the control group.(42) This could explain the smaller than expected difference between the intervention and control groups in respiratory effort, resulting in an underestimation of the effect. Although further larger trials are needed to test the effect of repetitive stimulation on clinical outcomes, the demonstrated positive effect on respiratory effort may reduce further our ability to attain clinical equipoise.(42)

Oxygenation

We reported the effect of oxygenation on respiratory effort at birth in a spontaneously breathing preterm rabbit model in **chapter 3** and in preterm infants in **chapter 5&6**.
In the model described in **chapter 3**, preterm rabbit kittens were breathing spontaneously while being supported non-invasively after birth. Kittens showed a more stable breathing pattern when FiO_2 1.0 was given when compared to FiO_2 0.21. Kittens receiving a FiO_2 of 0.21 suffered from apnea, and the breathing pattern was restored more stable after rescue ventilation was given with a FiO_2 of 1.0 compared to 0.21.

It is well established that, in utero, when oxygenation levels are reduced below normal (PaO_2 < 25 - 30 mmHg), fetal breathing movements are greatly reduced or even abolished. (46-50) On the other hand, hyperoxia can stimulate fetal breathing movements, but the stimulatory effect is not sustained.(51-53) After birth, it is now well established that the inhibitory effect of hypoxia (PaO_2 below 20 - 25 mmHg) on breathing persists for days - weeks, it diminishes with time and eventually switches to a stimulation of respiratory drive which persists for the remainder of our lives.(54) In addition, intermittent hypoxia during uterine contractions might elevate fetal plasma adenosine concentrations, which also could inhibit peripheral and central chemoreceptors and cause respiratory depression.(55)

Oxygenation is largely defined by the surface area available for gas exchange and the diffusion distance as well as the partial pressure gradient for oxygen between the alveoli and adjacent capillaries. It has become clear that in most very preterm infants, clinicians fail to create adequate lung aeration and thus have to use a higher FiO_2 to compensate for the suboptimal surface area created for gas exchange. Using the latest resuscitation guidelines, most preterm infants fail to reach the 25th percentile of the oxygen saturation (SpO_2) reference values in the first minutes after birth, despite the use of SpO_2-based titration.(56-62) Caregivers thereby appear to accept hypoxia and disregard the effect on respiratory effort. As increasing the FiO_2 can reduce the level of hypoxia, this would be expected to increase the respiratory effort in preterm infants. However, this has so far only been demonstrated in an observational study where an increase in respiratory drive was observed after switching fraction of inspired oxygen from 0.21 to 1.0.(63) Nevertheless, it is important to recognize that when hypoxia persists for longer than the first 5 minutes after birth, it is associated with a higher risk of mortality before hospital discharge and development of intraventricular hemorrhage. (64) Caregivers should aim for an optimal level of oxygenation in preterm infants directly after birth, while avoiding both hypoxia and hyperoxia.

Resuscitation with FiO_2 1.0 was shown to increase pulmonary blood flow in a preterm lamb model, equal to the increase occurring in term lambs, permitting optimal gas exchange, while this increase was not observed in lambs resuscitated with FiO_2 0.21.(65) Up until 2005, guidelines recommended that resuscitation of preterm infants commenced with a FiO_2 of 1.0 in order to improve oxygenation at birth. However, SpO_2 were not monitored consistently, which resulted in an increased risk for hyperoxia.(66) Excessive oxygen exposure should be avoided in infants during stabilization at birth, as hyperoxia increases free radical production thereby overwhelming the immature antioxidant capacity of the preterm infant, which might lead to damage to cells, enzymes, lipids, DNA and proteins.(67-69) Meta-analyses have found that resuscitation of term infants at birth with FiO_2 0.21 significantly reduced mortality compared with infants resuscitated with FiO_2 1.0.(70-72) Less data is available in preterm infants, although hyperoxia at birth likely increases the risk of bronchopulmonary dysplasia (BPD).(73, 74) For this reason, international resuscitation guidelines now recommend to initiate resuscitation with low FiO_2 levels, which should thereafter be titrated based on SpO_2 target ranges.(36) However, the SpO_2 target ranges are based on data from healthy term and preterm infants, who did not need extensive resuscitation.(75) As such, the optimal SpO_2 target ranges for compromised preterm infants are not clear, although it is possible that better oxygenation is needed for optimal stimulation of spontaneous breathing.

High versus low FiO_2 was compared in recent multi-center trials in human preterm infants.(58, 59, 61, 62, 76-79) Although a recent systematic review showed no differences in percentage of infants achieving SpO_2 target ranges between infants initiating stabilization with high or low FiO_2, the level of evidence of these results is low.(80) So far, the studies comparing different initial FiO_2 levels did not evaluate the effect on respiratory effort. We have conducted a trial to test the effect of initial high FiO_2 versus low FiO_2 with subsequent titration based on SpO_2, on respiratory effort in the first minutes after birth (**Chapter 5 & 6**). Respiratory effort was assessed by using a respiratory function monitor calculating minute volume and electromyography recordings of the diaphragm. In this trial, FiO_2 was titrated up from 0.3 to 0.5 and 1.0 when SpO_2 values are below reference ranges, and down from FiO_2 1.0 to 0.5, 0.3 and 0.21 if SpO_2 values are above reference ranges. Breathing effort was significantly higher in preterm infants who started initially with FiO_2 1.0 during stabilization as compared to infants initiating with FiO_2 0.3, with minute volumes that were almost twice as high in the FiO_2 1.0-group. Although other interventions described in this thesis had a positive effect on increasing breathing effort as well, the magnitude of the difference in MV that is shown between the FiO_2 1.0-group and the FiO_2 0.3-group in the trial described in

Chapter 6 was not demonstrated before in any other trial on breathing effort of preterm infants at birth. This indicates that oxygen might be the major contributor to improving breathing effort at birth. In addition, infants in the FiO_2 1.0-group were significantly better oxygenated in the first 5 minutes after birth, with a shorter duration of hypoxia without an increase in hyperoxia. The better oxygenation and breathing effort are also reflected by a shorter duration in mask ventilation given in the FiO_2 1.0-group. These results indicate that initiating with a high level of oxygen followed by careful titration to avoid hyperoxia, is a better option for stimulating breathing and decreases the need for positive pressure ventilation. Although not significantly different, intubation rates in the delivery room or within 24 hours after birth, as well as the incidence of intraventricular hemorrhage (IVH) and mortality, were higher in the FiO_2 0.3-group compared to the FiO_2 1.0-group. However, the current trial was not designed to detect significant differences in clinical outcomes. Because of the magnitude of the difference in breathing effort that was demonstrated, using this intervention in an appropriately sized clinical trial might result in significant differences in clinical outcomes.

It is important to note that, as we have described in **Chapter 4**, there appears to be a clear delay in obtaining the desired oxygen concentration when using a T-piece resuscitator in both a bench test and during stabilization of preterm infants at birth. As the international resuscitation guideline prescribes evaluation periods of 30 s, the clinical evaluation of the infant and physiological parameters might precede the effect of the performed intervention (e.g. titration of oxygen).(36) This delay was also present in the study by Follett et al.(81) who used different ventilators and different lengths of ventilation circuits, while we used the equipment that we use in the clinical setting. However, while both the current trial and the trial of Follett et al.(81) demonstrated that there appears to be a delay in achievement of desired FiO_2, the exact duration of delay might be dependent on multiple variables, including type of ventilator and length of ventilation circuits. In addition, the rate with which the desired FiO_2 is reached in the infant might also be dependent of the volume containing the different FiO_2 that needs to be replaced, which is influenced by the pressure administered (peak inspiratory pressure and positive end expiratory pressure), respiratory rate and type of respiratory support (CPAP vs positive pressure ventilation (PPV) vs sustained inflation).

Caffeine

In the study described in **Chapter 7** we evaluated the effect of administration of caffeine base (10 mg/kg, administered by the use of a butterfly needle (21 G) inserted in the umbilical vein) in the delivery room on respiratory effort of preterm infants in a small randomized controlled trial.(82) Infants who received caffeine in the delivery room had

a greater respiratory effort, with higher minute volumes, inspired tidal volumes and recruitment breaths (with a tidal volume > 8 mL/kg), as compared with infants receiving caffeine after admission to the NICU.(82) Although this trial consisted of a small number of infants with a gestational age of 24 - 30 weeks, the trial was able to demonstrate a significant positive correlation between minute volume and gestational age. The minute volume increased by 2.4 mL/min/kg with each day of gestational age. This association was even more pronounced when caffeine was administered in the first minutes after birth with an increase in minute volume of 4.1 mL/min/kg with each day of gestational age.(82) These results indicate that the stimulatory effect of caffeine is gestational age dependent, and different caffeine dosages per gestational age would be needed to gain the optimal effect on breathing effort.(82)

Caffeine is a methylxanthine that has a molecular structure similar to adenosine and works as an adenosine-receptor antagonist to reduce adenosine-induced respiratory depression.(83) The required dosage of caffeine might therefore be influenced by the level of adenosine. Inflammation leads to an increase in adenosine levels, and also the presence of hypoxia leads to an imbalance between adenosine synthesis and its breakdown.(84, 85)

Although the safety and effectivity of caffeine to prevent apnea of prematurity has been demonstrated in a large randomized trial, the optimal timing and dosage is still unclear.(86, 87) A systematic review comparing the effects of high versus low doses of caffeine in the first days after birth demonstrated that a high dose of caffeine led to a decrease in BPD, the combined outcome BPD or death and extubation failure, although the level of evidence was reported to be low.(88) However, these findings endorse the possible advantages of a higher dose of caffeine, which should be confirmed in a large randomized controlled trial. When caffeine is administered even earlier, within the first 2 days of life , it decreases the risk of developing BPD and improves both short- and long-term neurodevelopmental outcomes.(86, 89-92) When administered as soon as within 2 hours after birth, it decreases the incidence of CPAP failure, which in turn could lead to further improvement in outcome.(93) One possible explanation is increased diaphragm activity, which occurs after administration of a loading dose of caffeine, leading to higher tidal volumes that are indicative of an increase in respiratory effort. (94) Since we have described that caffeine administration in the first minutes after birth increases respiratory effort which might lead to better outcomes as well, the presence of the stimulatory effect of caffeine seems desirable as soon as the infant is born.

Because caffeine can freely pass the placenta by passive diffusion, administration to the mother before or during delivery could potentially lead to a direct stimulating effect on respiratory drive of the preterm infant at the time of birth.(95) This was demonstrated in the lamb-model of Binder-Heschl et al.(96), which showed that a loading dose of caffeine base administered to the ewe resulted in similar plasma caffeine concentration in the mother and the lamb, obtained immediately following infusion. In addition, the study of Binder-Heschl et al.(96) showed a significant decrease in SpO_2 after cord clamping in the lambs not receiving caffeine, while this was absent in the caffeine treated lambs. It is possible that when antenatal administration of caffeine leads to better aeration of the lung, this could decrease the occurrence of hypoxia after early clamping of the cord.

Delayed cord clamping

Before birth, the placental circulation contains approximately 30 – 50 % of the blood volume of the combined fetal/placental unit. While the lungs remain unaerated and the pulmonary circulation remains vasoconstricted, cardiac output is largely dependent on venous return from the placenta. Clamping the umbilical cord before lung aeration, therefore, causes umbilical venous return to cease which can lead to a sudden decrease in cardiac output. However, when the lungs aerate before cord clamping, the associated increase in pulmonary blood flow can replace umbilical venous return as the primary source of ventricular preload and as such cardiac output remains unchanged. As such, clamping the cord after ventilation onset has less impact on cardiac output and avoids the cardiovascular instability at the time of clamping.(97, 98) This has been demonstrated in a preterm lamb model showing a more stable heart rate and arterial pressure when the cord is clamped after lung aeration compared to clamping before lung aeration.(99) In addition, ventilation before clamping of the umbilical cord has shown to increase arterial and cerebral oxygenation.(100) Increasing the pulmonary blood flow also leads to a better ventilation/perfusion ratio, thereby optimizing the uptake of oxygen leading to better oxygenation. This will enhance respiratory effort even more.

It is currently unclear how delaying cord clamping affects the respiratory transition after birth. Keeping the cord intact should provide the newborn with a baseline PaO_2 that is no lower than that which occurred before birth, assuming that placental gas exchange is still functional. Delaying cord clamping could thereby help in the establishment of a continuous breathing pattern after birth, as severe breathing-inhibitory hypoxia due to cord clamping would be avoided. On the other hand, the placenta releases prostaglandins (of the E series) and adenosine into the fetal circulation which are known

to inhibit breathing.(101) As such, cutting the cord might be beneficial for breathing activity as it would reduce circulating prostaglandin (and adenosine) levels and thereby reduce any inhibitory effect on breathing.(102) However, as circulating prostaglandins are completely metabolized by circulation through the lung, the inhibitory effect of the prostaglandins may only be an issue in apneic infants.(103, 104) This makes it only more important that lung aeration occurs while delaying cord clamping. Also, it is possible that for those apneic infants, prostaglandin synthesis inhibitors might result in an increase in respiratory activity.(105)

So far, there are no studies assessing respiratory effort of infants receiving delayed cord clamping. However, Katheria et al.(106) showed that infants who did not receive respiratory support during delayed cord clamping needed significantly more stimulation to initiate breathing, and the duration of stimulation was longer to maintain spontaneous breathing. During delayed cord clamping, respiratory support could enhance spontaneous breathing by improving lung aeration, leading to better oxygenation and reducing hypoxia.(100, 107) Although timing of the first breath is a measure of respiratory effort, effectivity of spontaneous breathing was not objectively evaluated by using respiratory function parameters. By objectively evaluating respiratory effort during delayed cord clamping, one could determine to what extent circulating prostaglandin affects spontaneous breathing, and thus if there appears to be an indication for the use of prostaglandin synthesis inhibitors.

MAINTAINING SPONTANEOUS BREATHING IN THE FIRST HOURS AFTER BIRTH

CPAP

Applying CPAP can be used to facilitate respiratory transition at birth by increasing the pressure gradient which promotes alveolar fluid reabsorption and prevents end-expiratory alveolar collapse. This in turn increases the surface area available for gas exchange(108), leading to improved oxygen exchange and a decreased risk of hypoxia.(109) Also, functional residual capacity will increase by maintaining alveolar aeration during both inspiration and expiration, leading to a reduction in work of breathing.(110) In infants who breathe spontaneously at birth, CPAP is therefore recommended for use as the initial mode of respiratory support.(30, 36) Studies showed that the use of CPAP after the initial stabilization at birth led to decreased BPD rates when compared to elective intubation and positive pressure ventilation.(4, 111)

While the beneficial effects of CPAP use in the delivery room have been shown, the optimal CPAP level and strategy remain unclear. A recent review has shown that there is a wide variety of CPAP practices across different units, varying in pressure levels and titration strategies.(112) Although the international guidelines recommend the use of CPAP levels between 5 - 8 cm H_2O, experimental studies have shown that higher CPAP levels lead to better lung aeration.(24, 113) Instead of using a fixed CPAP level, we might need to adjust the level according to the phase of respiratory transition. It is likely that higher CPAP levels are initially needed to assist airway liquid clearance during lung aeration, whereas during the subsequent phase, the primary role of CPAP is to minimize airway liquid re-entry when the lung is at functional residual capacity (FRC).(112) On the other hand, sustained high CPAP levels might delay stiffening of the chest wall by opposing lung recoil. Therefore, CPAP levels should be weaned down after respiratory transition at birth and clearance of lung liquid from the interstitial space. However, more data are needed to define the optimal CPAP strategy for facilitating and maintaining lung aeration at birth in order to improve oxygenation without causing overdistension of the lung.(114)

Surfactant

After transition has been successfully established, respiratory distress syndrome (RDS) can cause difficulties in obtaining an appropriate level of oxygenation, which might lead to CPAP failure.(8, 10, 115) In this stage, oxygenation can be improved by treating RDS with exogenous surfactant, as this results in improved lung compliance and less work of breathing.(116) Recently, minimal or less invasive surfactant administration techniques have gained increasing favor.(31, 117) This involves administering surfactant via nasogastric tubes, angio-catheters or specially designed catheters positioned in the trachea, while the infant is spontaneously breathing on CPAP. These techniques have shown to be effective, resulting in increased breathing effort.(118-123) However, the terminology less or minimally invasive (MIST) is potentially misleading as the procedure still involves placement of a catheter in the trachea using a laryngoscope to visualize the vocal cords.(31, 117, 124) It is known that laryngoscopy is highly uncomfortable, and while this is performed in an awake infant, his/her attempts to resist this procedure might lead to negative cardiovascular responses.(125-127) As experiencing pain during procedures might affect neurodevelopment of preterm infants, efforts should be taken to reduce pain or discomfort during a procedure.(128) In addition, the use of sedation to enhance the comfort of the infant during MIST could increase the chance of an uneventful procedure. On the other hand, caution is needed as the use of sedation during the procedure might impair the infant's respiratory drive.

We assessed the effect of low-dose propofol as premedication for MIST in an observational study and a randomized controlled trial (**Chapter 8 & 9**).(129, 130) The number of infants who were comfortable (COMFORTneo score < 14) was significantly higher in the group who received low-dose propofol compared to no premedication. However, propofol led to significantly more desaturations and the need for nasal intermittent mandatory ventilation (nIMV) also increased, although temporarily.(129) This indicates a decrease in respiratory drive or respiratory effort, which might be counterproductive as the maintenance of spontaneous breathing is essential for successful administration of surfactant in a minimal invasive way. However, this effect was transient and did not lead to an increased need for intubation. In addition, the maturity of the respiratory center evolves during gestation, which might influence the response to premedication as well. Indeed, most infants with a gestational age < 32 weeks receiving propofol needed nIMV.(129) It might be because of this, that those infants with a low gestational age receive the lowest amount of analgesic interventions, while those infants undergo the largest amount of (painful) procedures.(131) Administration of low-dose propofol could therefore be considered in obtaining a better level of comfort during MIST.

The choice of sedative during MIST is dependent of the level of sedation/analgesia that can be achieved, counterbalanced by the negative effect it may have on respiratory drive. Remifentanil was thought to be promising sedative for procedures such as intubation-surfactant-extubation (INSURE) due to its rapid distribution and redistribution.(132) However, remifentanil is a potent respiratory depressant and the level of sedation during INSURE was not shown to be effective.(133) Recent trials have shown an adequate level of sedation when propofol is used and it is now widely used for intubation and other procedures.(134-136) However, side effects such as hypotension have been described.(137) There is much controversy whether propofol provides analgesia next to sedation.(138) Nevertheless, the sedative effect of propofol might result in better comfort and thus less stress, thereby avoiding possible negative effects of MIST on neurodevelopment.(129) An additional benefit of propofol is its short acting anesthetic properties which, in animals, minimizing the swallowing reflex allowing the larynx to relax and providing easier access to the upper trachea.

Taken altogether, available data indicate that sedatives can be used to decrease discomfort of infant receiving surfactant non-invasively, but dose finding and alternative drugs need to be investigated to decrease the side effect on respiratory effort.

LIMITATIONS

In **Chapter 1 & 2** the results of the studies on tactile stimulation are described. While the occurrence of tactile stimulation was evaluated by use of a respiratory function monitor, motivation to perform tactile stimulation could not be deduced. In addition, the incidence of stimulation appeared to increase during the conduction of the randomized trial. Therefore, the differences in treatment between the groups were likely smaller than expected, leading to smaller-than-expected differences in outcomes. However, although the majority of results were statistically not significant, we were able to demonstrate a significant difference in oxygenation – representing a clinically relevant effect.

In **Chapter 3** the positive effect of the use of 100% oxygen on respiratory effort was explicated. This study was limited as oxygenation of the preterm rabbits could not be confirmed. We administered 21% O_2 or 100% O_2, although we do not know what PaO_2 and oxygen saturation levels were achieved and, therefore, to what extent oxygenation of the kittens influenced the results. However, the optimal oxygen saturation range that we should aim for is not clear in those extreme preterm infants, as the internationally recommended target ranges are based on healthy term and preterm infants not requiring any resuscitation.

While the randomized trials described in part II of this thesis are limited by a small sample size, it is impossible to make clinically relevant treatment recommendations on the interventions studied. However, the aim of performing these trials was mainly to assess the direct effect of the interventions on respiratory effort, to guide a future larger trial focused on an important clinical outcome.

In part III of this thesis (**chapters 8 & 9**), the use of propofol as premedication for MIST was evaluated, but in both studies the control groups lacked the administration of a placebo. This might have influenced the results in terms of applied interventions for respiratory support. In case the infant received a low dose of propofol as premedication for MIST, the caregivers might have started to increase the level of respiratory support sooner, leading to a higher incidence of the use of nIMV. However, during analysis of the primary outcome of the randomized trial (level of comfort during the procedure), the researchers were blinded for the allocated treatment. Therefore, the conclusions made on this outcome are considered valid. However, in **Chapter 9**, the relatively small sample size precludes the recommendation of the use of low-dose propofol for different GA ranges.

FUTURE PERSPECTIVES

While we described different ways to stimulate breathing at birth, it is also important to recognize that these attempts should not take away the focus on applying adequate respiratory support (Figure 2). A possible solution could be to automate some of the described interventions to stimulate breathing. We described that tactile stimulation is often omitted. The development and use of a device for automated stimulation based on respiratory effort of the infant might assist the caregiver during resuscitation. Hereby, the caregiver can fully focus on applying optimal non-invasive ventilation by face-mask which might lead to less leakage or obstruction.

Normoxia is also an important determinant of respiratory drive. Ideally, both hypoxia and hyperoxia should be avoided. Automated oxygen titration used during resuscitation of preterm lambs led to similar time spent below and within SpO_2 target range compared to manual FiO_2 control, but the time spent above target range was significantly shorter when using automated control.(139) Optimizing automated oxygen titration in the delivery room by using narrower target ranges or devices with better algorithms could potentially lead to more time spent within the SpO_2 target range, resulting in improved respiratory effort.

We have shown in **Chapter 7** that the effect of caffeine is significantly related to GA of the infant. However, optimal caffeine dosages for infants in different GA ranges are not yet defined. In addition, those dosages might need to be defined based on other determinants of adenosine levels at birth as well, such as presence of inflammation or hypoxia. In addition, the stimulatory effect of caffeine on respiratory effort might be present as soon as the infant is born when caffeine would be administered antenatally. It has been shown that, using this approach, the required dose is reached immediately after birth. Thereby, less interventions might be required after birth focused on stimulation of spontaneous breathing. This, in turn, enables the caregiver to focus on applying non-invasive respiratory support. Altogether, respiratory transition might occur more smoothly, possibly leading to better outcomes.

In case of signs of RDS after admittance to the NICU, surfactant should be administered in a way that preserves respiratory drive and prevents discomfort. When we are able to determine the optimal dose of propofol in different gestational age ranges during this procedure, we might avoid adverse effects on neurodevelopmental outcome due to stress during the procedure.

For each of these interventions, large trials are needed to test these newly acquired hypotheses. However, since the subjects of these trials are infants born at the limit of viability which are considered most vulnerable, performing multiple trials on the same aim seems undesirable. Incorporating all interventions focused on stimulation of spontaneous breathing at birth into a bundle of care might reduce the number of patients needed to test the effect of our hypotheses. Using experimental studies to provide us with knowledge on the physiological basis of each intervention, a bundle of care might be composed comprising the optimized version of all interventions. Herewith, recommendations can be made on improving respiratory support during stabilization at birth.

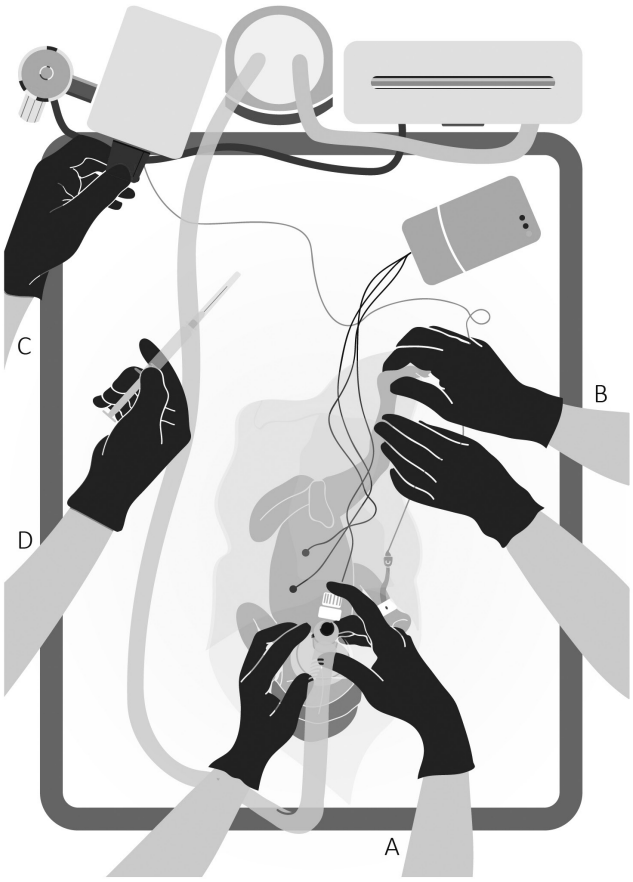


Figure 2 | Interventions focused on stimulation of spontaneous breathing during stabilization at birth. A: Application of continuous positive airway pressure, supplemented with inflations if indicated. B: Tactile stimulation. C: Administration of supplemental oxygen. D: Administration of a loading dose of caffeine via the umbilical vein.

CONCLUSION

The success of non-invasive ventilation depends on the effectiveness of spontaneous breathing both during transition and on the NICU. At birth, the importance of larynx function has been overlooked in the story of a successful transition of preterm infants. Thus, interventions which aide laryngeal patency could be a game changer when non-invasive ventilation for stabilization of preterm infants is desired. Therefore, the focus of the caregiver needs to shift towards stimulation instead of trying to take over the spontaneous breathing efforts of the infant with positive pressure ventilation. While different ways for supporting and stimulating breathing effort have been investigated separately, it is likely that combining these interventions in a bundle of care will increase the success in maintaining effective breathing of the preterm infant.

After admission to the NICU, maintaining spontaneous breathing leads to a reduction in mortality and morbidity.(111) The success of non-invasively administered surfactant has been shown before, and the focus has now shifted to the reduction of side-effects accompanied by this procedure. While the effect of non/less-invasive surfactant administration on morbidity and mortality still needs to be demonstrated, this procedure has already been adopted by many centers world-wide. Most would argue that the benefit has already been shown by decreasing the need for mechanical ventilation. In this thesis we demonstrated that using a low-dose sedation increases comfort of the infant, which has the potential to reduce the risk for neurodevelopmental complications due to stress of the procedure. Dose finding and comparison with different agents is still needed to find the right balance between comfort and effect on respiratory drive. Nevertheless, maintaining spontaneous breathing while avoiding stress during the procedure could increase the success and benefit of non/less-invasive surfactant administration.

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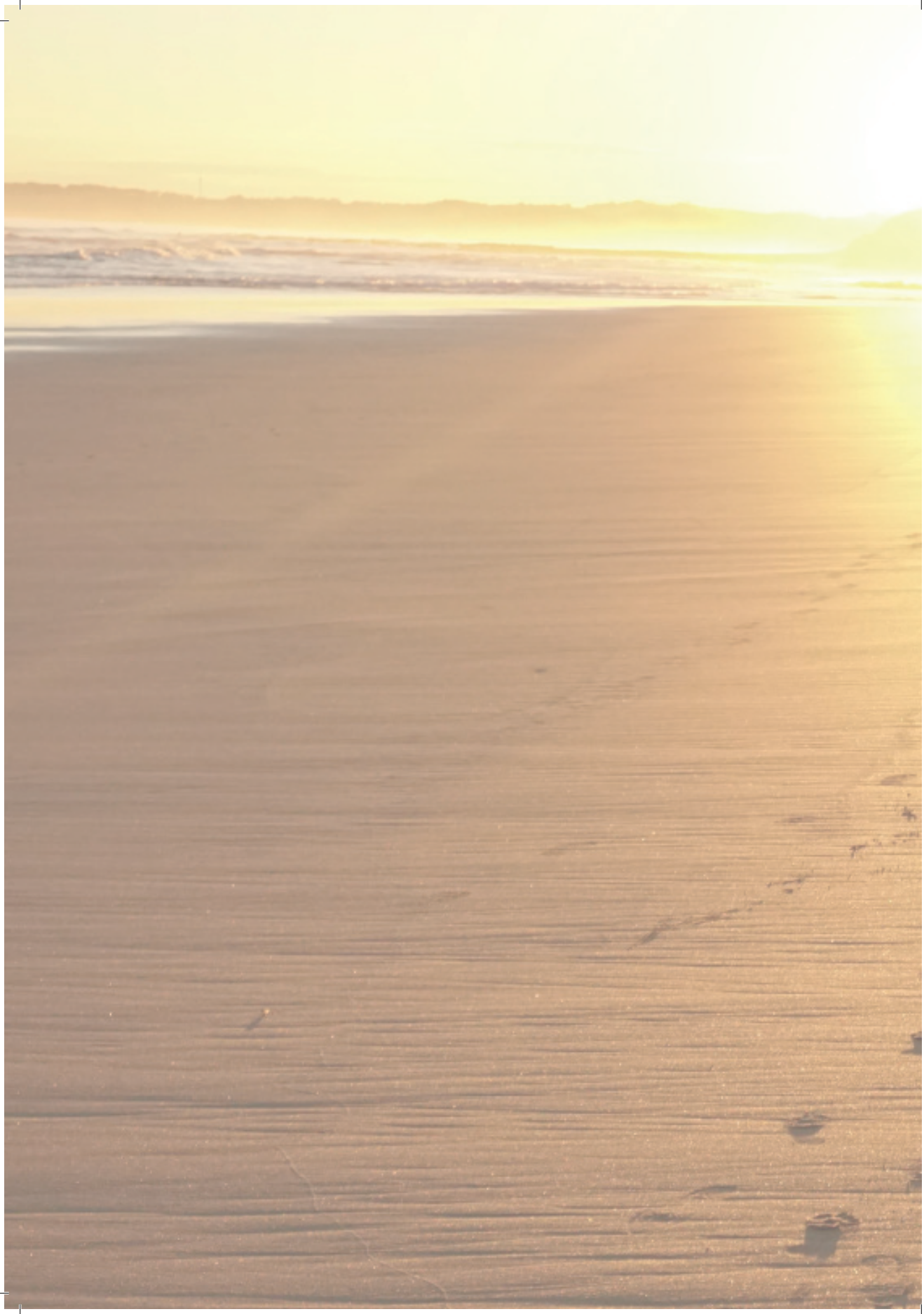


SUMMARY

Most preterm infants breathe at birth, but need additional respiratory support due to immaturity of the lung and respiratory control mechanisms. To avoid lung injury, the focus of respiratory support has shifted from invasive towards non-invasive ventilation. However, applying effective non-invasive ventilation is difficult due to mask leak and airway obstruction. The larynx has been overlooked as one of the causes for obstruction, preventing face mask ventilation from inflating the lung. The larynx remains mostly closed at birth, only opening briefly during a spontaneous breath. Stimulating and supporting spontaneous breathing could enhance the success of non-invasive ventilation by ensuring that the larynx remains open. Maintaining adequate spontaneous breathing and thereby reducing the need for invasive ventilation is not only important directly after birth, but also in the first hours after admission to the Neonatal Intensive Care Unit (NICU). Respiratory distress syndrome (RDS) is an important cause of respiratory failure. Traditionally, treatment of RDS required intubation and mechanical ventilation to administer exogenous surfactant. However, new ways have been implemented to administer surfactant and preserve spontaneous breathing while maintaining non-invasive support.

The general aim of this thesis was to evaluate the effect of interventions on the respiratory effort of preterm infants performed directly after birth in the delivery room and shortly after arrival on the neonatal intensive care unit. The interventions evaluated in this thesis are targeted on stimulating and maintainaning spontaneous breathing. This thesis comprises experimental studies, observational clinical studies and randomized clinical trials.

STIMULATING SPONTANEOUS BREATHING AT BIRTH

Tactile stimulation

We evaluated the use and effect of tactile stimulation on respiratory effort at birth.

In **chapter 1**, we evaluated the incidence and methods of tactile stimulation during stabilization of preterm infants at birth. In total 245 recordings of video and physiological parameters of the stabilization at birth of preterm infants with a gestational age < 32 weeks were retrospectively analyzed, and details of tactile stimulation during the first 7 minutes after birth were noted. We observed that tactile stimulation was applied in 164/245 (67%) of infants during stabilization at birth. Stimulation was clinically indicated in 67% of stimulation episodes, but a proportion of infants did not receive stimulation while this was indicated. In addition, the starting time, duration and method of tactile

stimulation varied between infants. The observed variation in practice warrants further studies to investigate the most optimal duration and type of tactile stimulation, which will then lead to a more specific recommendation of the use of tactile stimulation in resuscitation guidelines.

In **chapter 2**, we evaluated the direct effect of repetitive tactile stimulation on breathing effort of preterm infants at birth in a randomized controlled trial. Preterm infants with a gestational age < 32 weeks were randomized to receive repetitive stimulation, defined as gently rubbing the back or the soles of the feet during 10 s, alternated with 10 s of rest, or standard stimulation, defined as gently rubbing the back or the soles of the feet when clinicians considered the breathing to be insufficient or absent. In both groups, physiological parameters, including respiratory function monitoring (RFM) were recorded during stabilization at birth and analyzed to evaluate respiratory effort. Although not significantly different, all respiratory effort values (minute volume (MV), tidal volumes (Vt) and rate of rise to maximum tidal volume (RoR)) were higher in the repetitive stimulation group. Considering that all measured parameters point to the same direction, the findings are likely to be clinically relevant. The study created more awareness for tactile stimulation as infants in the standard stimulation group were much more frequently stimulated when compared to a cohort in our previous observational study (96% vs 67%). Infants in the standard group were often stimulated without a clear clinical indication (37%). This could have resulted in reducing the difference in outcomes between the two groups. Nevertheless, we observed better oxygenation (SpO_2 values 87.6 ± 3.3% vs 81.7 ± 8.7%, p=0.01) while less extra oxygen was needed at the end of resuscitation (28.2 (22.8 – 35.0) % vs 33.6 (29.4 – 44.1) %, p=0.04) in the repetitive stimulation group when compared to the standard group, indicating that the non-statistical differences in respiratory function between the two groups were biologically significant.

Oxygen

To evaluate the effect of the administration of oxygen on hypoxia-induced breathing inhibition at birth, we have performed an experimental study and subsequently a randomized controlled trial. To inform the resuscitation guidelines also in a more practical way, we evaluated the duration of achievement of desired oxygen concentration at the infant while using a T-piece ventilator during stabilization at birth.

The experimental study comparing using initially 100% O_2 with 21% O_2 for the stabilization of preterm rabbits at birth is described in **chapter 3**. This experiment consisted of two phases. Rabbit kittens who were spontaneously breathing on continuous positive

airway pressure (CPAP) were divided into two groups and were initially breathing in either 21% O_2 or 100% O_2, while their breathing pattern was analyzed (phase 1). All kittens started on CPAP as means of respiratory support, commencing with a pressure of 15 cmH_2O, which was titrated down to 8 cmH_2O, with a rate of 2 cm H_2O/30 seconds. If kittens became apneic, a rescue intervention was performed with positive pressure ventilation (PPV) using a ventilation rate of 60 breaths per minute, peak inflation pressure (PIP) of 25 cm H_2O and positive end-expiratory pressure (PEEP) of 8 cm H_2O. The oxygen concentration during the rescue ventilation depended on the group. Kittens that commenced in 21% O_2 were randomized to receive either 21% O_2 or 100% O_2, whereas kittens that commenced in 100% O_2 remained in 100% O_2. Whenever ventilation alone was not sufficient for regaining a stable breathing pattern, physical stimulation was applied. After rescue intervention, breathing pattern was again analyzed (phase 2). In both phases, functional residual capacity (FRC) was measured using high resolution phase-contrast X-ray imaging as a parameter of lung aeration. We observed that initiating resuscitation of preterm kittens with 21% O_2 resulted in a more unstable breathing pattern (higher variability in inter-breath interval: 68.5 ± 11.9 % vs 40.1 ± 4.2 %, p=0.042), a lower respiratory rate (29 ± 4 breaths/minute vs 42 ± 3 breaths/minute, p=0.038) and a higher incidence of apnea (11/12 (92%) vs 1/8 (13%), p=0.001) compared to kittens starting resuscitation in 100% O_2. Furthermore, the kittens who were rescued with 21% O_2 after apnea, also had a higher inter-breath variability (83.6 ± 32.2 % vs 9.5 ± 1.3 %, p=0.014) and lower respiratory rate (27 (18 – 31) vs 45 (39 – 50) breaths/minute, p=0.007) than kittens rescued with 100% O_2. The degree of lung aeration, as assessed by measuring FRC, was not affected by the O_2 concentration used for stabilization or rescue from apnea. The degree of lung aeration was similar between the two groups, despite a much higher respiratory activity, which could be influenced by the moment of start of resuscitation. As the kittens are very fragile, providing them with medication, an oesophageal tube, a face mask and attaching them to the ventilator is a delicate process, which can vary the delay between birth and imaging onset. However, while this delay is not well defined in our study, human preterm infants likely experience similar variations in the delay between birth and resuscitation onset. However, these data indicate that avoidance of apnea and stability of breathing are predominantly determined by oxygenation in the newborn immediately after birth.

During stabilization of human preterm infants at birth, oxygen is titrated based on oxygen saturation reference ranges described by Dawson et al.(2010) to reduce the risk for hypoxia and hyperoxia. During stabilization after birth, infants are evaluated every 30 s, which guides the amount of support and titration of additional oxygen. In

chapter 4, we described an observational study consisting of a bench test and clinical observations of stabilization of preterm infants, which aimed to determine the time between adjustment of FiO_2 at the oxygen blender and the desired FiO_2 reaching the preterm infant. A Neopuff™ T-piece Resuscitator attached to a 50 mL test lung or a face mask was used to administer CPAP or PPV. The circuit was set up with a flow of 8 L/min, PEEP of 8 cm H_2O, and a PIP of 20 cm H_2O in case of PPV. FiO_2 was measured at two distinct positions at the circuit, using oxygen analyzers: proximally - at the outlet of the Neopuff™, and distally – between the T-piece of the Neopuff™ and the test lung or face mask. This study shows a clear delay in obtaining the desired oxygen concentration at the distal part of the Neopuff™ circuit in both the bench test (34.2 (21.8 – 69.1) s) and during stabilization of preterm infants at birth (19.0 (0.0 – 57.0) s). As the international resuscitation guideline prescribes evaluation periods of 30 s, the clinical evaluation of the infant and physiological parameters might precede the effect of the performed intervention (e.g. titration of oxygen). This is demonstrated by the finding that in half of all titration episodes, the desired FiO_2 was not yet reached at the distal part of the Neopuff™ circuit. In addition, the time between two titration episodes was less than 30 s in 31% of titrations performed in this study. This delay is clinically relevant when aiming for adequate mask ventilation, whereby over- and under titration of oxygen might result in an increased risk for hypoxia and hyperoxia. Caregivers should be aware of this delay when proceeding titration if the effect is not forthcoming. With this delay in mind, trials should focus on determining the optimal titration steps to achieve normoxia.

In **Chapter 5** we described the study protocol of our randomized controlled trial on initiation stabilization with a FiO_2 of 0.3 or 1.0, with subsequent titration of FiO_2 based on oxygen saturation. Previously reported trials comparing high versus low levels of oxygen did not report on how oxygenation levels influence respiratory effort. Although the level of respiratory support during stabilization at birth was reported in most trials and shown to be not significantly different, the effectiveness of spontaneous breathing in both infants receiving higher or lower FiO_2 was not evaluated. Gaining an adequate level of spontaneous breathing during stabilization at birth to improve the success of non-invasive respiratory support could potentially lead to a lower incidence of intubation and mechanical ventilation in the delivery room, contributing to a lower risk of lung injury in high-risk preterm infants. Therefore, our study protocol aims to evaluate respiratory effort during stabilization at birth.

In **chapter 6**, the results of our randomized trial on stabilization with an initial FiO_2 of 0.3 or 1.0 are shown. In total 52 infants were randomized, and physiological parameters on breathing effort and oxygenation could be recorded of 44 infants (20 infants in 100%

O_2-group, 24 infants in 30% O_2-group). Average minute volume/kg was significantly higher in the 100% O_2-group (146.34 ± 112.68 mL/kg/min vs 74.43 ± 52.19 mL/kg/min, p=0.014). Average tidal volumes and rate of rise to maximum tidal volumes in the first 5 minutes after birth were significantly higher in the 100% group, while the duration of mask ventilation given was significantly shorter. Oxygenation was significantly higher in infants in the 100% O_2-group (85 (64 – 93) % vs 58 (46 – 67) %, p<0.001). The duration of hypoxia was significantly shorter in the 100% O_2-group, while the duration of hyperoxia (SpO_2 > 95%) was not different between groups. These results indicate that initiating with a high level of oxygen followed by careful titration to avoid hyperoxia, is a favourable option for stimulating breathing and decreasing the need for positive pressure ventilation.

Caffeine

In **chapter 7** we evaluated the effect of administration of caffeine base (10 mg/kg, administered by the use of a butterfly needle (21 G) inserted in the umbilical vein) in the delivery room on respiratory effort of preterm infants. We randomized 30 infants with a gestational age < 30 weeks to receive caffeine in the delivery room or after arrival at the NICU. To evaluate the effect on respiratory effort, we used the RFM to measure MV, Vt, RoR, respiratory rate and recruitment breaths. We observed that MV at 7 – 9 minutes after birth was significantly greater when caffeine was administered in the delivery room, after correction for gestational age (189 ± 74 mL/kg/min vs 162 ± 70 mL/kg/min, p<0.05). While respiratory rate did not increase, all other parameters assessing respiratory effort increased significantly by caffeine administration, including Vt_i, RoR and the percentage of recruitment breaths. Furthermore, in caffeine treated infants, we found that the relationship between MV and gestational age was highly significant (p=0.0008), with MV increasing at a rate of 4.1 mL/min/kg/day. In contrast, in control infants the relationship between MV and gestational age just failed to reach statistical significance (p=0.09), due to the small number of observations, with MV tending to increase at only 2.3 mL/min/kg/day. These findings indicate that caffeine enhances the gestational age-related increase in MV and that the stimulatory effect of caffeine on MV increases with gestational age. These results indicate that caffeine at birth could play a role in stimulating breathing during transition at birth.

MAINTAINING SPONTANEOUS BREATHING AFTER BIRTH

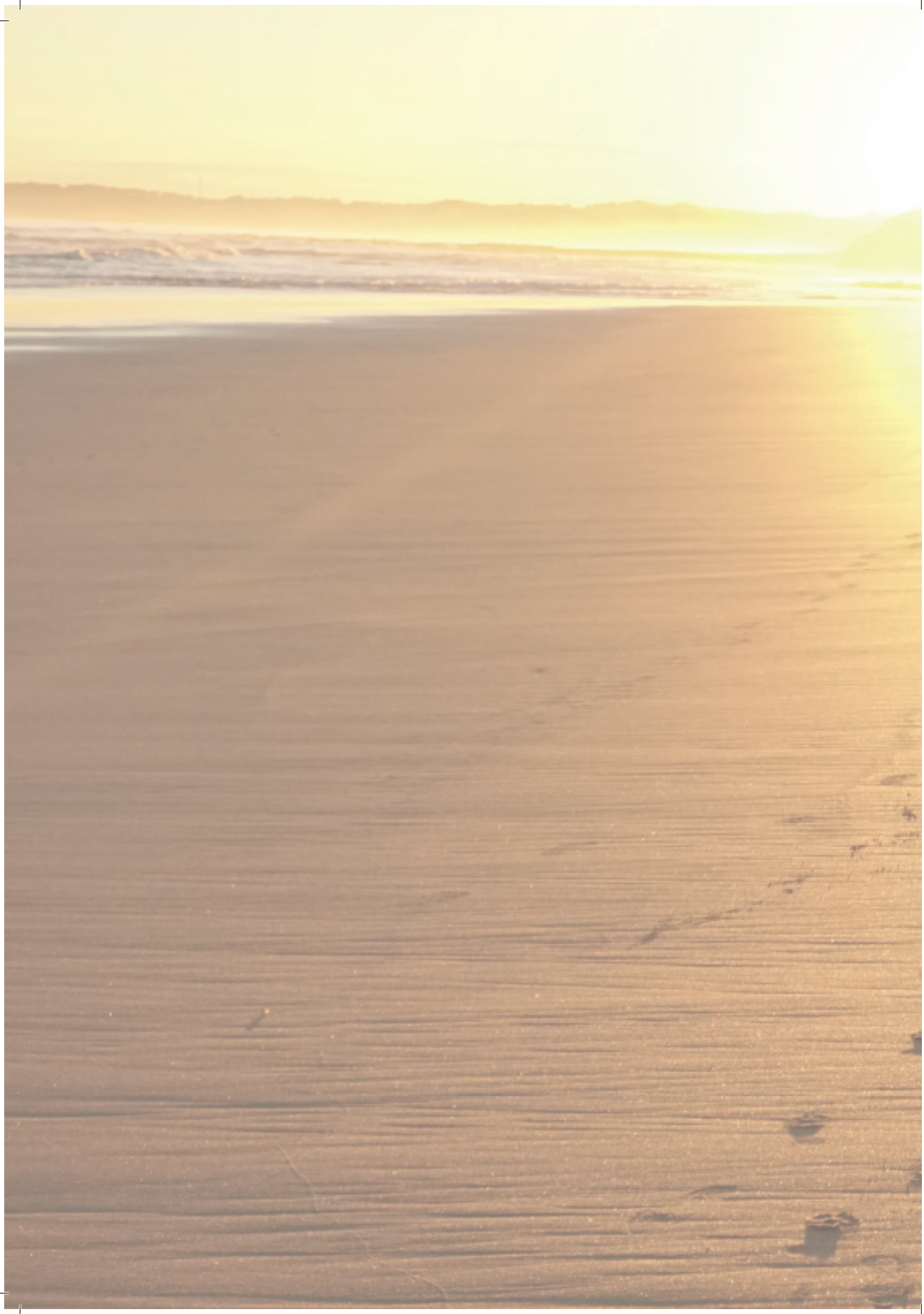
While the majority of very preterm infants leave the delivery room supported by non-invasive ventilation, in a large proportion their spontaneous breathing will be compromised by development of respiratory distress syndrome, for which administration of exogenous surfactant might be needed. Traditionally, intubation and subsequently mechanical ventilation was required to administer surfactant, increasing the risk for ventilation-induced-lung-injury. However, recent trials have demonstrated the feasibility and efficacy of surfactant administration in a minimal invasive way, thereby maintaining spontaneous breathing and avoiding intubation. A stable respiratory drive is a prerequisite to make this procedure successful in avoiding intubation and mechanical ventilation.

In **Chapter 8** we described our retrospective study on the effect of sedation during minimal invasive surfactant therapy (MIST) in preterm infants. MIST was performed in 38 infants with a gestational age of 26 - 37 weeks, using the method as described earlier by Dargaville et al.(2011), in which the vocal cords are visualized using a laryngoscope where after a semi-rigid angiocatheter was orally introduced to catheterize the trachea. Sedation (propofol 1 mg/kg) was optional and left to the discretion of the caregiver. Standardized COMFORTneo scores were compared and COMFORTneo < 14 was considered comfortable. We observed that preterm infants were more comfortable when they received a low dose of propofol as premedication for MIST (COMFORTneo score sedated group 12 (9 – 17) vs non-sedated group 20 (15 – 23), p=0.002), but the occurrence of respiratory complications was increased (incidence of nasal intermittent mandatory ventilation (nIMV) 23/23 (100%) vs 5/15 (33%), p<0.001) and non-significantly more intubations took place (8/23 (35%) vs 2/15 (13%), ns). These results needed to be confirmed in a randomized controlled trial to determine whether the benefit of sedation in comfort outweighs the risks for complications.

We therefore conducted a randomized trial on the use of sedation during MIST, which is described in **chapter 9**. In this randomized controlled trial, 78 infants with a gestational age between 26 - 37 weeks were randomized to receive either low dose sedation (1 mg/kg propofol iv) or no premedication during MIST procedure using the same technique as in the observational study. Standard comfort care was given in both groups, which consisted of administering sucrose in the cheek pouch of the infant and containment. We assessed the percentage of infants that were comfortable during the procedure (COMFORTneo-score < 14), as well as complications of both the MIST procedure and low dose sedation administration. We observed that preterm infants were more often

comfortable during the MIST procedure (percentage of infants with COMFORTneo score < 14: 32/42 (76%) vs 8/36 (22%), p<0.001). Although more often desaturation occurred (38/42 (91%) vs 25/36 (69%), p=0.023) and nIMV was given (39/42 (93%) vs 17/36 (47%), p<0.001), there was no increase in failed MIST procedure for which intubation was needed. We concluded that the low dose sedation could help increase comfort during MIST and might reduce the risk for neurodevelopmental complications due to stress of a painful stimulus. However, because of the relatively small sample size we are not able to distinguish the effect on complications between different gestational age strata. Therefore, a large and adequately powered randomized trial is warranted in order to guide recommendations for infants of different gestational ages.

In conclusion, the success of non-invasive ventilation depends on the effectiveness of spontaneous breathing both during transition and on the NICU. Therefore, the focus of the caregiver needs to shift towards stimulation instead of trying to take over the spontaneous breathing efforts of the infant with positive pressure ventilation. While different ways for supporting and stimulating breathing effort have been investigated separately, combining these interventions in a bundle of care will potentially increase the success in maintaining effective breathing of the preterm infant, which could improve important clinical outcomes.



NEDERLANDSE SAMENVATTING

De meeste kinderen die te vroeg (prematuur) geboren worden ademen zelfstandig bij de geboorte. Doordat hun longen en ademhalingssysteem onderontwikkeld zijn, is ondersteuning van hun ademhaling noodzakelijk om voldoende zuurstofopname te garanderen. Om schade aan hun onderontwikkelde longen te voorkomen, wordt deze ondersteuning niet standaard via een beademingsbuisje gegeven, maar wordt er een masker geplaatst over neus en mond (non-invasieve ondersteuning). Via dit masker voorziet een continue luchtstroom de longen van positieve druk en eventueel een verhoogde zuurstofconcentratie. Deze ondersteuning is echter alleen effectief als de luchtstroom de longen kan bereiken – in het geval van een open luchtweg. Bij de geboorte zijn de stembanden voornamelijk gesloten, tenzij een spontane ademhalingsteug wordt genomen. Ten gevolge hiervan is de ademhalingsondersteuning slechts effectief wanneer er spontane ademhaling plaats vindt. Stimulatie van de spontane ademhaling kan daardoor het succes van de non-invasieve ondersteuning bevorderen.

Het behouden van effectieve spontane ademhaling, en daarmee het voorkomen van de noodzaak tot het plaatsen van een beademingsbuisje (intubatie) en het geven van beademing, is niet alleen belangrijk bij de geboorte, maar ook na opname op de neonatale intensive care unit (NICU). Een veelvoorkomend probleem in de eerste uren na de geboorte, is het respiratoir distress syndroom (RDS). Bij dit ziektebeeld is er een tekort aan de oppervlakteverlagende stof surfactant, waardoor longblaasjes makkelijk dichtvallen tijdens de uitademing. Hierdoor kan er minder zuurstof worden opgenomen in het bloed. Het opnieuw openen van de longblaasjes kost veel kracht, iets waar het een prematuur kind nu juist aan ontbreekt. Om deze problemen te verhelpen, kan surfactant worden toegediend aan de longen. Deze toediening gebeurde lange tijd via een beademingsbuisje. Echter, om intubatie en beademing te voorkomen, zijn nieuwe methoden ontwikkeld om surfactant te kunnen toedienen terwijl de spontane ademhaling behouden blijft.

Dit proefschrift had tot doel om het effect te evalueren van verscheidene interventies die vlak na de geboorte en na opname op de NICU worden toegepast om de spontane ademhaling te stimuleren en behouden. Dit proefschrift omvat een experimenteel onderzoek, observationele klinische studies en gerandomiseerd klinische trials.

STIMULATIE VAN DE SPONTANE ADEMHALING BIJ GEBOORTE

Tactiele stimulatie

Het gebruik van tactiele stimulatie om de ademhalingskracht te bevorderen is de eerste interventie die wordt geëvalueerd in dit proefschrift.

Hoofdstuk 1 beschrijft het gebruik van tactiele stimulatie tijdens de opvang van premature kinderen, geboren na een zwangerschapsduur van ten hoogste 32 weken, en de methoden die hiertoe worden gebruikt. In totaal werden 245 opnames geanalyseerd, bestaande uit videobeelden van de opvang en opnames van een respiratoire functie monitor (RFM) die gebruikt wordt om fysiologische metingen, zoals de longfunctie, te beoordelen. Details met betrekking tot tactiele stimulatie in de eerste 7 minuten na geboorte werden genoteerd. Aan 164/245 (67%) kinderen werd tactiele stimulatie gegeven tijdens de opvang na de geboorte. Bij 67% van deze kinderen was hier een klinische reden voor, zoals het ontbreken van een effectieve spontane ademhaling, een lage hartslag of een lage zuurstofsaturatie in het bloed. Er was echter ook een groep kinderen waarbij wel een klinische indicatie bestond voor het gebruik van tactiele stimulatie, maar waarbij dit vervolgens niet werd toegepast. We hebben verschillen geobserveerd in het tijdstip waarop tactiele stimulatie werd gestart, de duur van tactiele stimulatie en de gebruikte methode. Vervolgonderzoek is noodzakelijk om specifieke aanbevelingen te kunnen doen met betrekking tot de meest optimale duur en methode van tactiele stimulatie om de spontane ademhaling te stimuleren.

Vervolgens werd een gerandomiseerde klinische trial opgezet, welke wordt beschreven in **hoofdstuk 2**. Door middel van loting werden kinderen, geboren na een zwangerschapsduur van ten hoogste 32 weken, toegewezen aan 1 van de behandelgroepen. Eén groep kinderen ontving herhaalde tactiele stimulatie, waarbij er afwisselend 10 seconden werd gewreven over de voetzool of de rug van het kind, en 10 seconden geen stimulatie plaats vond. De andere groep kinderen ontving standaard tactiele stimulatie, waarbij er over de voetzool of rug van het kind gewreven werd wanneer de zorgverlener hier een klinische reden toe zag. In beide groepen werd de ademhalingskracht beoordeeld door middel van de RFM. Ook al konden er geen statistisch significante verschillen worden aangetoond, waren alle parameters van ademhalingskracht (ademminuut volume (AMV), teugvolumes (Vt) en snelheid waarmee de maximale teugvolumes werden bereikt) hoger in de herhaalde stimulatie-groep. Omdat alle gemeten parameters dezelfde kant op wijzen, is het waarschijnlijk dat de bevindingen klinisch relevant zijn. Door het uitvoeren van deze studie werd

het bewustzijn onder de zorgverleners vergroot met betrekking tot het nut van tactiele stimulatie. Dit bleek uit het feit dat in 96 % van de kinderen in de standaard stimulatie-groep tactiele stimulatie werd toegepast, een percentage dat aanzienlijk hoger is dan het percentage kinderen dat tactiele stimulatie ontving in het onderzoek beschreven in **hoofdstuk 1**. Mogelijk zijn de verschillen in uitkomsten tussen de groepen hierdoor kleiner dan verwacht. Desondanks was de zuurstofsaturatie (SpO_2) van het bloed significant hoger in de herhaalde stimulatie-groep (SpO_2 87.6 ± 3.3% vs 81.7 ± 8.7%, p=0.01), terwijl de concentratie zuurstof die werd toegediend aan het einde van de opvang significant lager was in deze groep (28.2 (22.8 – 35.0) % vs 33.6 (29.4 – 44.1) %, p=0.04). Ook al was de ademhalingskracht niet statistisch significant hoger in de herhaalde stimulatie-groep, zijn de gevonden verschillen biologisch wel significant.

Zuurstof

Een te laag zuurstofgehalte in het bloed (hypoxie) heeft een remmende werking op de ademhaling bij geboorte. Zowel een experimenteel onderzoek alsmede een gerandomiseerde klinische trial zijn opgezet om te evalueren of het zuurstofgehalte in het bloed kan worden verhoogd indien extra zuurstof wordt gegeven bij geboorte, teneinde de ademhalingskracht te stimuleren. Daarnaast hebben we in een observationele studie geëvalueerd hoelang het duurt totdat de ingestelde zuurstofconcentratie daadwerkelijk het kind bereikt.

Hoofdstuk 3 beschrijft het experimentele onderzoek waarbij de ademhaling van premature konijnen bij geboorte wordt onderzocht wanneer zij worden ondersteund met 21% of 100% zuurstof. Hierbij werden zowel de stabiliteit van ademhaling gemeten, alsmede de luchthoudendheid van de long (gemeten door het longvolume dat in de long achterblijft na een uitademing (de functionele residu capaciteit (FRC)). Het FRC werd gemeten door middel van röntgenstraling met hoge resolutie, waarvoor de synchrotron in Japan gebruikt werd.

Wanneer tijdens de start van het experiment 21% zuurstof werd gegeven, leidde dit tot een instabieler ademhalingspatroon (hogere variatie in lengte tussen 2 ademteugen, lagere ademhalingsfrequentie) dan wanneer het experiment werd gestart met 100% zuurstof. Tevens trad er vaker een apneu op (21% zuurstof-groep: 11/12 (92%) vs 100% zuurstof-groep: 1/8 (13%), p=0.001). Wanneer vervolgens ten tijde van de apneu beademing werd gegeven met 21% zuurstof, leidde dit na hervatting van de spontane ademhaling opnieuw tot een meer instabiel ademhalingspatroon, dan wanneer er beademing werd gegeven met 100% zuurstof. Ondanks het verschil in ademhalingspatroon, was de mate van luchthoudendheid van de long (gemeten door FRC) vergelijkbaar tussen

de groepen. Concluderend kunnen we stellen uit de resultaten van dit onderzoek dat het voorkomen van apneu en stabiliseren van de ademhaling hoofdzakelijk beïnvloed worden door de verzadiging met zuurstof vlak na geboorte.

Tijdens de opvang van premature kinderen wordt de SpO_2 continu gemeten, en vergeleken met referentiewaarden opgesteld door Dawson et al. (2010). Na de geboorte wordt iedere 30 seconden geëvalueerd of de toegediende zuurstofconcentratie moet worden aangepast, teneinde het risico op zowel een te lage (hypoxie) als een te hoge zuurstofconcentratie in het bloed (hyperoxie) te voorkomen. In de observationele studie die beschreven wordt in **hoofdstuk 4** wordt de tijd onderzocht die verstrijkt tussen het aanpassen (titratie) van de zuurstofconcentratie op de zuurstofblender en het moment waarop de gewenste concentratie het kind bereikt. Deze tijd is geëvalueerd tijdens zowel bench tests, als tijdens klinische observaties van de opvang van premature kinderen. Hieruit bleek een duidelijke vertraging in het bereiken van de gewenste zuurstofconcentratie bij het kind (bench tests: 34.2 (21.8 – 69.1) s, klinische observaties: 19.0 (0.0 – 57.0) s). Indien tijdens de opvang na geboorte, volgens de internationale richtlijnen, iedere 30 seconden wordt geëvalueerd of de zuurstofconcentratie dient te worden getitreerd, heeft mogelijk de zuurstofconcentratie van de vorige titratie het kind nog niet bereikt – iets wat in de helft van de titratiemomenten werd aangetoond in dit onderzoek. Zorgverleners dienen zich bewust te zijn van de vertraging die optreed na titratie van de zuurstofconcentratie op de zuurstofblender indien de titratie geen verschil in SpO_2 tot gevolg heeft.

In **hoofdstuk 5** wordt het studieprotocol beschreven voor een gerandomiseerde klinische trial waarbij tijdens de opvang na de geboorte premature kinderen worden ondersteund met non-invasieve ondersteuning met 100% of 30% zuurstof. Na deze initiële concentratie zal de zuurstofconcentratie worden getitreerd op basis van SpO_2. Ondanks dat er eerdere onderzoeken zijn gedaan naar klinische uitkomsten bij verschillen in initiële zuurstofconcentratie, is de effectiviteit van de spontane ademhaling nooit eerder geëvalueerd.

De resultaten van deze gerandomiseerde klinische trial worden weergegeven in **hoofdstuk 6**. In totaal werden 52 kinderen door middel van loting toegewezen aan 1 van de behandelgroepen: een initiële zuurstofconcentratie van 100%, dan wel 30%. Fysiologische metingen van ademhalingskracht en SpO_2 werden verkregen van 44 kinderen (20 kinderen in de 100% zuurstof-groep, 24 kinderen in de 30% zuurstof-groep). AMV, Vt en snelheid waarmee de maximale Vt werden bereikt waren significant hoger onder kinderen in de 100% zuurstofgroep. Tevens was de tijd dat deze kinderen

tijdens de opvang maskerbeademing kregen significant korter. SpO_2 was significant hoger in kinderen in de 100% zuurstof-groep (SpO_2 100% O_2-groep: 85 (64 – 93)% vs 30% O_2-groep: 58 (46 – 67)%, $p<0.001$). De duur van hypoxie tijdens de opvang was lager in de 100% zuurstof-groep, terwijl er geen verschillen werden aangetoond in de duur van hyperoxie. Deze resultaten laten zien dat een hoge initiële zuurstofconcentratie tijdens de opvang na geboorte, gevolgd door nauwlettende titratie van zuurstof, een gunstige methode is om de ademhalingskracht te stimuleren en de noodzaak voor masker beademing te verminderen.

Cafeïne

In **hoofdstuk 7** wordt het effect van het toedienen van cafeïne vlak na de geboorte op de ademhalingskracht van premature kinderen geëvalueerd. In deze gerandomiseerde klinische trial werd bij 30 kinderen op basis van het lot bepaald of zij een gift cafeïne kregen toegediend tijdens de opvang vlak na geboorte, of na opname op de NICU (standaard zorg). Om de ademhalingskracht tijdens de opvang na geboorte te meten, werd de RFM gebruikt. Het AMV bleek significant hoger 7 - 9 minuten na geboorte, indien kinderen bij geboorte een gift cafeïne toegediend kregen. Vt en snelheid waarmee de maximale teugvolumes werden bereikt waren ook significant hoger in de groep kinderen die cafeïne kreeg bij geboorte. Hiernaast vonden we ook dat in de cafeïnegroep een significante relatie bestond tussen het AMV en de zwangerschapsduur ($p<0.001$), waarbij het AMV toenam met 4.1 mL/min/kg voor elke dag dat de zwangerschapsduur toenam. Hoewel er in de groep die geen cafeïne kreeg ook een relatie leek te zijn tussen AMV en de zwangerschapsduur, was deze toename kleiner dan in de cafeïnegroep (2.3 mL/min/kg per dag dat de zwangerschapsduur toenam), en tevens niet significant ($p=0.09$). Deze resultaten tonen aan dat het stimulerende effect van cafeïne toeneemt met de zwangerschapsduur. Het geven van cafeïne tijdens de opvang vlak na de geboorte kan een rol spelen in het stimuleren van de spontane ademhaling, en daardoor mogelijk de effectiviteit van de non-invasieve ondersteuning bevorderen.

BEHOUDEN VAN DE SPONTANE ADEMHALING

Het merendeel van de premature kinderen verlaat de opvangkamer met non-invasieve ademhalingsondersteuning. Echter wordt de spontane ademhaling van een groot gedeelte van deze kinderen belemmerd door RDS, wat toediening van surfactant noodzakelijk maakt. In de laatste jaren werd aangetoond dat het toedienen van surfactant terwijl het kind non-invasief wordt ondersteund, haalbaar en effectief is. Deze procedure is echter alleen succesvol in het voorkomen van intubatie en beademing in

het geval van een stabiel spontaan ademhalingspatroon. Omdat bij deze procedure gebruik wordt gemaakt van een laryngoscoop om de luchtweg in beeld te brengen, kan er mogelijk discomfort optreden.

In **hoofdstuk 8** wordt een observationele studie beschreven waarbij het comfort van premature kinderen tijdens minimaal invasieve surfactanttherapie (MIST) werd geëvalueerd. Surfactant werd toegediend aan 38 kinderen, geboren na een zwangerschapsduur tussen 26-37 weken, volgens de methode die eerder werd beschreven door Dargaville et al. (2011). Voorafgaand aan de procedure kon een lage dosis sedatie (propofol, 1 mg/kg) worden gegeven op basis van de beoordeling van de zorgverlener. COMFORT scores werden vergeleken tussen gesedeerde kinderen en kinderen die geen sedatie ontvingen. Een COMFORT score < 14 werd gezien als een indicator voor comfort. In deze studie bleek dat kinderen meer comfortabel waren wanneer zij een lage dosis sedatie kregen voorafgaand aan de MIST procedure (COMFORT score sedatiegroep: 12 (9 – 17) vs de niet gesedeerde groep: 20 (15 – 23), p=0.002). Echter werd er wel een toename in complicaties gezien die betrekking hebben op de spontane ademhaling. De incidentie van tijdelijke non-invasieve positieve druk beademing (nIPPV) was significant hoger in de groep kinderen die een lage dosis sedatie ontving (23/23 (100%) vs 5/15 (33%), p<0.001), en hoewel deze bevinding niet significant is, werden er meer kinderen in de sedatie groep geïntubeerd (8/23 (35%) vs 2/15 (13%), ns). Om te kunnen aantonen of het voordeel met betrekking tot comfort zwaarder weegt dan het nadeel met betrekking tot het risico op complicaties, is er een gerandomiseerde klinische trial noodzakelijk.

Deze gerandomiseerde klinische trial werd opgezet, en de resultaten hiervan worden beschreven in **hoofdstuk 9.** Tijdens deze trial werden 78 premature kinderen (zwangerschapsduur 26 - 37 weken) door middel van loting toegewezen aan de sedatie groep of de geen-sedatie groep. Kinderen in de sedatie groep ontvingen een lage dosis sedatie (1 mg/kg propofol iv) voorafgaand aan de procedure, terwijl kinderen in de niet gesedeerde groep geen medicatie kregen. Beide groepen kregen standaard zorg om het comfort te verhogen, bestaande uit het toedienen van sucrose en containen, waarbij het kind wordt vastgehouden op een wijze die vergelijkbaar is aan de positie in de baarmoeder. De mate van comfort werd, net als in **hoofdstuk 8**, geëvalueerd door middel van de COMFORT score. De bevindingen van deze gerandomiseerde klinische trial zijn vergelijkbaar aan die van de observationele studie: het percentage kinderen met een COMFORT score < 14 was significant hoger in de sedatie groep (sedatie groep: 32/42 (76%) vs geen-sedatie groep: 8/36 (22%), p<0.001). In de sedatie groep trad vaker desaturatie op waarvoor nIPPV werd gegeven (sedatie groep: 39/42 (93%) vs

niet gesedeerde groep: 17/36 (47%), p<0.001), echter was er geen significant verschil in de incidentie van intubatie. Naar aanleiding hiervan hebben we geconcludeerd dat een lage dosis sedatie kan helpen om het comfort van premature kinderen tijdens MIST te verhogen. Door het verminderen van stress ten gevolge van een (pijnlijke) stimulus kan het geven van een lage dosis sedatie tijdens MIST daarmee mogelijk leiden tot een verminderd risico op een verstoorde neurologische ontwikkeling. Mogelijk kunnen de uitkomsten variëren, afhankelijk van de zwangerschapsduur bij geboorte. Vervolgonderzoek is nodig om dit aan te kunnen tonen.

Concluderend, het succes van non-invasieve ondersteuning van de ademhaling hangt af van de effectiviteit van de spontane ademhaling, zowel tijdens de opvang na geboorte, als na opname op de NICU. Stimuleren en ondersteunen van de spontane ademhaling zou daarom de voornaamste focus moeten zijn van de zorgverlener, om intubatie te voorkomen. Ook al is de effectiviteit van verschillende interventies afzonderlijk van elkaar aangetoond door de studies in dit proefschrift, kan het combineren van deze interventies in één zorgbundel mogelijk leiden tot een verdere toename in effectiviteit van de spontane ademhaling, waardoor klinische uitkomsten zullen verbeteren.

PART FIVE

APPENDICES

LIST OF ABBREVIATIONS

AUC	Area Under the Curve
AUMC	Amsterdam University Medical Center
BPD	BronchoPulmonary Dysplasia
CPAP	Continuous Positive Airway Pressure
ECG	ElectroCardioGram
EMG	ElectroMyoGraphy
FiO_2	Fraction of inspired Oxygen
FRC	Functional Residual Capacity
INSURE	INtubation-SURfactant-Extubation
(I)PPV	(Intermittent) Positive Pressure Ventilation
IVH	IntraVentricular Hemorrhage
LUMC	Leiden University Medical Center
MIFR	Mean Inspiratory Flow Rate
MIST	Minimal Invasive Surfactant Therapy
MV	Minute Volume
NEC	Necrotizing EnteroColitis
NICU	Neonatal Intensive Care Unit
nIMV	nasal Intermittent Mandatory Ventilation
PEEP	Positive End-Expiratory Pressure
PIP	Peak Inspiratory Pressure
PVL	PeriVentricular Leucomalacia
RDS	Respiratory Distress Syndrome
REM	Rapid Eye Movement
ROP	Retinopathy Of Prematurity
RoR	Rate of Rise to maximum tidal volumes
RFM	Respiratory Function Monitor
SpO_2	Oxygen Saturation
Vt	tidal Volume
WMO	Wet Medisch-wetenschappelijk Onderzoek met mensen (Medical Research Involving Human Subjects Act)

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CURRICULUM VITAE

Janneke Dekker was born on the 23rd of August, 1988 in Alphen aan den Rijn. She completed secondary school at the Groene Hart Lyceum in Alphen aan den Rijn in 2005. After not being selected to enter the Medicine Bachelor study twice, she decided to finish her Bachelor of Nursing at the Leiden University of Applied Sciences. During the last year of her Nursing study she started an internship at the neonatal high care unit of the Leiden University Medical Center. After graduation, she successfully completed training in High Care and Intensive Care Neonatal Nursing. Concurrently, she started a master of Clinical Health Sciences, from which she graduated in 2013.

Janneke started her PhD curriculum in December 2014 under supervision of prof.dr. A.B. te Pas (Department of Pediatrics, Leiden University Medical Center, the Netherlands) and prof.dr. S.B. Hooper (Hudson Institute of Medical Research, Monash University, Melbourne, Australia). While she started her PhD curriculum part-time next to her work as a NICU nurse at the Leiden University Medical Center, she dedicated her time fully to research in her final year. During her PhD curriculum, she visited the SPring-8 synchrotron in Japan three times, where she was involved in preclinical experimental projects aimed at imaging the preterm rabbit lung during transition at birth. Janneke has visited many national and international conferences, where she has presented her research using poster and oral presentations. She was awarded with the yearly award for nursing research at the Leiden University Medical Center in 2016. She became a member of the European Scientific Collaboration of Neonatal Resuscitation (current chair: dr. G. Lista) in 2015, and the European Society for Paediatric Research (current chair: dr. C.C. Roehr) in 2019.

Janneke married Simon in May 2015 and on the 22nd of March 2018, they welcomed their daughter Anna-Sophie to their family.

ACKNOWLEDGEMENTS

Zonder de hulp van vele betrokkenen zou het niet mogelijk zijn geweest dit proefschrift tot stand te brengen. Een aantal van hen wil ik daarom persoonlijk bedanken. Mijn dank gaat allereerst uit naar alle kinderen en ouders die hun medewerking hebben verleend aan één van de de onderzoeken die in dit proefschrift worden beschreven.

Prof.dr. A.B. te Pas, beste Arjan. Vanaf het moment dat ik aangaf graag onderzoek te willen doen, heb je je ingezet om dit mogelijk te maken. Ook al was er het eerste jaar nog geen budget voor een nieuwe promovendus, zijn we toch gestart met het opzetten van een gerandomiseerde studie. Na deze volgden er al snel meer projecten, waarbij jouw begeleiding steeds kon worden omschreven door het thema van dit proefschrift: stimuleren en ondersteunen, in plaats van overnemen. Dit werkte voor mij heel prettig, waardoor ik me op heel veel gebieden heb kunnen ontwikkelen. Daarnaast heb jij me ook doen inzien dat het moeten opgeven van een droom niet leidt tot het einde van je toekomst, maar juist tot een open deur. Dankzij jou kan ik na mijn promotie een verdiepende stap maken in het onderzoek. Bedankt voor alles de afgelopen jaren! Ik had me geen betere promotor kunnen wensen.
Prof.dr. S.B. Hooper, dear Stuart. Even though I did not have any experience in experimental research or physiology, you have introduced me into this fascinating part of research. You made me feel very welcome in your research group, and always assured that my nursing background is more a benefit than a disadvantage. Thank you for teaching me the in-depth understanding of the findings of the studies described in this thesis. And thank you for providing me with the opportunity to continue performing research as a postdoc at your lab!

Stafleden van de neonatologie, ik heb heel veel bewondering voor jullie openheid met betrekking tot onderzoek. De grote hoeveelheid studies die zich afspelen tijdens de transitie na de geboorte heeft nooit geleid tot weerstand, maar juist tot interesse voor het onderzoek. Ik wil jullie bedanken voor jullie kritische beoordeling van studie opzet, hulp bij counselen en randomisatie en feedback tijdens presentaties.
Verpleegkundigen van de NICU, veel van de interventies die zijn onderzocht in de studies beschreven in dit proefschrift betreffen verpleegkundige handelingen. Jullie betrokkenheid bij het onderzoek was daarom noodzakelijk voor een succesvolle uitvoering hiervan. Bedankt voor jullie interesse, zowel in het onderzoek als in mijn persoonlijke ontwikkeling. Ik ben dankbaar dat ik onderdeel mocht zijn van zo'n fijn team!

Rianne, Jeanette en Kim, bedankt voor jullie support in mij als verpleegkundige en onderzoeker. Door jullie vertrouwen in mij was ik in de mogelijkheid om een gedeelte van mijn dienstverband te kunnen besteden aan het onderzoek.

Co-authors, thank you all for your valuable input in the design and analysis of the studies, and your critical evaluation of the reporting.

Scientists of Monash University, thank you all for welcoming me into your research group. You have learned me a lot about science and physiology, and I cannot wait to continue learning from you.

PhDummies, bedankt voor jullie steun, luisterend oor, interesse, maar ook het verzinnen van nieuwe mogelijkheden om successen te kunnen vieren met bijzondere baksels. Carolien, Dian, Henriëtte, Lisanne, Isabelle, Vivian, Emma, Marieke, Hylke, Lisette, Hilda, Amber, Sophie G, Thijs, Yolentha, Kristel en Patricia, bedankt voor jullie gezelligheid en support. Sophie C, bedankt voor het maken van illustraties voor een aantal publicaties.

Mijn paranimfen, jullie zijn voor mij van onschatbare waarde geweest tijdens mijn promotietraject, zowel onderzoekswise als persoonlijk. Ik ben dan ook blij en trots dat jullie naast mij staan tijdens mijn verdediging!
Lieve Ilona, vanaf het moment dat ik startte als promovenda heb jij mij op onderzoeksgebied overal bij betrokken. Naast onze gezamenlijke interesse in onderzoek delen we ook op persoonlijk gebied een heleboel, en ik ben blij dat we vriendinnen geworden zijn! Ik kijk op tegen jouw vermogen om je academische carriere te combineren met je sociale leven. Bedankt dat je een voorbeeld voor mij bent!
Lieve Tessa, als student begon je met het verzamelen van data voor één van de studies beschreven in dit proefschrift. Later werden we PhD-collega's, en naast dat we het altijd heel gezellig hebben, heb ik ook veel geleerd over het analyseren van de data van de respiratoire functie monitor door jouw kritische vragen hierover. Sindsdien zijn onze onderzoeken enorm in elkaar verweven en kunnen we daarom als de beste onze successen en obstakels delen. Bedankt dat je altijd optimistisch bent en meedacht in oplossingen!

Mijn beste vriendinnetjes; Christel, Paulien, Jolanda, Janet, Selma, Vivian, Ryanne, Sanne, Marleen en Patricia, bedankt dat ik altijd bij jullie terecht kon voor een beetje afleiding door middel van een goed gesprek, etentje, wijntje of een playdate met de kindjes.

Lieve familie en schoonfamilie, bedankt voor jullie onvoorwaardelijke steun en interesse in mijn onderzoek. Ik ben blij dat ik het afronden van dit proefschrift met jullie kan vieren! Papa was de eerste die lang geleden tegen mij zei: “Is promoveren niet iets voor jou?”. Op dat moment voelde dat als een enorme ver-van-mijn-bed show, maar dit boekje bewijst dat het toch dichter bij was dan ik dacht. Helaas heeft hij niet meegemaakt dat ik dit traject gestart ben, ik zou hem graag willen laten weten dat hij gelijk had. Daarom draag ik dit proefschrift aan hem op.

Lieve Simon, mijn steun en toeverlaat ten allen tijde! Bedankt dat ik iedere presentatie opnieuw voor jou mocht oefenen, terwijl het merendeel van de tekst klinkt als abracadabra. Alle reizen die ik heb kunnen maken tijdens dit promotietraject begonnen met jouw taxiritjes naar Schiphol, maar nog leuker is dat ze ook weer eindigden met een taxiritje met jou, terug naar huis. En het allerleukst is dat we in het laatste jaar een extra reiziger hebben toegevoegd aan de taxiritjes. Allerliefste Anna-Sophie, jij hebt me met jouw komst doen laten inzien dat er dingen zijn in het leven die nóg belangrijker zijn dan het doen van onderzoek.