Noninvasive surfactant administration to the newborn - Experimental studies

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– Experimental studies

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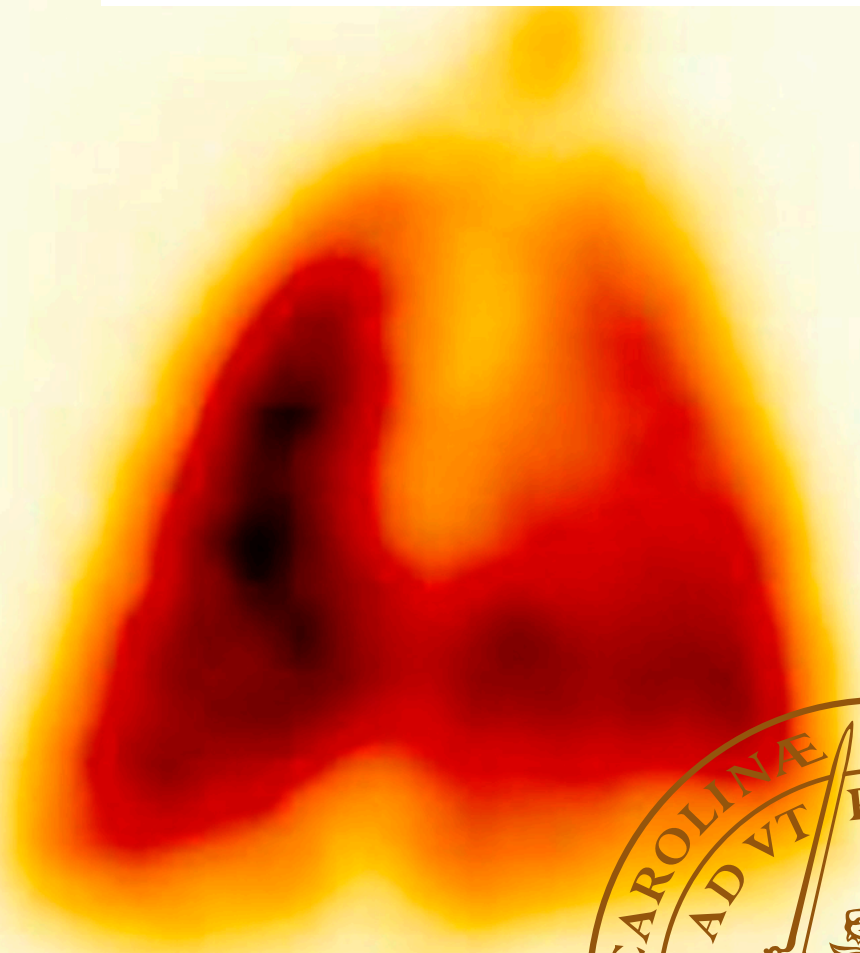

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Anders Nord

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Noninvasive surfactant administration to the newborn–Experimental studies

Surfactant replacement therapy and nasal continuous positive airway pressure (nCPAP) are the core of treating the respiratory distress syndrome (RDS) of the prematurely born baby. The surfactant is usually administered via endotracheal instillation assisted by direct visualization by laryngoscopy, a procedure that can cause hemodynamic changes and discomfort. Further, at least a short period of positive pressure ventilation is often required. There is always a risk of needing prolonged invasive mechanical ventilation, which is also harmful to the immature lungs. Therefore, attempts were initiated to find a way to administer surfactant in a less invasive way.

In five experimental studies, we explored various aspects of the noninvasive surfactant administration to spontaneously breathing healthy newborn piglets. Using gamma scintigraphy, we evaluated the efficiency of delivering surfactant by different noninvasive administration techniques. The natural surfactant poractant alfa was mixed with ^{99m}Technetium nanocolloid, and the deposition in the lungs and airways was evaluated in each study.

A new supraglottic delivery system for surfactant atomization was employed in the first **study (I).** Using this device, we achieved a lung deposition of 40%, almost half of the amount in the tracheal instillation control group.

In the second **study (II)**, we investigated how two different noninvasive ventilatory support modes (nCPAP and nasal intermittent pressure support ventilation, nIPPV), widely used in neonatal intensive care, would affect the total lung deposition of surfactant. In both groups, the piglets lied on their side, and we found no difference in surfactant deposition between the two groups. The deposition was 18% with nCPAP and 23% with nIPPV. The amount of surfactant reaching the lungs in both groups was still relatively high, and probably enough to yield a clinical effect.

In **study III**, using the eFlow-Neos Investigational nebulizer, we assessed if body posture during nebulization would affect the surfactant deposition and distribution in the lungs. We studied nebulization in the prone, supine, and lateral positions. The highest deposition was achieved in the prone position (32.4 ± 7.7%), and we confirmed the critical role of gravity in the distribution of surfactant while on lateral positions. Most of the surfactant was found in the dependent lung.

Neonatal guidelines recommend tracheal instillation of 200 mg/kg of exogenous surfactant. We estimated it would be necessary to nebulize 600 mg/kg of surfactant to reach up to the estimated surfactant pool of the term baby, i.e. 100 mg/kg. In **study IV**, we found it possible to nebulize 600 mg/kg and achieved a mean phospholipid deposition of 138 mg/kg, not different from 172 mg/kg obtained with the instillation of 200 mg/kg.

Finally, the fifth **study (V)** investigates the administration of surfactant via two prototypes of laryngeal mask airway (LMA). We evaluated if an LMA with an integrated camera and a dedicated catheter channel facilitated catheter placement below the vocal cords. The surfactant deposition via both LMAs (delivery catheter above or below the vocal cords) was lower than that obtained via instillation. Albeit not statistically significant, placing the catheter below the vocal cords under visual control with an integrated camera in the LMA improved the surfactant delivery by 65%.

Key words: Surfactant, Nebulization, Noninvasive ventilation, RDS, Experimental

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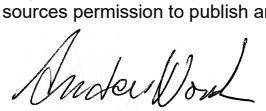
Signature  Date 2021-04-14

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To Alma, Johana and Susanne

Contents

Abbreviations

BPD	Bronchopulmonary dysplasia
$CrSO_2$	Regional cerebral oxygen saturation
DPPC	dipalmitoyl-phosphatidylcholin
Dv50	Median particle size distribution
FDA	United States Food and Drug Administration
FFP	Fine particle fraction value
FiO_2	Fraction of inspired oxygen
HR	Heart rate
InSurE	Intubation- Surfactant administration-extubation
LISA	Less invasive surfactant administration
LMA	Laryngeal mask airway
MAP	Mean arterial blood pressure
MIST	Minimal invasive surfactant administration
MMD	Mass median diameter
MV	Minute volume
nCPAP	Nasal continuous positive airway pressure
nIPPV	Nasal intermittent positive pressure ventilation
$PaCO_2$	Partial pressure of carbon dioxide
PaO_2	Partial pressure of oxygen
PCV	Pressure controlled ventilation
PEEP	Positive end expiratory pressure
PIP	Peak inspiratory pressure
pMDI	Pressurized metered dose inhaler
RDS	Respiratory distress syndrome
RR	Respiratory rate
TV	Tidal volume

List of papers

I **Nord A**, Linner R, Milesi I, Zannin E, di Castri M, Bianco F, Dellacá RL, Cunha-Goncalves D, Perez-de-Sa V. A novel delivery system for supraglottic atomization allows increased lung deposition rates of pulmonary surfactant in newborn piglets. *Pediatr Res. 2020*

II **Nord A**, Linner R, Salomone F, Bianco F, Ricci F, Murgia X, Schlun M, Cunha-Goncalves D, Perez-de-Sa V. Lung deposition of nebulized surfactant in newborn piglets: Nasal CPAP vs Nasal IPPV. *Pediatr Pulmonol. 2020*

III Cunha-Goncalves D, **Nord A**, Bianco F, Salomone F, Ricci F, Schlun M, Linner R, Perez-de-Sa V. Impact of body position on lung deposition of nebulized surfactant in newborn piglets on nasal continuous positive airway pressure. *Neonatology. 2020*

IV **Nord A**, Bianco F, Salomone F, Ricci F, Schlun M, Linner R, Cunha-Goncalves D. Nebulization of high-dose poractant alfa in newborn piglets on nasal continuous positive airway pressure yields therapeutic lung doses of phospholipids. *Am J Perinatol. 2020*

V **Nord A,** Cunha-Goncalves D, Linnér R, Bianco F, Salomone F, Ricci, F, Lombardini, M, Massimo Micaglio M, Trevisanuto D, Perez-de-Sa V. Lung deposition of surfactant delivered via a dedicated laryngeal mask airway in piglets. *In manuscript, submitted for publication.*

Disclosure statement

Chiesi Farmaceutici SpA (Parma, Italy) funded the studies and supplied the animal-derived surfactant poractant alfa (Curosurf®). PARI Pharma GmbH, Starnberg, Germany, owns the patent of the mesh-nebulizer and provided the nebulizer units used in studies II, III, IV. The authors reinforce that the delivery of surfactant by atomization and nebulization is off-label product use.

Background

Introduction

The number of babies born prematurely is increasing worldwide. Even though more babies survive their first month of life, many children suffer from various lethal diseases. According to the World Health Organization [1], despite a decrease in neonatal mortality from 37 deaths per 1000 live births in 1990 to 18 per 1000 in 2017, 2.5 million children still died in the first month of life in 2017. There are many reasons for premature babies to develop breathing difficulty at birth. The preterm baby is especially at risk of developing neonatal respiratory distress syndrome (RDS) due to lung immaturity and insufficient surfactant amounts in the lungs. The lower the gestational age, the more pronounced is the surfactant deficit. Surfactant is a surface tension lowering substance that is essential to keep the lungs' alveolar sacks open. Its deficiency manifests itself through increased oxygen demand, respiratory acidosis secondary to carbon dioxide retention, and increased work of breathing with symptoms of nasal flaring, tachypnoea/apnoea, retractions, and grunting. Pulmonary X-ray pictures show a typical image with bilateral, diffuse ground-glass fields and air bronchogram secondary to diffuse atelectasis [2].

Besides antenatal steroids, the treatment of RDS consists mainly of respiratory support and exogenous surfactant administration [3]. The development of surfactant treatment for RDS is considered the most significant medical achievement in neonatal care in the last century [4].

Short surfactant history

Kurt von Neergaard described in 1929 the importance of surfactant for lung compliance and suggested the need to keep a low surface tension in the lungs to facilitate the newborn infant's first breath. Two decades later, three independent researchers (Chris Macklin in Canada 1954[5], Richard Pattle in the UK 1955[6], and John Clements in USA 1957[7]) described the existence of surfactant in the lungs. They observed that the bubbles originated during lung edema after nerve gas exposure remained stable for many hours, and they concluded that a stabilizing substance from the lungs' lining layers covered the bubbles. In his following experiments, John Clements described that lung extracts from animals lowered

surface tension [3]. By doing so, he established the importance of the alveoli's surfactant lining in the reduction of lung surface tension. In 1959 Mary Ellen Avery and Jere Mead [8] made the historical association between respiratory distress syndrome or, as it was named before, hyaline membrane disease and the absence of surfactant. Neergaard died in 1949 and did not see the application of his findings.

Surfactant composition, development, and clinical application

Surfactant has a complex structure including phospholipids (predominantly dipalmitoyl-phosphatidylcholine–DPPC), and surfactant proteins (SP)-A, B, C, and D. Surfactant proteins B and C are two hydrophobic proteins that play an essential part in the distribution and adsorption of DPPC [9,10].

The search for an exogenous surfactant alternative started in the 1960s. Throughout history, there is no other neonatal medication that has been studied in so many well-designed and large randomized controlled studies before approval by the American Food and Administration agency (FDA)[11-15]. The synthetic surfactant Colfoscerilpalmitate (Exosurf®) was approved in 1990, and the bovine-derived surfactant Beractant (Survanta®) was approved in 1991. Poractant alfa (Curosurf®), the surfactant we have used in our studies, is extracted from porcine lungs. Its first pilot clinical trial in Europe was started in 1985[11]. Curosurf was approved by the FDA in 1999. There has been an increasing number of surfactants on the market, even though a few have already been withdrawn. The most studied surfactants are shown in Table 1 below.

Table 1–Different surfactant preparations

Substance	Year	Origin	Additional proteins	Trademark	Konc. mg/ml
Lucinactant	2012	Synthetic	KL4 SPB analog	Surfaxin	
Calfactant	1998	Calf lung	PB+PBC	Infasurf	35 mg/ml
Poractant alfa	1999	Porcine	PB+PBC	Curosurf	80 mg/ml
Beractant	1992	Bovine	PB+PBC	Survanta Alveofact Beraksurf	25 mg/ml
Colfosceril palmitate	1990	Synthetic	None	Exosurf	
Surfactant-TA		Bovine		Surfacten	25 mg/ml
bLES	2002	Bovine	PB+PBC	Neosurf Liposurf	27 mg/ml

Endogenous pulmonary surfactant reduces surface tension at the alveoli's air-liquid interface during ventilation and stabilizes the alveoli against collapse at resting

transpulmonary pressures. It is estimated that the term newborn baby has a surfactant pool of about 100 mg/kg at birth. In contrast, preterm babies have only around 4–5 mg/kg of surfactant in their lungs [16]. Traditionally, the exogenous surfactant is administered by instillation in the trachea under controlled pressure ventilation with a ventilator. Surfactant treatment history goes back to the 12th January 1980, when a seminal paper was published in The Lancet by Tetsuro Fujiwara [17]. Ten severely ill pre- term babies with the hyaline-membrane disease were treated with a mixture of natural lipids, synthetic dipalmitoyl lecithin, and phosphatidylcholesterol given endotracheally. The clinical picture changed dramatically with the breathing disorder's reversal, improvements in blood gases, and concomitant radiographic enhancement. This landmark study was the start of a magnificent series of investigations for surfactant treatment in RDS. Initially, most of the trials were carried out on already intubated children, and the short-term results were excellent. However, positive pressure ventilation increases the risk of developing chronic lung disease of prematurity or bronchopulmonary dysplasia (BPD)[18]. Therefore, new less invasive surfactant administration modalities were introduced in the last decades to further improve outcomes by avoiding or shortening the exposure to invasive mechanical ventilation.

The timing of surfactant treatment and the initial modality of respiratory support for RDS has also changed over time. At the beginning of the 1990s [19], prematurely born babies were immediately intubated and given surfactant, and in some centers, surfactant was administered even before the baby's first breath. With the introduction of nCPAP in neonatal care, this prophylactic approach to surfactant administration started to change. The use of intubation *vs.* nCPAP for very preterm infants was investigated in 2008 by Morley in the COIN study [20]. The authors looked specifically at the incidence of BPD and mortality. Although there was an increase in air-leaks with the CPAP and selective surfactant technique, the need for ventilator treatment decreased, and fewer babies received supplemental oxygen at 28 days. These findings were supported by Finer *et al.* in a randomized controlled study showing no significant differences between death or BPD, but a lower rate of intubation and a shorter duration of respiratory support with CPAP [21]. Finally, it was concluded that early stabilization on CPAP with selective surfactant administration given only to babies who develop RDS reduced the risk of chronic lung disease and mortality. Prophylactic surfactant treatment showed no advantages; on the contrary, there was a trend towards a higher incidence of BPD and increased mortality [22].

Evolution of surfactant administration techniques–the search for less invasive procedures.

Several less invasive techniques of exogenous surfactant administration have been developed over the years in an effort to preclude tracheal intubation and prolonged mechanical ventilation. They have been investigated in many studies, both in-vivo and in randomized clinical trials.

INSURE technique.

Verder *et al*. described in 1992 [23] the intubation- surfactant-administration and extubation technique (In-Sur-E), a method aimed to be used in babies with mild to moderate RDS who were supported by nasal CPAP. These babies were briefly intubated, received surfactant instillation, and were then extubated to continue with nasal CPAP. For babies with established RDS, one single dose of surfactant in association with the avoidance of prolonged mechanical ventilation with the In-Sur-E technique was advantageous compared to CPAP alone, as confirmed in other trials [24]. One of the critical issues when using intubation-extubation modalities is how to give adequate comfort to the baby during the procedure without incurring the drawback of needing to provide prolonged mechanical ventilation due to excessive sedation. Verder *et al*.[25] used 0.1 mg/kg morphine and 10 ug/kg atropine as premedication in the South-Scandinavian randomized multicenter trial. They reported that naloxone given before extubation improved the success rate of early extubation. Even Bohlin *et al*. [26] achieved good results using 2 mg/kg pentobarbital combined with morphine 200 ug/kg followed by 0.1 mg/kg naloxone before extubation. Because fast recovery after premedication is essential, it is crucial to use short-acting, safe, and tolerable premedication with the InSurE technique. One example is the use of short-acting opioids, such as remifentanil [27]. Propofol is used with caution due to its adverse hemodynamic effects in the preterm [28]. Nevertheless, extraordinarily little is known about which premedication or drug doses are nontoxic and safe during the INSURE procedure [29].

LISA and MIST–Less Invasive Surfactant Techniques

In 2007 Kribs *et al*. [30] administered exogenous surfactant to spontaneously breathing preterm babies through a flexible catheter, a technique they named less invasive surfactant administration (LISA). The procedure is performed without or with less need for analgesia or sedatives, and the babies receive continuous support with nCPAP. Besides laryngoscopy, the introduction of the flexible catheter in the trachea often requires the use of a Magill forceps. Dargaville *et al.* [31] further developed the technique by introducing a semirigid catheter, which eliminated the

need for a Magill forceps, a procedure they named minimal invasive surfactant therapy (MIST).

Improvements in outcome with the less invasive surfactant techniques (LIST) of surfactant administration

To reduce the incidence of BPD, it is essential to minimize mechanical ventilation in premature babies. Göpel *et al*. [32] conducted a multicenter study in Germany on the LISA technique's ability to decrease the need for mechanical ventilation in preterm infants aged 26-28 weeks gestational age. They found a reduction in mechanical ventilation from 46% to 28% with LISA. In 2013 Kanmaz *et al*. [33] published another randomized controlled study, the "Take care" study. They found a similar reduction in the need for mechanical ventilation and a decrease in the rate of BPD with the thin catheter technique. Angela Kribs *et al*. [34] investigated in 2015 the impact of the LISA technique on survival and BPD. They found an association between LISA and survival without major complications but no significant reduction in the rate of BPD or total survival. A systematic review published by Rigo *et al*. [35] consisting of six RCTs concluded that surfactant instillation through a thin catheter in spontaneously breathing infants decreases the need for invasive ventilation and reduces BPD and death risk.

Other less invasive investigational techniques of surfactant delivery

Despite representing a considerable improvement, the LISA and MIST techniques still require laryngoscopy, which can be painful and is considered a stressful moment for the neonate. Excessive airway manipulation runs the risk of causing increases in blood pressure and possibly intracranial hemorrhage. To avoid laryngoscopy, additional less invasive methods have been investigated since the 1990s. One of these is the intrapartum installation of surfactant into the nasopharynx before the baby takes his first breath [36]. Others are surfactant nebulization [37], surfactant atomization [38], and surfactant administration through a laryngeal mask airway [39].

Pharyngeal instillation of surfactant during delivery

Surfactant administration into the oropharynx during preterm delivery has the advantage of totally excluding laryngoscopy and other painful interventions in the

first minutes of life. When the baby's head appears, the mouth and stomach are suctioned, and surfactant is given via a catheter into the pharynx. As soon as the shoulders and the rest of the body are delivered, the baby is stimulated to breathe. Theoretically, the baby would aspirate the surfactant into the lungs with the first breaths of life.

Animal studies in preterm lambs and rabbits support the idea that deposition of surfactant in the posterior pharynx before the first breath allows aspiration of surfactant at the initiation of ventilation and improves the aeration of the lungs [40]. Further, the surfactant is delivered to fluid-filled instead of air-filled lungs, which probably makes the distribution even more uniform [41]. It has been shown that at least in animal models, satisfactory pulmonary function is maintained for a longer time [42]. Direct administration of surfactant into the pharynx of newborn babies was studied in the late 1980s in the ten-center trial of artificial surfactant study [43]. The administration of a protein-free synthetic surfactant into the pharynx of prematurely born babies reduced mortality from 27% to 14%. The authors also noticed less severe RDS and a reduction in ventilation requirements. In 2004, Kattwinkel *et al*. [36] ran a feasibility study on surfactant administration in the oropharynx at birth. They concluded that it was feasible and safe to administer surfactant into the pharynx of premature babies. The authors studied both vaginal and cesarean delivery; they suggested that the surfactant was aspirated into the lungs in babies born vaginally but had some concerns about using the technique in babies with breech presentation and those delivered by cesarean section. In a Cochrane review from 2011 on the pharyngeal instillation of surfactant at birth before the first breath, Abdel Latif *et al*. [44] reported encouraging data from animal studies and human observational studies but found no randomized controlled study and concluded that well-designed trials are still needed. An ongoing trial is currently examining the efficacy of pharyngeal surfactant administration to premature infants less than 29 weeks of gestation; the POPART study [45] is an unblinded multicentre study that compares administration of poractant alfa in addition to CPAP versus CPAP alone. Interestingly, the amount of surfactant administered is decided upon gestational age and not on weight, which results in an approximated dose ranging from 150 to 267 mg/kg. The primary outcome is the incidence of respiratory failure in the first five days of life, but there are, to date, no results published.

Atomization of surfactant

Atomization could easiest be explained with the help of an old perfume dispenser. If you pressurize a liquid through a nozzle, it will appear in small particles. In the Wagner *et al*. study from 2000 [38] on surfactant atomization in tracheotomized lung-lavage rabbits, this was called "a fog," which contained aerosol with droplet sizes 120 ± 4 um. Wagner reported comparable lung doses of surfactant and equivalent

respiratory function improvements in the tracheal atomization and the tracheal instillation groups in his study. Rey-Santano *et al*. [46] used an Aero-Probe catheter connected to a pneumatic compressor in surfactant depleted rabbits and compared the effects of aerosolized poractant alfa to bolus instillation finding similar improvements in oxygenation index and mean dynamic compliance. However, there was a significant increase in cerebral blood flow and $PaCO_2$ in the surfactant bolus group. Using the same supraglottic atomization device that we used, Milesi *et al*. [47]administered atomized poractant alfa with particle sizes of 40–60 um to spontaneously breathing premature lambs on nCPAP and observed improved oxygenation with 22–43% of the surfactant being deposited in the lungs as measured by non-radioactive labeling with samarium-oxide.

Surfactant nebulization

Following the updated consensus guidelines on RDS management [48], most preterm babies are currently stabilized on CPAP and receive surfactant only if they need intubation or develop clinical signs of RDS. Nevertheless, it is estimated that around 50% of these premature babies on CPAP, especially those that are very preterm, still need delayed surfactant administration. If the goal during surfactant administration is to avoid the harms of airway manipulation and mechanical ventilation, surfactant nebulization appears as the only genuinely noninvasive method of surfactant delivery.

In 1964 Robillard *et al*. [49] reported the results of a small study including 11 newborn babies suffering from RDS who were treated with a nebulized mixture of synthetic dipalmitoyl lecithin. They found a decrease in respiratory rate and retraction score in eight of the 11 children. To my knowledge, this was the first published report of treating newborn children suffering from respiratory distress with nebulized lipids. In the 1960–1970s Chu *et al*. [50], Shannon [51], and Bunnell [52], investigated the nebulization of DPPC in small clinical trials with disappointing results. However, as animal studies started to show some beneficial effects of surfactant nebulization, clinical studies began to appear. Most studies were done on mechanically ventilated children, but with the increasing use of noninvasive breathing support, nebulization became an appealing alternative. Jorch *et al*. [53] reported in 1997 the first clinical pilot study of nebulized surfactant on spontaneously breathing babies. Bovactant (Alveofact®) was administered by jet nebulization to 20 premature babies supported with bubble-CPAP. They reported that surfactant nebulization was feasible and caused an immediate improvement in blood gases, but the costs were high. A year later, Arroe *et al*. [54] used a synthetic surfactant (cosforceril palmitate, Exosurf®) in a pilot study of 22 infants on their second day of life. There was no improvement in the a/A ratio, and six out of 22 infants were intubated. In 2000 Bergren *et al*. [37] ran a Swedish multicenter study on poractant alfa nebulization in 34 spontaneously

breathing preterm babies supported by nCPAP. The authors found no beneficial effects of aerosolized surfactant, and the need for further improvements in technology was addressed in the paper. Finer *et al.* [55] did an open-label feasibility study of lucinactant (Aerosurf®) with a mesh-nebulizer in seventeen children. He reported it was feasible and safe to nebulize lucinactant, further raising issues on the type of surfactant and the appropriate patient interface to be used. In 2013 Timersma *et al.* [56] compared jet nebulizers, pMDI-holder chambers, and MESH nebulizers reporting that mesh-nebulizers delivered aerosols more efficiently. Minnochieri *et al*. [57] used the eFlow-Neos vibrating membrane nebulizer to study premature babies on CPAP at 29^0–33^6 weeks of gestational age. In their study, poractant alfa nebulization reduced intubation needs for infants at 32^0–33^6 gestational age. Recently, Cummings *et al*. [58], using a modified Solary device and a pacifier adaptor as an interface, gave 210 mg/kg of aerosolized calfactant (Infasurf®) directly into the mouth of 457 infants at 23–41 weeks of gestational age (median 33 weeks). Like Minnochieri, he observed a marked decrease in intubation from 50% to 26% in the aerosol group for infants with mild to moderate RDS.

Surfactant nebulization given to spontaneously breathing premature newborn babies seems to work well, at least for mild prematurity. However, there is a shortage of data on surfactant deposition in the lungs with this new modality.

Laryngeal mask airway

The laryngeal mask airway (LMA) was designed by Archie Brain in 1981 [59]and has since then been used to keep a patent airway during adult and pediatric anesthesia [60] for surgical procedures. The LMA is recommended in the pediatric and neonatal resuscitation guidelines [61] when bag-mask ventilation is not possible or insufficient. The easiness of placing the laryngeal mask, the low-pressure seal (maximally 20–25 cm H_2O), and the patient's superior comfort favors the use of LMA compared to intubation. In contrast, compared to tracheal intubation with cuffed tubes, a possible disadvantage is that the LMA's maximum seal pressure can be a limitation with a risk for ineffective ventilation during neonatal resuscitation.

Other advantages are that the newer developed LMAs have a suction channel allowing passage of a gastric catheter and emptying of the stomach. Besides, the development of smaller LMAs gives the opportunity to administer surfactant to smaller premature babies suffering from respiratory distress syndrome. Five small clinical trials have been published on surfactant administration via LMAs.

Table 2–Studies of surfactant delivery via LMA.

Study	Attridge et al. 2013[62]	Sadeghnia et al 2014[63]	Pinheiro et al 2016[64]	Barbosa et al 2017[65]	Roberts et al 2018[66]
Population	BW>1.200 gram 32 GA	BW > 2.000 gram 33–36 GA	BW > 1.945 gram 29+0 – 36+6 GA	BW > 1.450 gram 32(28–35) GA	BW > 1.950 gram 33(28+0 – 35+6) GA
Surfactant/LMA	Calfactant 105 mg/kg Classic LMA Catheter + nCPAP	Survanta 100 mg/kg i–gel + 5Frcatheter	Calfactant 105 mg/kg LMA Classic + 5Fr catheter	Poractant alfa 200 mg/kg ProSeal LMA + 6Fr Catheter	Poractant alfa 200 mg/kg LMA Unique 1 + suction catheter
LMA group	n=13 AS 54%	n =35 AS 51%	n=30 AS 50%	n= 6 AS 54%	n=50 AS 72%
Control group	n=13 AS 46% CPAP , no surfactant	n=35 InSurE Protocol AS 65%	n=30 InSurE protocol AS 53%	n=22 InSurE Protocol AS 77%,	n=53 AS 64% CPAP , no surfactant
Comments/ Sedation			Atropin both groups , +Morphine InSurE	Remifentanil + midazolam for InSurE 46% Mechanical ventilation LMA	Atropin + sucrose for LMA insertion
Results	Decrease in FiO_2 1h 12 h Feasible	Improvement in oxygenation, higher a/APO_2 in LMA group	Decrease of mechanical ventilation in LMA group	Study interrupted, no differences in the primary outcome	Decrease rate of intubation and mechanical ventilation

Experimental models used for the study of surfactant

Animal models that are suitable for the study of surfactant metabolism and its effects in vivo require at least some degree of surfactant deficiency. There are mainly two different surfactant deficiency models: one includes relatively surfactant deficient animals due to preterm delivery, where the lamb model [67] is historically the most used one and considered the gold standard model of surfactant deficiency. Premature rabbits can also be used in the setting of surfactant deficiency studies [68]. Another model of surfactant deficiency is to perform lung lavage with saline until most lung surfactant is "washed out" from the lungs, as Lachmann [69] described in adult guineapigs. This technique has also been used in studies with other animals, for instance, rabbits [70]and piglets [71] that are then maintained on nCPAP.

The neonatal piglet model is an excellent model for studying respiratory failure [72] because of many reasons. The pig is an animal that resembles humans in many ways, both anatomically and histologically. The cost for an animal is affordable, and the availability of piglets is good in Sweden. Most laboratories have broad experience in handling piglets. Lund's facilities are excellent, with the opportunity to perform gamma scintigraphy investigations within a few minutes after completing the animals' interventions.

Nonetheless, other animals are also suitable for RDS studies, to mention a few, the macaque [73], the sheep [74], and the rabbits [38].

Our studies aimed to investigate surfactant deposition in the lungs, and we opted for a model of newborn piglets on their first day of life. There is no surfactant deficiency in this model, and no conclusions can be drawn on surfactant clinical efficiency. However, piglets can be maintained on spontaneous ventilation on CPAP under light sedation for a few hours, allowing lung deposition studies of inhaled drugs with minimal animal wastage, which is always an essential ethical concern in the experimental laboratory. This model's advantages for respiratory research are that it reasonably reflects the anatomic and dynamic variations in breathing observed in newborn babies. Like neonates, the piglets have small airway diameters and a wide variation in breathing pattern with characteristic rapid and shallow breathing. Alternatives for deposition studies in the newborn baby are limited, and animal translational models are suitable for deposition studies of inhaled drugs in this age group.

Gamma scintigraphy

Gamma scintigraphy was used to assess the lung deposition of surfactant in our piglets. This method is considered the gold standard for quantifying the deposition of inhaled drugs in the lungs [75]. However, ethical aspects restrain the use of radioactive tracers in newborn babies, and therefore the evaluation of the lung deposition of inhaled drugs in neonates often needs to be performed in animals. When using radioactive tracers, it is vital to make sure that the tracer mixes well with the drug's aerosol particles so that tracer readings from the gamma chamber mirror the drug deposition. The most used isotope is ^{99m}Tc-human serum albumin (Nanocoll). Dijk *et al*. have previously reported that they found a 0.97 correlation coefficient between mixing ^{99m}Tc Nanocoll with surfactant and the technique of labeling surfactant with carbon-14 [76].

The choice of surfactant–poractant alfa

Our inhalational studies were done with poractant alfa, a sterile naturally occurring surfactant extracted from porcine lungs. It contains 80 mg of surfactant extract per ml of the drug. Of these, 99% are polar lipids, including around 76 mg of phospholipids, especially dipalmitoyl-phosphatidylcholine (DPPC); 1% consists of low molecular weight hydrophobic proteins, including surfactant proteins SP-B and SP-C. Because the amount of phospholipids/ml is so high, it is possible to deliver a

high amount of surfactant in small volumes, an advantage during inhalation studies. Moreover, lower volumes decrease the airway obstruction risk [77], which can be a problem with surfactant treatment.

The first generations of synthetic surfactants were protein-free, contained mainly DPPC, and were not as effective as natural surfactants [78]. Whenever there is an indication for surfactant treatment in RDS, the European Consensus Guidelines on the Management of RDS recommend using an animal-derived surfactant for RDS treatment as the third-generation synthetic surfactants containing surfactant proteins have not yet been thoroughly investigated in clinical trials. Equivalent doses of natural surfactants seem to be equally effective. Still, better survival has been observed using 200 mg/kg of the porcine-derived surfactant poractant alfa (Curosurf®) compared to 100 mg/kg of poractant alfa or 100 mg/kg of the bovine-derived surfactant beractant (Survanta®). The total dose of phospholipids delivered to the lungs is an important factor for RDS outcome [79]. Surfactant dosage is usually calculated in mg phospholipids/kg. Among all the currently available surfactant preparations, poractant alfa has the advantage of containing the highest amount of phospholipids/ml.

Aims of the studies

Study I

In this feasibility study, we aimed to quantify the amount of poractant alfa deposited in the lungs when surfactant was given via an orally introduced new supraglottic delivery system for surfactant atomization. For this study, we used an experimental model of spontaneously breathing newborn piglets on nasal mask CPAP. A control group was intubated and received intratracheally administered surfactant.

Study II

In this study, we hypothesized that the eFlow-Neos investigational nebulizer system (PARI Pharma GmbH, Gräfelfing, Germany) would deliver reasonable amounts of nebulized surfactant to the lungs of spontaneously breathing newborn piglets on noninvasive ventilation support. Further, we compared the amount of surfactant deposited in the lungs when nebulized under the two most common modes of noninvasive ventilatory support used in the neonatal intensive care unit (NICU), that is, nasal CPAP and nasal IPPV (intermittent positive pressure ventilation).

Study III

This study aimed to determine whether body positioning during surfactant nebulization influences surfactant distribution and deposition in the lungs. Spontaneously breathing newborn piglets on nCPAP via prongs received nebulized surfactant either in the prone, supine, or in one of the lateral positions. Surfactant deposition in the lungs was assessed by gamma scintigraphy.

Study IV

We investigated the feasibility of nebulizing a high-dose of poractant alfa (600 mg/kg) with the eFlow-Neos investigational vibrating-membrane nebulizer in newborn piglets on nasal continuous positive airway pressure (nCPAP). We hypothesized that this dose would result in about 100 mg/kg of phospholipids being delivered to the lungs, an amount that is associated with the stabilization of respiratory function in surfactant deficient subjects.

Study V

We hypothesized that delivering surfactant by instillation through a catheter that was placed below the vocal cords and under visual guidance through an LMA that was customized to include an integrated camera and a dedicated channel for the surfactant delivery catheter would improve lung deposition of surfactant when compared to bolus administration in the central lumen of a blindly placed common LMA. Lung deposition of surfactant in these two groups was compared to the one obtained with the standard surfactant instillation method with a catheter placed via an endotracheal tube.

Methods

Shared protocol for all the experiments.

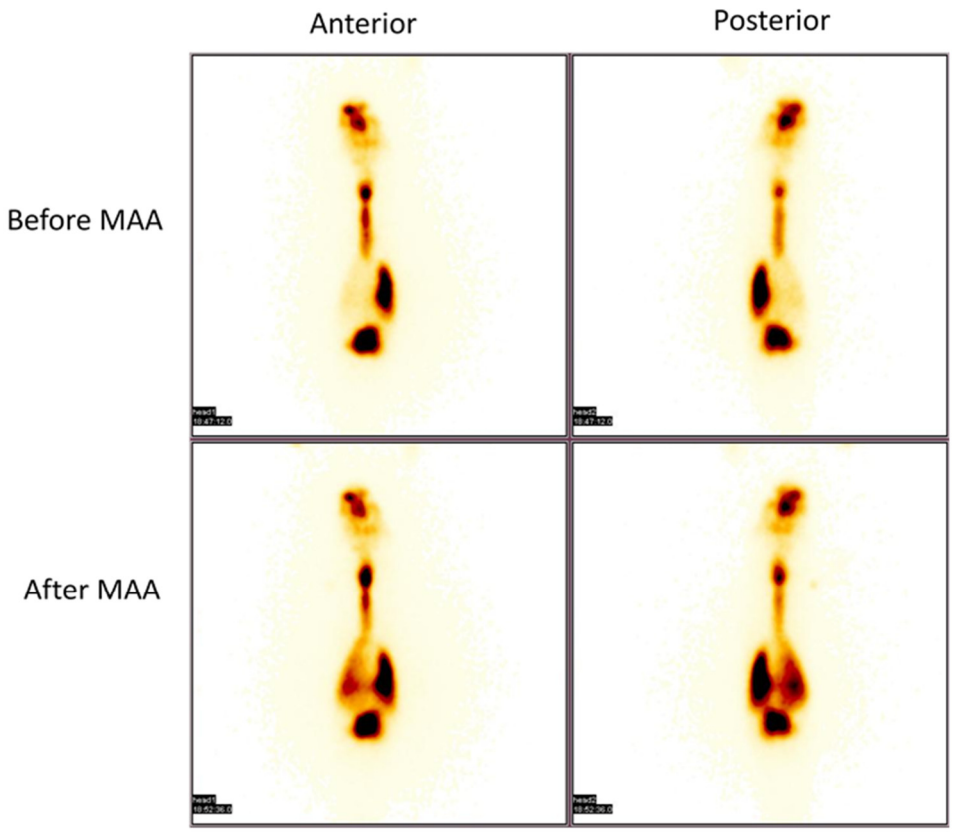
All five studies were done at the facilities of Lund University (BMC) between 2015 –2019. The local committee on animal research ethics of Lund University approved the studies (Diary number M64–14). The animals received care according to the European Parliaments Directives on the protection of animals used for scientific purposes (Directive 2010/63/EU).

Ninety-two full-term piglets that were 8–36 hours old and had a weight of 1.2–2.6 kg were used in the five studies. Premedication was given before instrumentation of the animals in the same way in all five studies and it consisted of intramuscular injection of ketamine (6 mg), midazolam (4 mg), and atropine (0.2 mg). During the different interventions, the analgesia and sedation were titrated as needed to ascertain animal comfort. Local anesthesia was used when supposed painful interventions were performed, like intubation or vascular access. All through the experiments, the animals received continuous sedation with an infusion of 1–3 ug/kg/h dexmedetomidine, supported with intermittent propofol (1–3 mg/kg) or ketamine (1–3 mg/kg/h) as needed.

The piglets were taken from their mother sow shortly after birth, and to secure water and glucose balance, an infusion of 10 ml/kg /h of 50mg/ml Glucose with 70 mmol potassium, 45 mmol chloride, and 25 mmol acetate per liter was started as soon as an intravenous access was established. This description applies to all five studies, and particular interventions in each study are further explained in the following sections.

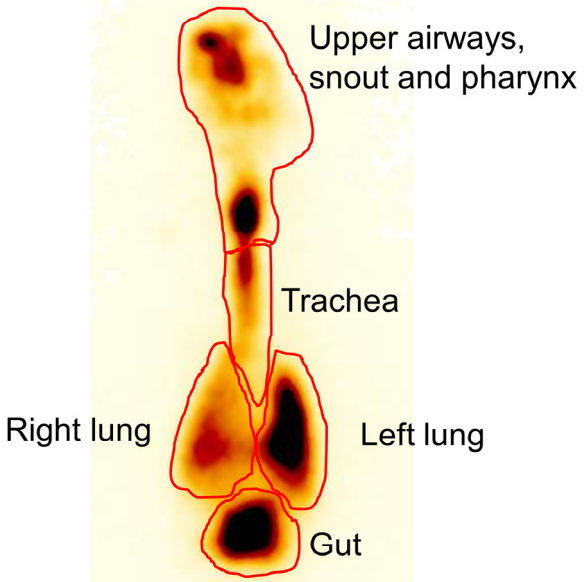
The deposition of surfactant was evaluated with gamma scintigraphy, the most used method for measuring regional deposition of different aerosolized drugs in vivo, which is, unfortunately, not suitable for newborn babies [75]. The deposition measurements in the gamma chamber were done according to our group's protocol that has been previously described by Linner *et al*. in 2015 [80]. The piglets were placed in a Philips Skylight, dual-head chamber (Philips AB, Stockholm), and posterior and anterior images were taken before and after the i.v. injection of 25 MBq of ^{99m}Tc-labelled macroaggregated human serum albumin (^{99m}Tc-MAA). As the ^{99m}Tc-MAA passes the lung circulation, it is completely retained in the lung

capillaries, delineating both lung fields and defining the boundaries of the regions of interest (ROIs) for the analysis of the gamma camera pictures. The ^{99m}Tc-MAA was also used for calibrating the images. In this way, the amount of ^{99m}Tc-labelled nanocolloid deposited in the lungs could be determined, and, by inference, even the amount of deposited surfactant. The procedure is illustrated in the next series of pictures.



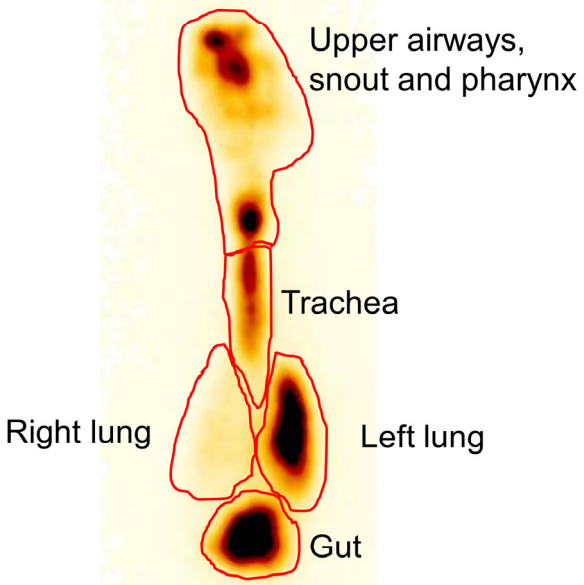
This pig was lying on its left side.

Step 1 – ROIs of interest are delineated in images after MAA



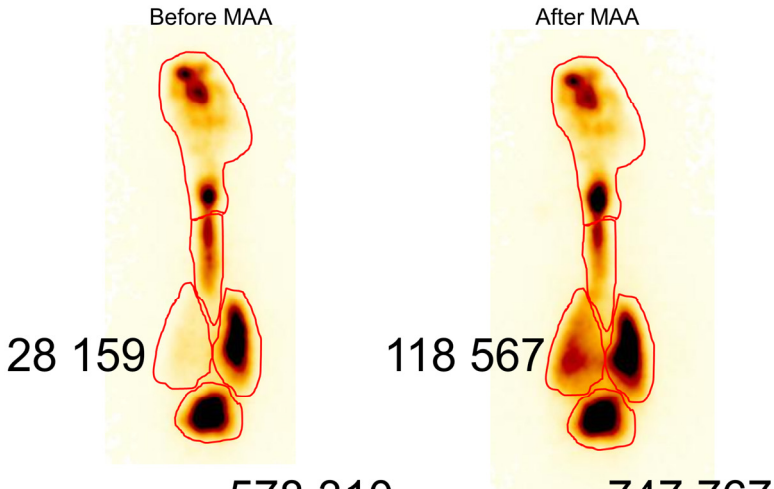
Region of interest (ROI)

Step 2 – ROIs are copied to images before MAA



Region of interest (ROI), Macroaggregated albumine (MAA)

Step 3 – Counts in ROI and image matrix before and after MAA



Region of interest (ROI), Macroaggregated albumin (MAA)

How deposition is calculated

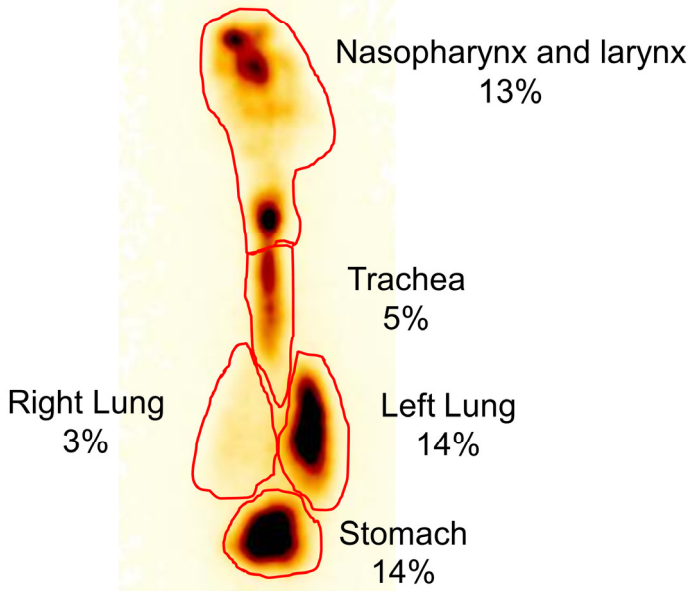
Step 4 – All counts are normalized to the same time due to the 6.01 h half-life of ^{99m}Technetium.

Step 5 – The formula below is used to calculate the deposit in the ROI (Droi).

$$Droi = \frac{100 \times Croi}{(Cwif(2) - Cwif(1)) \times \frac{TcMAA\ dose}{TcNanocolloid\ dose}}$$

Croi: ROI before ^{99m}TcMAA dose
Cwif(1): Whole image field before ^{99m}TcMAA dose
Cwif(2): Whole image field after ^{99m}TcMAA dose

Deposition in different regions



Pig 295, mask, lying on the left side during inhalation

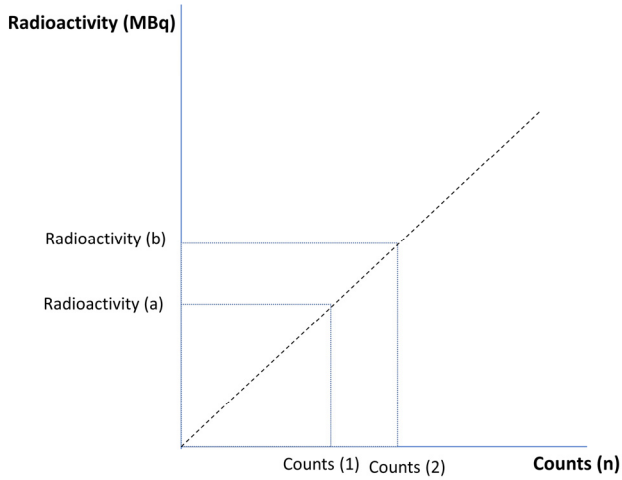


Figure 1–Estimation of surfactant deposition with the gamma camera.

To calculate the lung deposition in a subject and to calibrate the gamma camera readings, we start by measuring the total whole body counts in a first run in the gamma camera ("counts 1", which reflects the "radioactivity a", i.e., the total amount of surfactant-^{99m}Tc mixture in the subject) (Figure 1). Then we inject a known amount of ^{99m}Tc-macroaggregated albumin (^{99m}Tc-MAA) and repeat the measurement in the gamma camera ("counts 2" which is the total amount of surfactant-bound radioactivity in the piglet plus the total amount of radioactivity coming from the injected ^{99m}Tc-MAA). In each experiment, by performing this procedure, we can calibrate the gamma camera readings to a known amount of radiation. This is possible because there is a linear relationship between the gamma camera counts and the total amount of radioactivity the piglet receives, which is illustrated in the picture above. Using a Geiger counter, we also correct for residuals lost during manipulation (vials, syringes, interfaces, cannulas, etc), which are subtracted from the total amount of radioactivity administered. Finally, before calculating the deposition for each region of interest (ROI), we correct all readings for the decay of ^{99m}Tc, which has a half-life of approximately 6-hours. The calculation of the deposition in each ROI is done using the equation described in the pictures above, where counts (ROI) is the number of counts in a specific area before the injection of ^{99m}Tc-MAA (after correction for ^{99m}Tc half-life and count adjustment for residuals)[80].

Detailed procedures for each series of experiments

Study I, Atomization

In this study, twelve one-day-old piglets received analgesia and sedation as previously described and were randomized into two groups. In the control group, 200 mg/kg poractant alfa was mixed with ^{99m}Technetium-nanocolloid and given by standard tracheal instillation to intubated piglets on pressure-support ventilation. In the intervention group, the same surfactant dose was delivered to spontaneously breathing piglets by atomization above the vocal cords using a novel instrument that delivers surfactant at the retro-pharynx in synchronization with the inspiratory effort [81] (Figure 2–4).

In the intervention group (atomization), the piglets received a customized snout-mask interface. They breathed spontaneously with 3 cm H_2O CPAP delivered by a Servo-i ventilator. A humidifier was enclosed in the respirator ventilatory circuit and the FiO_2 set at 0.4. The surfactant mixture was atomized in four aliquots interpolated by five minutes of pressure support ventilation (inspiratory pressure of 4–8 cm H_2O above 4 cm H_2O PEEP). Under the atomization procedure, the FiO_2 was adjusted as needed to keep the SaO_2 over 85%. Fifteen minutes after the atomization, the animals were intubated and transferred to the gamma camera. In the control group (instillation), the animals were intubated and received pressure support ventilation (inspiratory pressure 4–8 cm H_2O, PEEP 4 cm H_2O and humidified gases with FiO_2 0.5). The surfactant was administered as a bolus via a catheter placed 0.5 cm past the cuffed endotracheal tube's tip. After instillation, the animal was reconnected to the Servo-i ventilator, and PEEP was increased to 10 cm H_2O for 1 minute. The previous ventilator settings were then resumed, and 15 minutes later, the animals were transferred to the gamma camera.

The atomization device consists of a modified infusion pump connected to an atomizing air-blasting catheter mounted into a customized oropharyngeal cannula which is placed in the subject's pharynx. The atomizing catheter has a central lumen where the surfactant flows and several outer atomization lumens connected to a source of pressurized air that is adjusted to produce an atomizing flow of 0.5 L/min. Atomization occurs at the tip of the catheter, where the surfactant liquid jet is gently fragmented by the pressurized airflow and then driven towards the trachea by the inspiratory flow. The catheters we used produced particles with a median particle size distribution (Dv50) ranging between 40–60 µm. To avoid wasting of surfactant, the delivery is synchronized to the subject's inspiratory effort; the inspiratory effort sign is obtained from the pressure signal recorded at the pharynx using a thin fluid-filled catheter that is attached to the device's oropharyngeal cannula.

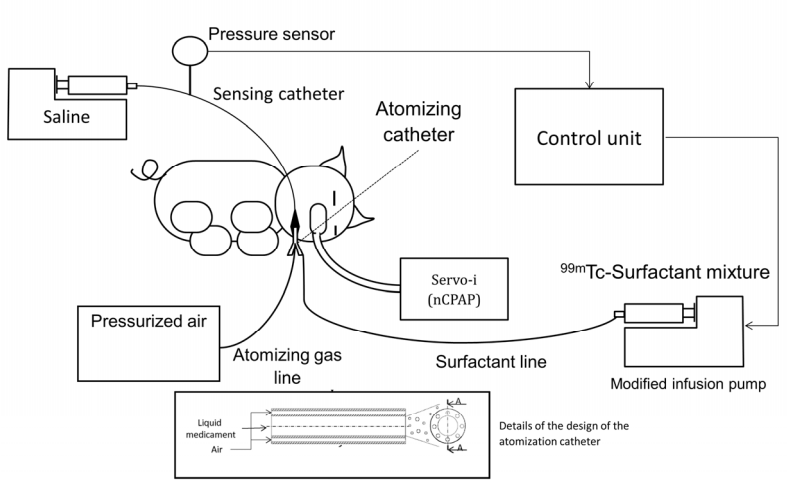


Figure 2–Schematics of the delivery system. Reproduced with permission from Springer Nature. Nord A, Linner R, Milesi I, Zannin E, di Castri M, Bianco F, Dellacá RL, Cunha-Goncalves D, Perez-de-Sa V. A novel delivery system for supraglottic atomization allows increased lung deposition rates of pulmonary surfactant in newborn piglets. Pediatr Res 87, 1019–1024 (2020).

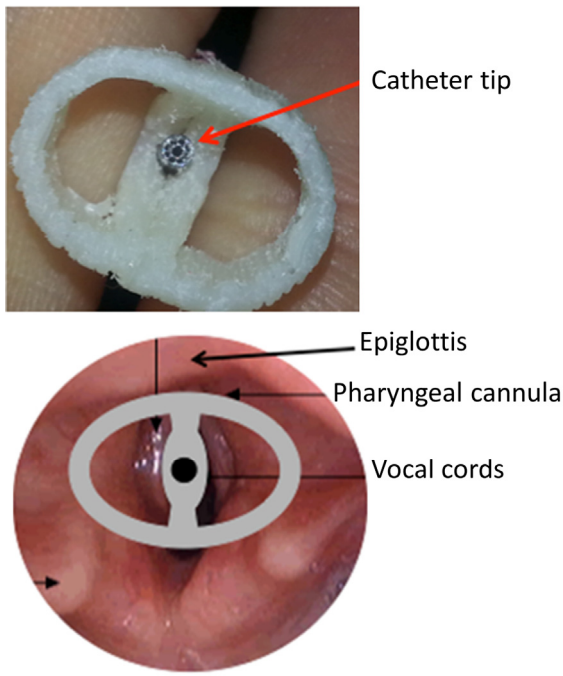


Figure 3–Oropharyngeal cannula and catheter tip and its position at the laryngeal entrance. Reproduced with permission from Springer Nature. Nord A, Linner R, Milesi I, Zannin E, di Castri M, Bianco F, Dellacá RL, Cunha-Goncalves D, Perez-de-Sa V. A novel delivery system for supraglottic atomization allows increased lung deposition rates of pulmonary surfactant in newborn piglets. Pediatr Res 87, 1019–1024 (2020).

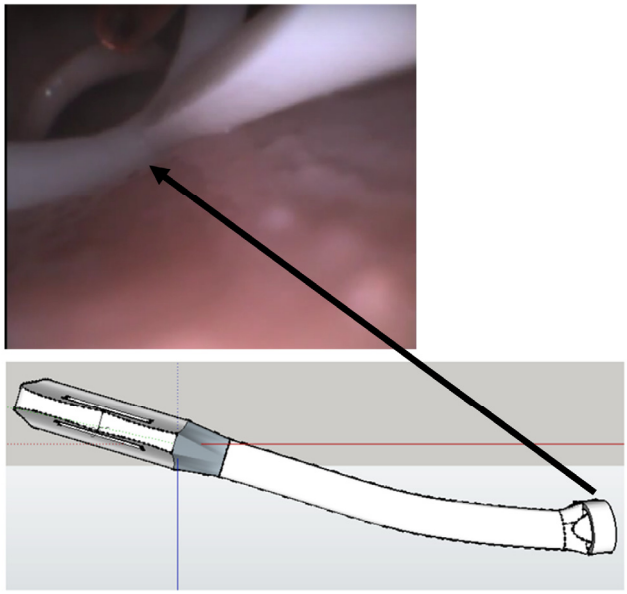


Figure 4–Picture taken with a fiberscope confirming the correct position of the cannula in relation to the larynx entrance and a technical drawing of the cannula's lateral view. Reproduced with permission from Springer Nature. Nord A, Linner R, Milesi I, Zannin E, di Castri M, Bianco F, Dellacá RL, Cunha-Goncalves D, Perez-de-Sa V. A novel delivery system for supraglottic atomization allows increased lung deposition rates of pulmonary surfactant in newborn piglets. Pediatr Res 87, 1019–1024 (2020).

Nebulization (Studies II-IV)

In these studies, we used our animal model of newborn piglets that breathe spontaneously on nasal CPAP support. We investigated the deposition of nebulized surfactant in the lungs with two different ventilatory support modes (Study II), the effect that animal positioning during nebulization has on surfactant deposition and distribution (Study III), and the feasibility of nebulizing higher surfactant doses to reach lung deposition rates equivalent to the ones obtained by surfactant instillation (Study III). We used the customized investigational eFlow-Neos vibrating membrane nebulizer system from Pari (Pari Pharma Starnberg Germany) in all three studies. Nebulization was unsynchronized, and we kept the nebulizer between the Y-piece of the ventilation circuit and the subject's interface to decrease the loss of surfactant to the expiratory limb of the circuit due to the bias flow (Figure 5 and Figure 6).

Vibrating mesh nebulizers show several clinically significant advantages over standard nebulizers. The technology allows the engineering of small-size units with

high nebulizing efficiency, which can shorten treatment time, leave minimal residual volume, and are quietly operated [82]. Among other concerns, to assure that the nebulized drug can reach the lungs, it is vital during nebulizer investigations to optimize and determine the delivered particle's properties. The PARI eFlow-Neos was explicitly developed for poractant alfa (Curosurf 80 mg/ml) administration, and the particle size distribution obtained by this nebulizer was determined in vitro by laser diffraction. The mean MMD (mass median diameter) of the nebulized surfactant was 3.0 +/- 0.1 um at 90% relative humidity, close to human lung physiologic conditions at 37 °C. The fine particle fraction value (FPF) was 93.7% and represented the fraction of respirable particles below 5 µm contained in the aerosol cloud.

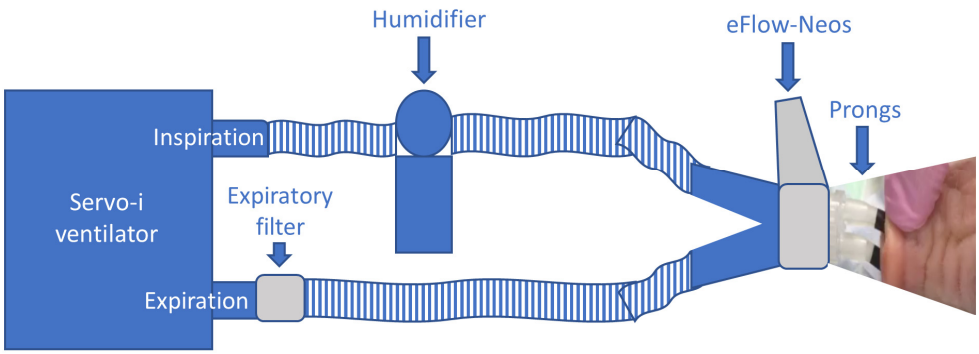


Figure 5–Schematic of the nebulization circuit in studies I–III. Nord A, Bianco F, Salomone F, Ricci F, Schlun M, Linner R, Cunha-Goncalves D. Nebulization of high-dose poractant alfa in newborn piglets on nasal continuous positive airway pressure yields therapeutic lung doses of phospholipids. *Am J Perinatol. 2020 Copyright ©2020, Georg Thieme Verlag KG Stuttgart·New York*

Figure 6–eFlow-Neos Investigational Nebulizer directly connected to the custom-made prongs.

Study II

After premedication and initiation of the analgesic and sedation infusions, arterial and venous cannulations were performed. The 25 piglets were then randomized into two treatment groups in either the right or left lateral decubitus: 1 – group nCPAP, 12 subjects treated with 3 cm H_2O of nasal continuous positive airway pressure, and 2 – group NIPPV, 13 subjects treated with 3 cm H_2O of nasal intermittent positive pressure ventilation over 3 cm H_2O of positive end-expiratory pressure (PEEP), thus peak inspiratory pressure (PIP) of 6 cm H_2O. Inspired oxygen was 0.4 in both groups. The piglets received custom-made nasal prongs connected to the ventilatory circuit (Servo-i including a ventilatory circuit-interposed Fisher Paykel FP 850 humidifier). The Pari eFlow-Neos nebulizer was placed between the Y-piece of the dual-limb breathing circle and the interface (Figure 5). 200 MBq of ^{99m}Tc Nanocoll was mixed with 200 mg/kg poractant alfa (Curosurf 80 mg/ml) and continuously nebulized until the nebulizer chamber was completely empty. After an additional 15 min on nCPAP, the piglets were transported to the gamma camera for scintigraphy as described in the previous section.

Study III

In earlier studies, we noticed a significant side difference (right vs. left lung) in the deposition of surfactant depending on the animals' positioning during nebulization. There are only a few investigations referring to the impact of body positioning during nebulization. We decided to investigate if positioning would influence the pattern of surfactant deposition in the lungs. Twenty-four 12–36-hour old full-term piglets weighing 1.3–2.2 kg were randomized into four groups (n=6 in each group): 1. Lateral right decubitus, 2. Lateral left decubitus, 3. Prone position, and 4. supine position. All animals were supported with 3 cm H_2O of nCPAP via customized nasal prongs and received humidified gases with an inspired oxygen fraction of 0.4 via a Sevo-i ventilator. Each piglet received a nebulization of 200 mg of poractant alfa mixed with 200 MBq of ^{99}Tc-nanocolloid. Blood gases were taken, and hemodynamic and respiratory parameters, as well as the nebulization time interval, were recorded. After nebulization, gamma scintigraphy was performed.

Study IV

Intratracheal surfactant instillation has been the gold standard method for treating babies suffering from RDS since a long time ago. In this study, we investigated if it

would be possible to nebulize a sufficiently high dose of poractant alfa that would yield a surfactant lung dose equivalent to the one obtained by intratracheal instillation. Twelve piglets were supported with 3 cm H_2O nasal CPAP via a Servo-i ventilator. They received humidified air/oxygen mixture with an inspired oxygen fraction of 40%. Via the eFlow-Neos nebulizer, the piglets received 600mg/kg of poractant alfa mixed with 200 MBq of ^{99m}TC nanocolloid. Six animals were used as a control group and received 200 mg/kg of intratracheally instilled synthetic surfactant (CHF 5633, 80 mg·mL^{-1}, Chiesi Farmaceutici S.p.A., Parma, Italy) mixed with 100 MBq of ^{99m}TC nanocolloid. After surfactant instillation, the piglets received pressure support ventilation for five minutes (4–8 cm H_20 and 4 cm H_20 PEEP) and then 20 minutes of CPAP via the endotracheal tube. The total amount of phospholipids deposited in each animal's lungs was calculated from the gamma camera pictures.

Study V, Laryngeal Mask Airway

In this fifth study, divided into two parts, we first randomized 25 piglets into two groups (Insertion Attempt, Figure 7) to investigate the success rate of inserting a surfactant delivery catheter tip below the vocal cords without the aid of laryngoscopy. One LMA type had a channel dedicated to introducing the surfactant catheter. In contrast, the other LMA did not have such a channel, and the catheter was introduced into its central lumen. All procedures were video-recorded by one of the researchers for posterior assessment, but the LMA-operator was blinded throughout the insertion attempts. The weight of the animals ranged from 1.2 kg to 2.3 kg. The LMAs we used were LMAs of type Unique, size 1.0, specifically modified for this study. The LMA with a catheter channel had even an incorporated endoscopic camera to which the LMA-operator did not have access in this first part of the study. The time and easiness for placement of the LMA were recorded, and the success rate of positioning the catheter tip was analyzed by reviewing the video recordings afterward.

When all recordings were finished, the LMAs were withdrawn, and the animals were kept on nCPAP for five minutes when they were randomized into three groups for the second part of the study (Main Study). All animals in the main study received 100 mg/kg poractant alfa (Curosurf) mixed with 100 MBq ^{99m}Tc-nanocolloid. In the first group (LMA Camera), nine piglets breathing spontaneously with no respiratory support received surfactant through a catheter passed beyond the vocal cords via a dedicated catheter channel; the procedure was performed under visual guidance (LMA Camera with a fixed camera and catheter channel, Figure 8). In the second group (LMA Standard without fixed a camera or a catheter channel, Figure 9) with eight piglets, the placement of the catheter for surfactant delivery was done blindly;

the catheter was introduced via a swivel connector into the central lumen of the LMA, with the intention to position it just above the vocal cords, i.e., with the tip just outside the distal opening of the LMA (this distance was measured ahead of time and the catheter length was shortened accordingly). The surfactant was administered as a bolus followed by positive pressure support ventilation via a Servo-i ventilator (10 cm H_2O of inspiratory pressure, 4 cm H_2O PEEP, and a respiratory rate of 40 per minute) to assist the distribution. Just before surfactant instillation, with the aid of a flexible scope, an external observer did a video recording of the LMA and catheter positioning in the supraglottic area. In the third group (InSurE, Figure 10), eight piglets received surfactant instillation according to the INSURE method. The catheter was placed one cm below the endotracheal tube, and surfactant was instilled as a bolus followed by 5 minutes of pressure-controlled ventilation. Then the animals were extubated to CPAP.

All LMA procedures, even those performed with the LMA without an integrated camera, were video recorded by another researcher (not the LMA-operator). These videos were analyzed afterward to assess where the catheter tip was located. The surfactant deposition was measured with gamma scintigraphy as described earlier.

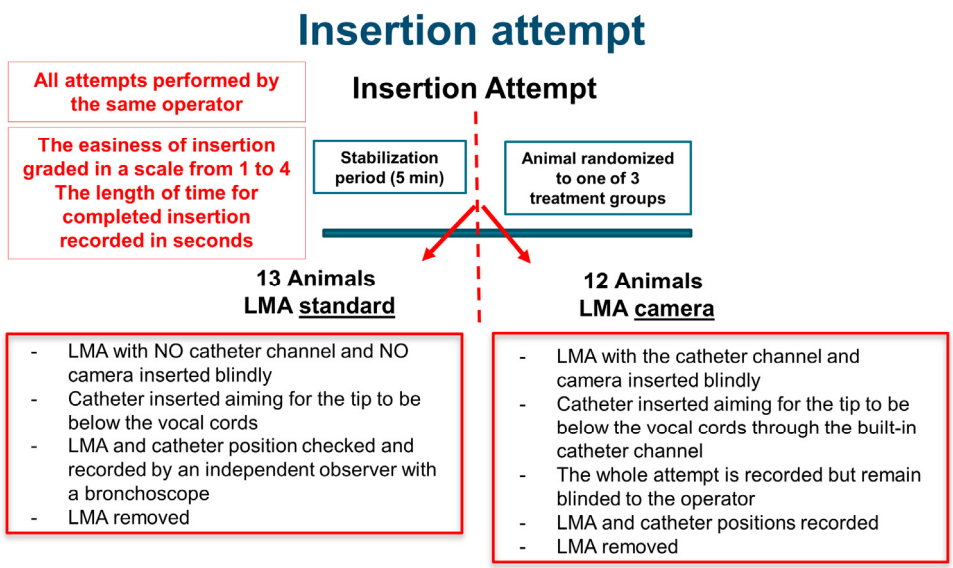


Figure 7–Insertion attempt

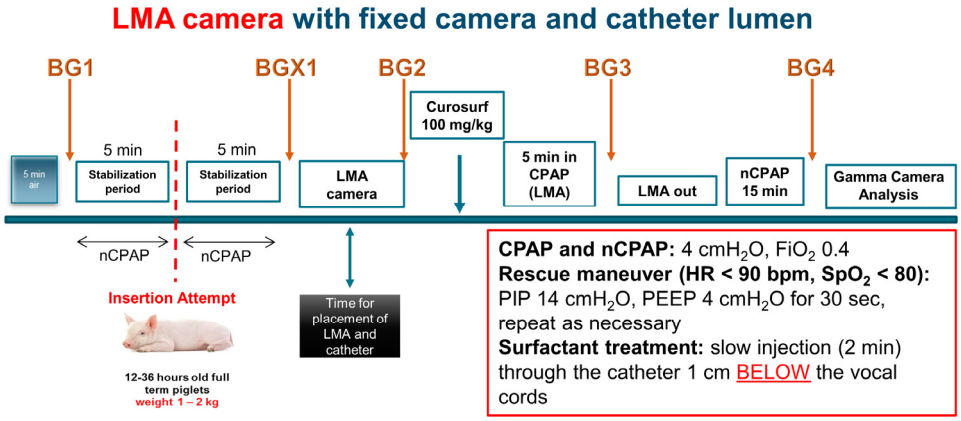


Figure 8–LMA camera protocol

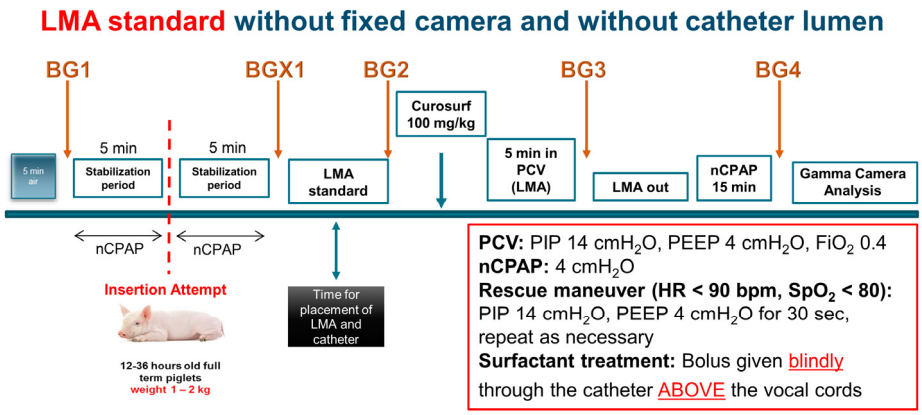


Figure 9–LMA standard protocol

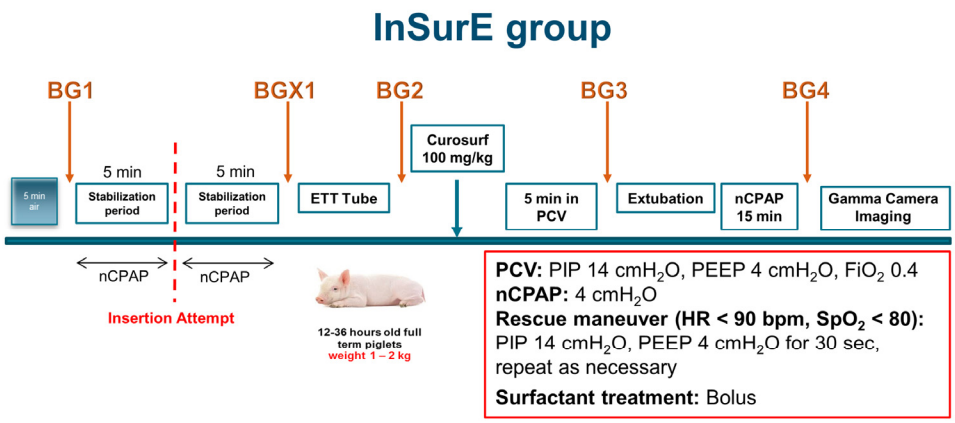


Figure 10–InSurE protocol

Main Results

Study I

The percentage of the administered surfactant found in the lungs in the atomizer group was 40% (24–68%), which was significantly lower than in the InSurE group 87% (55–95%), $p<0.001$(Table 3). Deposition above the trachea in the atomizer group was 13% vs 5.4% in the intubated group (n.s). As we have observed before, surfactant deposition in the dependent lung was higher in both groups, $p < 0.001$, with no difference in the ratio of distribution between upper and lower lungs between the two groups.

Table 3–Deposition of surfactant in various locations

Group	Upper lung %	Dependent lung %	Both lungs %	Rati of upper/both lungso	Nasopharynx-ET tube %	Trachea %	Gut %
Atomizer	4.7 (0.8–19)	32 (23–50)	40 (24–68)	14 (3.3–35)	13 (5.5–16)	4 (3–6)	1.9 (1–17)
Instillation	9.3 (1.9–29)	66 (53–92)	87 (55–95)	11 (3.5–31)	5.4 (4.3–15.6)	3 (1–8)	2.1 (1.1–2.5)
***p*-value**	0.41	<0.001	<0.001	0.8	0.057	0.965	0.937

Deposition median (range) as a percentage of total atomized dose or total instilled dose. n= 6 in both groups. *p*-values are calculated using *t*-test or Mann-Whitney test.

Table 4–Arterial blood gases

Group	Baseline	Start	15 min	End
PaO_2 ,*kPa*				
Atomizer	19 (13 – 22)	9.2 (6.1 – 19)*,§	5.7 (3.7 – 13)§	7.2 (6.8 – 12)*,§
Instillation	16 (14 – 26)	18 (14 – 24)	9.6 (5.3 – 13)§	14 (13 – 21)
$PaCO_2$,*kPa*				
Atomizer	5.8 (4.9 – 6.7)	5.4 (4.8 – 6.4)	6.8 (5.0 – 8.5)	8.6 (5.7 – 11) *§
Instillation	7.5 (4.6 – 12)	5.1 (4.5 – 5.8)	6.8 (4.9 – 9.4)	6.1 (5.6 – 7.2)
pH				
Atomizer	7.49 (7.39 – 7.55)	7.42 (7.33 – 7.49)	7.41 (7.33 – 7.49)	7.29 (7.24 – 7.44)*,§
Instillation	7.44 (7.29 – 7.53)	7.52 (7.47 – 7.65)	7.42 (7.24 – 7.58)	7.47 (7.41 – 7.50)

Values presented as median (range). Differences within groups were analyzed with RM ANOVA on ranks followed by Dunn's *post hoc* test when indicated. Differences between groups at the different stages were analyzed with a *t-test* or Mann-Whitney test. *P* <0.05 was considered significant. * denotes significant changes between groups. § denotes significant changes from Baseline.

The animals in the atomizer group were smaller, 0.9 kg (0.8–1.1) compared to the animals in the InSurE group, 1.2 kg (1–1.7), $p = 0.014$. Heart rate (HR), mean arterial blood pressure (MAP), regional cerebral oxygen saturation ($CrSO_2$), and blood gases were similar at baseline.

The atomizer group animals were kept on CPAP and received pressure support ventilation intermittently for short periods as per protocol. During atomization, PaO_2 gradually decreased, and $PaCO_2$ increased compared to baseline. (Table 4)

The total time for atomizing 200 mg/kg of surfactant was 28 min (17–52 min) with a total delivered volume of 3.3 mL (2.7– 4.4), equivalent to an output rate of 0.1–0.2 mL/min.

The animals were hemodynamically stable during treatment. Notable is a momentary decrease in $CrSO_2$ about one minute after the instillation of surfactant in the InSurE group (Figure 11).

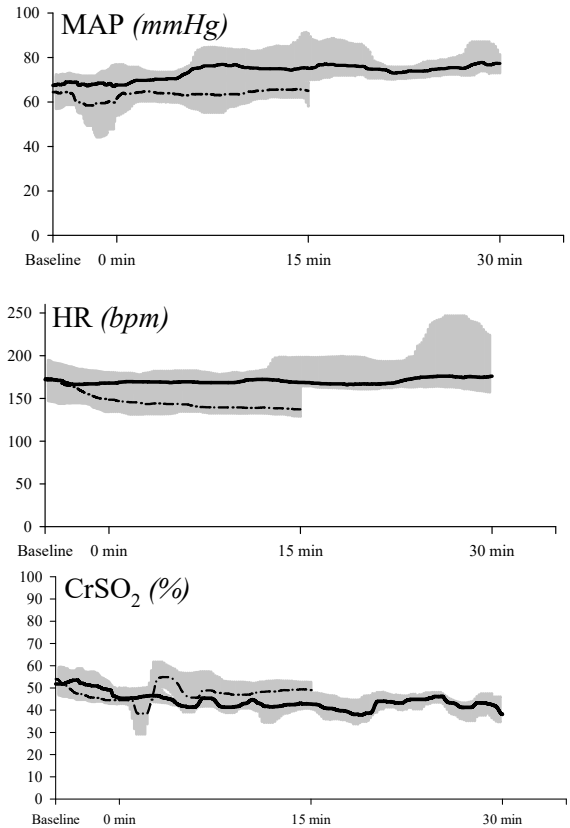


Figure 11–Hemodynamics and cerebral oxygenation during surfactant administration. The solid line depicts median values for the atomizer group and the interrupted line depicts the median values for the instillation group. The shadow areas are the interquartile variation. MAP, mean arterial pressure; HR, heart rate; $CrSO_2$, regional cerebral oxygen saturation.

Study II

The studied groups were not different at baseline regarding the observed parameters (body weight, HR, MAP, RR, $CrSO_2$, blood gases). The mean weight for both groups was 1.7 ± 0.3 [1.6, 1.8] kg. There was no difference in the performance of the eFlow-Neos investigational nebulizer system with the different types of ventilatory assistance. The nebulizer mean output for both groups was 0.24 ± 0.04 [0.22, 0.26] mL/min. There was no difference in nebulization times. It took 17.8 ± 3.6 [15.5, 20.1] min to nebulize 200 mg/kg of poractant alfa with nCPAP, and 21.1 ± 7.4 [16.6, 25.6] min with NIPPV respectively. Circuit leakage as displayed on the Servo-i user interface was larger in the NIPPV group, 7.1 ± 4.0 [4.7, 9.5] % vs. 2.2

± 2.1 [0.8, 3.6] % in the nCPAP group respectively (P<0.001). As expected, the weight indexed minute volume (MV) was higher in the NIPPV group at 0, 5, and 15 min (Figure 12).

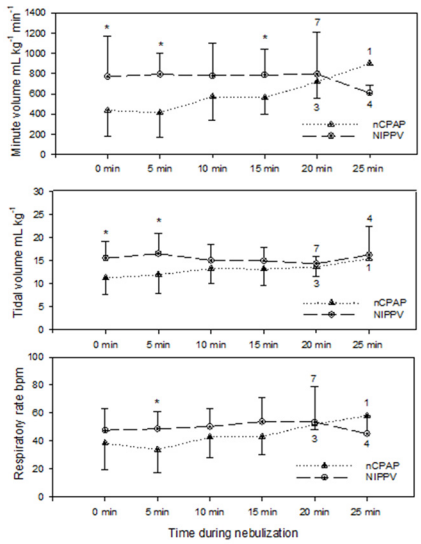


Figure 12–Indexed minute volume, indexed tidal volume, respiratory rate. Values are shown as mean ± SD. * *P* < .05 between groups. There were no inside-group differences between stage and 0 min. nCPAP, nasal continuous airway positive pressure; NIPPV, nasal intermittent positive pressure ventilation. **Nord A**, Linner R, Salomone F, Bianco F, Ricci F, Murgia X, Schlun M, Cunha-Goncalves D, Perez-de-Sa V. Lung deposition of nebulized surfactant in newborn piglets: Nasal CPAP vs Nasal IPPV. *Pediatr Pulmonol. 2020, with permission.*

Likewise, the mean MV value for the entire nebulization time was higher in the NIPPV group, 780 ± 241 [634, 926] mL/kg/min *vs* 489 ± 195 [365, 613] mL/kg/min for the nCPAP group, p=0.004. The mean indexed TV (TV/kg) under the entire nebulization period was higher in the NIPPV group 15.3 ± 2.5 [13.8, 16.8] mL/kg vs 12.4 ± 2.5 [10.8, 14.0] mL/kg in the nCPAP group, p=0.009. The mean RR during nebulization was lower in the nCPAP group, that is, 39.1 ± 14.7 [29.7, 48.5] vs 50.8 ± 13.1 [42.9, 58.7] in the NIPPV group, $p < 0.05$.

The changes in PaO_2 and $PaCO_2$ are shown in Figure 13.

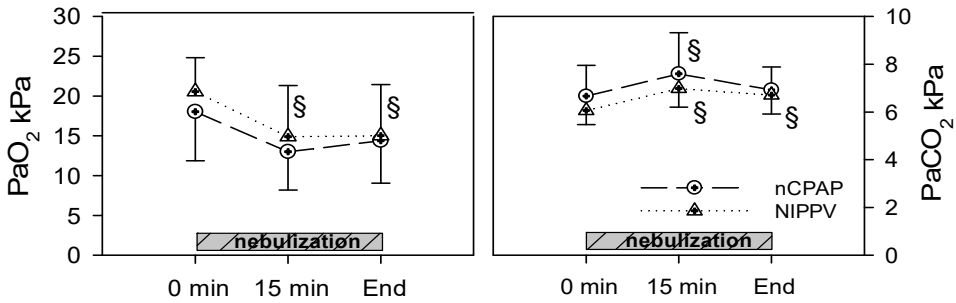


Figure 13–**PaO_2 and $PaCO_2$**. Values are shown as mean ± SD. § denotes within-group differences; P < 0.05 (stage *vs* 0 minutes). No between-group differences were observed. nCPAP, nasal continuous positive airway pressure, NIPPV, nasal intermittent positive pressure ventilation. **Nord A**, Linner R, Salomone F, Bianco F, Ricci F, Murgia X, Schlun M, Cunha-Goncalves D, Perez-de-Sa V. Lung deposition of nebulized surfactant in newborn piglets: Nasal CPAP vs Nasal IPPV. *Pediatr Pulmonol. 2020 with permission.*

There were no significant changes in the regional cerebral oxygen saturation during treatment (Figure 14).

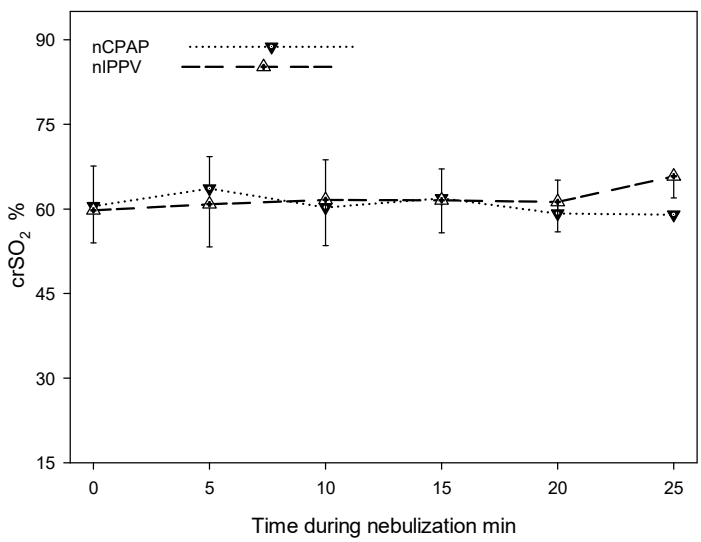


Figure 14 – Regional cerebral oxygen saturation. Values are shown as mean ± SD. There were no significant changes neither within- nor between groups during the nebulization period.

Total lung deposition of surfactant was similar in both groups: 15.9 ± 11.9 [8.3, 23.5] % with nCPAP and 21.6 ± 10.0 [15.6, 27.6] % with NIPPV, *P*=0.21. Deposition in the nondependent lung was higher with NIPPV than with nCPAP.

Study III

We studied 24 piglets, median weight 1.7 (1.3 – 2.2) kg, with no difference between groups. It took between 16 and 19 min to nebulize 200 mg/kg (2.5 mL/kg) of poractant alfa. There was no visually detectable residual volume in the nebulizer chamber at the end of nebulization. The remaining radioactivity in the nebulizer and prongs was usually under 1% of the nebulized dose in all subjects.

At baseline, before starting nCPAP, there were no differences between groups for blood gases, respiratory rate (RR), regional cerebral oximetry ($CrSO_2$), and hemodynamics. During nebulization, mean arterial pressure (MAP) and HR were stable in all groups.

The lung dose of surfactant was highest in the prone position (Figure 15). In this position, more surfactant was found in the right lung (Table 5). In the lateral positions, most of the surfactant was found in the dependent lung (Table 5).

The mean tidal volume (TV) during nebulization was higher in the prone group, but similar in the other three groups (Figure 16, Table 6).

There were no between-group differences in blood gases during treatment, but PaO_2 decreased and $PaCO_2$ increased in the prone group (0 min *vs* end of nebulization).

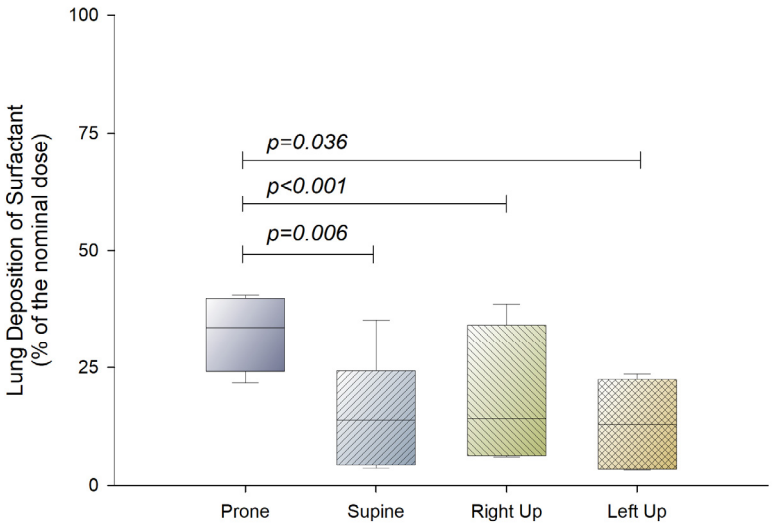


Figure 15 – **Lung deposition of surfactant as a percent of the nominal dose**. Box plots and whiskers depict the median, 5th and 95th percentiles for total lung deposition in each group. The prone group was significantly different from the other groups (One-way ANOVA with Student-Newman-Keuls post hoc test). P values are shown in figure.

Table 5 – Surfactant deposition

Anatomical site	**Prone** ***n=6***	**Supine** ***n=6***	**Right Side Up** ***n=6***	**Left Side Up** ***n=6***
Right Lung	21.0 ± 8.6 [12.0, 30.1]	10.7 ± 11.4 [0.0, 22.7]	3.4 ± 1.0 [2.4, 4.4]	11.2 ± 9.8 [0.9, 21.5]
Left Lung	11.3 ± 5.7 [5.4, 17.3]	4.5 ± 2.4 [2.0, 7.0]	15.3 ± 13.4 [1.2, 29.3]	1.8 ± 0.7 [1.1, 2.6]
p-value (t-test)	*0.04*	*0.22*	*0.06*	*0.04*
Nasophaynx	12.2 ± 1.6 [10.5, 13.9]	28.0 ± 8.4 [19.2, 36.9]	15.8 ± 8.5 [6.9, 24.8]	16.4 ± 4.5 [11.6, 21.1]
p-value (ANOVA)	*0.002 (supine vs. all)*			
Trachea	5.1 ± 1.9 [3.1, 7.1]	5.2 ± 3.2 [1.9, 8.6]	3.7 ± 2.4 [1.1, 6.2]	3.1 ± 2.3 [0.7, 5.5]
p-value (ANOVA)	*0.384*			
Stomach	6.6 ± 7.4 [0.0, 14.4]	6.8 ± 7.6 [0.0, 14.8]	21.5 ± 13.4 [7.5, 33.5]	19.1 ± 11.7 [6.8, 31.3]
p-value (ANOVA)	*0.034*			

Data presented as mean ± SD, 95% CI [min, max]. ANOVA denotes differences among all groups; the *t-test* (paired) denotes differences between the right and left lungs within each group; and *n* is number of subjects. % of the administered dose found in the animals.

Table 6 – Ventilatory parameters

Parameter	Prone *n*=6	Supine *n*=6	*p-value*	Right Side Up *n*=6	Left Side Up *n*=6	*p-value*	*ANOVA p-value*
Minute volume ***mL·kg^{-1}***	834 ± 245 [576, 1091]	618 ± 85 [529, 706]	*0.07*	620 ± 185 [426, 814]	362 ± 103 [255, 470]	*0.01*	*0.001*
Respiratory rate ***breaths·min^{-1}***	49 ± 18 [30, 67]	57 ± 14 [43, 72]	*0.36*	50 ± 13 [37, 64]	29 ± 7 [21, 37]	*0.01*	*0.01*
Circuit leakage ***%***	1.7 ± 1.3 [0.4, 2.9]	2.7 ± 1.0 [1.6, 3.8]	*0.16*	3.3 ± 4.2 [0.0, 7.7]	8.5 ± 11.6 [0.0, 20.7]	*0.70*	*0.56*
Nebulizing time ***min***	18.2 ± 3.9 [14.0, 22.3]	17.0 ± 3.4 [13.6. 20.4]	*0.37*	19.2 ± 4.6 [14.4, 24.0]	16.5 ± 1.9 [14.5, 18.5]	*0.22*	*0.58*
Nebulizer output ***mL·min^{-1}***	0.23 ± 0.03 [0.20, 0.26]	0.27 ± 0.03 [0.24, 0.31]	*0.03*	0.24 ± 0.05 [0.19, 0.29]	0.27 ± 0.01 [0.27, 0.29]	*0.03*	*0.10*

Data presented as mean ± SD, 95 % CI [min, max]. Minute volume, respiratory rate, and leakage (as measured by the Servo-i ventilator and displayed in the user interface) are the mean values during the whole nebulization time. We compare prone *vs.* supine and Right-Up *vs.* Left-Up (t-test) and all groups (ANOVA). *n* is the number of subjects in each group.

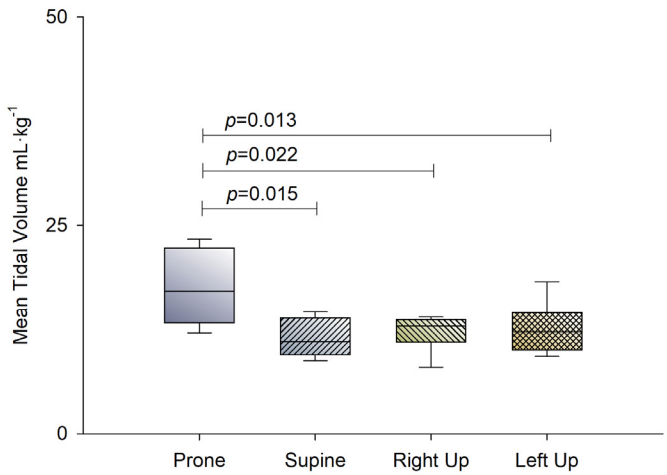


Figure 16 – Mean indexed tidal volume during nebulization. Box plots and whiskers depict the median, 5th, and 95th percentiles for total lung deposition in each group. The prone group was significantly different from the other groups (One-way ANOVA with Student-Newman-Keuls post hoc test). *p* values are shown in the figure.

Study IV

We studied twelve piglets on nCPAP in the nebulization group and six piglets in the instillation group. There was no between-groups difference in subject size and the median weight of 1.5 (1.0 – 1.9) kg. It took 58 ± 12.4 min (mean ± SD) to nebulize 600 mg/kg (7.5 mL/kg) of poractant alfa and the nebulizer output rate was 0.2 ± 0.05 mL/min.

The total lung deposition in % of the nominal dose in the nebulization group was 23 ± 16 % (13, 33) (mean ± SD, CI) and 86 ± 12 % (73, 99) in the instillation group, $p < 0.001$. The resulting lung dose of phospholipids was 138 ± 96 (78, 199) mg/kg and 172 ± 24 (147, 197) mg/kg in the nebulization and instillation group, respectively p=0.418. In both groups, most of the surfactant was found in the dependent lung (Figure 17).

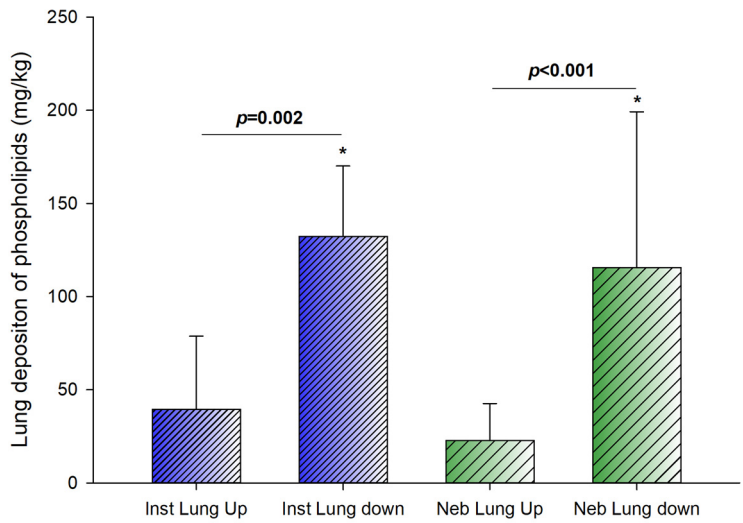


Figure 17 – Deposition and distribution of surfactant phospholipids in the lungs. Mean values with whiskers representing standard deviation. Inst, instillation; Neb, nebulization.

Table 7 shows the surfactant deposition in the different anatomic sites or regions of interest.

Table 7: Deposition at the different sites in % of the administered dose (mean ± SD)

Group	Trachea %	Nasopharynx %	Stomach %	Lung up %	Lung down %
Nebulization (n= 12)	1.9 ± 1.1	10.7 ± 2.5	18.1 ± 11.7	3.8 ± 3.3	19.3 ± 13.9
Instillation (n= 6)	3.6 ± 2.2*	1.9 ± 1.1*	1.4 ± 0.7*	19.7 ± 19.7*	66.2 ± 18.8*

n is number of subjects in each group, * denotes *p* <0.05 for differences between groups.

Hemodynamics (MAP, HR) and regional cerebral oxygen saturation were stable throughout the nebulization period.

PaO_2 gradually decreased during treatment in the nebulization group. In the same group, $PaCO_2$ increased during treatment but was not statistically significant different from 0 minutes at the end of nebulization (Figure 18).

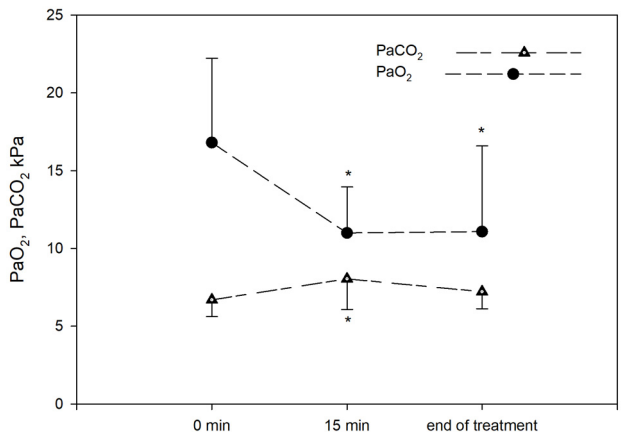


Figure 18 – PaO_2 and $PaCO_2$ during nebulization. Values are mean ± SD. *denotes p < 0.05 for within-groups changes from 0 minutes (RM-analysis of variance with *post hoc* Bonferroni test).

Respiratory rate, tidal volume, and circuit leakage in the nebulization group are shown in Figure 19 below.

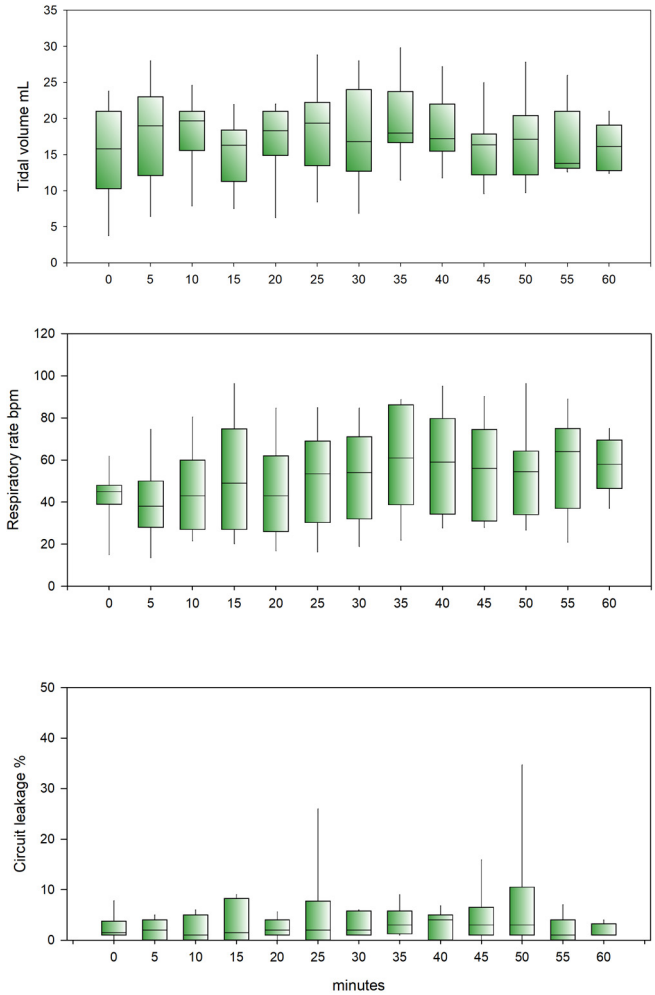


Figure 19 – Tidal volume, respiratory rate, and circuit leakage in the nebulization group. Box plots depict median and whiskers 5th and 95th percentiles.

Study V

25 piglets with a median weight of 1.8 kg (1.4 – 2.3 kg) were studied. There were no significant differences between the groups neither regarding the easiness nor the time for device placement. All animals tolerated well the insertion of the LMA with no significant changes in hemodynamic parameters or oxygenation (MAP, HR, SaO_2,) during the insertion procedure (fig 20).

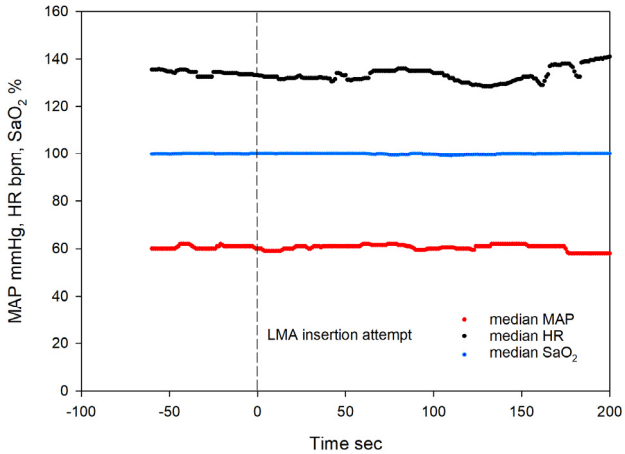


Fig 20- Hemodynamics and oxygen saturation during LMA insertion at "Insertion Attempt".

The success rate for correct placement of the delivery catheter below the vocal cords, using the integrated camera was 92%. There were no significant differences between groups in blood gases taken at baseline and at the end of the study, before transferring the animals to the gamma camera.

The lung deposition of surfactant given via the LMAs was lesser than that obtained with endotracheal instillation (Table 8). Compared to the LMA-group with the catheter introduced in the main lumen and the tip placed above the vocal cords (blind insertion), the deposition improved by 65 % (not statistically significant) in the group with a customized LMA with the integrated camera and catheter channel (catheter tip placed below the vocal cords under visual assistance).

Table 8–Surfactant distribution as a percentage of the total administered dose

Group	Trachea	Nasopharynx	Stomach	Left lung	Right lung	Both lungs
LMA-camera %	5.6 (1.2 – 17.2)	5.4 (2.3 – 12.5)	1.8 (1 – 78.9)	23.9 (1.7 – 61.7)	26.8* (5.3 – 50.5)	68.5* (9.8 – 84.6)
LMA-standard %	8 (2.9 – 12.8)	8.7* (3.3 – 41.1)	28.6* (1.6 – 37.2)	26 (1.7 – 55.6)	17.6* (2.9 – 42.7)	41.2* (4.6 – 88)
InSurE %	6 (2.3 – 9.1)	1.7 (0.5 – 16.2)	1.6 (1.2 – 3.9)	32.8 (8.2 – 53.7)	52.5 (35.7 – 79.6)	87.7 (67.5 – 92.4)

Data are presented as median (range). * denotes $p<0.05$ between the group and InSurE with One Way Analysis of Variance and all pairwise multiple comparison procedure with the Dunn's *post hoc* test.

Discussion

Study I, Atomization

As stated in the most recent European Consensus Guidelines on the Management of Respiratory Distress Syndrome in the neonate (2019), "surfactant replacement therapy is a crucial part of the management of RDS" together with the avoidance of invasive mechanical ventilation. Emphasis is placed on the use of noninvasive techniques of ventilatory support while still offering a rescue-maneuver with the timely administration of surfactant to the babies still requiring higher inspired oxygen fractions despite adequate CPAP/NIPPV titration.

As suggested by previous animals (rabbits [68], lambs [74], baboons [73,83]) and human studies [84], it is believed that to elicit the expected physiological effect, one needs to administer at least 50 mg/kg of phospholipids to the alveolar surface. Even though a few pilot trials were undertaken in neonates, there were only a few experimental studies assessing lung deposition of surfactant aerosolized above the glottis (Table 9).

Table 9 – Animal studies on nebulization and atomization of surfactant

Author	Year	Method	Deposition	Surfactant	Breathing	Ventilation	Animal
Lewis *et al.* [85]	1991	Nebulization	2.7%	Survanta	Intubated	IPPV	Lamb
Lewis *et al.* [86]	1991	Nebulization	3.6%	Survanta	Intubated	IPPV	Rabbit
Lewis *et al.* [87]	1993	Nebulization	6.1%	Survanta	Intubated	IPPV	Sheep
Dijk *et al.* [88]	1997	Nebulization	8.4%	Alveofact	Intubated	IPPV	Rabbit
Dijk *et al.* [89]	1998	Nebulization	9.8%	Alveofact	Intubated	HFV	Rabbit
Fok *et al.* [90]	1998	Nebulization	0.1–1%	Survanta/Exosurf	Intubated	IPPV	Rabbit
Wagner *et al.* [38]	2000	Atomization	86.5%	Poractantalfa	Intubated	IPPV	Rabbit
Rahmel *et al.* [91]	2012	Aerosolized	1%	rSP-C	Spontaneous	nCPAP	Lamb
Rey-Santano *et al.* [46]	2013	Aerosolized	Not reported	Poractantalfa	Intubated	IPPV	Lamb
Linnér *et al.* [80]	2015	Nebulization	14 %	Poractantalfa	Spontaneous	nCPAP	Pig
Milesi *et al.* [74]	2016	Atomization	Not reported	Poractantalfa	Intubated	IPPV	Lamb
Hutten *et al.* [92]	2015	Nebulization	Not significant	Poractantalfa	Spontaneous	nCPAP	Lamb
Milesi *et al.* [47]	2017	Atomization	32%	Poractantalfa	Spontaneous	nCPAP	Lamb
Gregory *et al.* [73]	2019	Nebulization	11.4%	Lucinactant	Spontaneous	nCPAP	Macaque

Wagner *et al*. [38] demonstrated that it was possible to atomize surfactant inside the trachea at the tip of a modified endotracheal tube. With breath synchronization, the average lung deposition was 86% using a radio-labeling technique. Nonetheless intubation was still necessary. Using the same system we tested in our study but with another type of labeling for the administered surfactant, Milesi *et al*. [74] observed a median lung deposition of 32% of the total atomized surfactant dose (200 mg/kg) in spontaneously breathing lambs. Using the same device during spontaneous breathing and nCPAP, we demonstrated, for the first time ever, a lung surfactant deposition well above 50 mg/kg, as assessed by the gold standard technique for measuring lung deposition with radioactive tracers. This device was built and refined to avoid intubation, reduce dead space, and work synchronously with inspiration. It thereby reduces the amount of surfactant lost to the surrounding environment.

Aerosol delivery to the lungs is affected by many different factors, intrinsic and extrinsic, to the subject to be treated. Amongst the extrinsic elements is the choice of the aerosol generator, its placing inside the ventilatory circuit, the drug formulation, the aerosol particle size distribution, the choice of ventilatory support modality, and the type of patient/device interface. The optimal particle size for reaching the distal airways and alveoli during nebulization is around 1–3 μm. Still, in the study by Wagner *et al*. [38], with a Sauter Mean particle Diameter >100 μm, a similar lung deposition and distribution as that obtained with the instillation technique was observed. It is known that the surfactant deposited in the large, central airways distributes to non-expanded alveoli by capillarity and surface tension gradients (Marangoni effect) [93]. The mean particle size in the present study was smaller than Wagner's, i.e., 40–60 μm. Still, the generated particles were most undoubtedly big enough to prompt the initial deposition in the central airways, reducing the losses during expiration. The effective breath synchronization obtained by placing the atomizing catheter close to the glottis opening was also an important factor in reducing the loss of surfactant during expiration and improving the deposition in the larger airways.

A special oropharyngeal cannula adapted to the piglet's specific anatomy was developed to support the atomization catheter and the pressure sensing catheter during the atomization process. As the cannula did not perfectly match the oropharyngeal dimensions of the piglets, there was the possibility of dislocation of the cannula and thus the catheters, during treatment. We believe this is one of the reasons for the large intersubject variability we have observed. There was also a wide range in the time necessary for completing atomization of the 200 mg/kg of poractant alfa, 17–52 min. The varying respiratory rate and inspiratory:expiratory ratio clearly influenced the time to atomization, which was breath triggered. There was, however, no correlation between the time to complete atomization and the total lung deposition observed.

The hemodynamic profiles observed during the study were inherent to the model itself (Figure 11). That the instillation group had lower blood pressure at baseline can be explained by the need for more profound analgesia needed for the intubation procedure itself. There were no significant changes in neither mean arterial pressure nor heart rate during the surfactant atomization (inside-group). The dramatic changes in hemodynamics and oxygenation, often described during surfactant instillation in premature babies, were not observed in this study, probably because of the level of sedation and the fact that the animals were healthy, term newborns.

There was a significant increase in $PaCO_2$ and a decrease in PaO_2 in the atomizer group. As with hemodynamics, the between-group differences observed in the blood gases (Table 4) are partly explained by the intrinsic dissimilarities in ventilatory strategies linked to the study design and the different techniques for delivering the surfactant. Besides more sedation for the performance of laryngoscopy and intubation, the control group was kept on pressure support ventilation throughout the intervention. We also think that the progressive accumulation of fluid in the large airways associated with a low CPAP amount contributed to the worsening ventilation status in the atomizer group.

Following the results obtained during pilot studies, we chose to divide the surfactant dose into four aliquots and introduce a period of pressure support ventilation to mitigate this problem.

We did not observe any significant complications of the atomizing system itself. It is possible to use different gas flow rates to create the aerosol, but in our model, flows above 0.75 L/min induced breath-holding in the piglets (Heuring-Breuer reflex). We do not know the significance of this finding for the sick newborn child.

Studies II, III, and IV

Nebulization offers an alternative approach for surfactant delivery during spontaneous breathing. It is most likely the least invasive method entirely deterring airway instrumentation. Earlier attempts[37] were unsuccessful mainly due to technological constraints, but the development of more efficient nebulizing systems has renewed interest in this treatment strategy.

To our knowledge, there are no other reports of in vivo deposition of a nebulized radioactive tracer-surfactant mixture in a clinically relevant experimental neonatal model with spontaneous breathing and noninvasive ventilatory support.

Due to obvious ethical reasons, there is extremely modest data on in vivo lung deposition of aerosols in human neonates. The in vitro models have many constraints and can hardly reproduce the variability in the respiratory physiological

profile of sick premature surfactant deficient babies. Among other intrinsic factors affecting aerosol deposition in this population, high respiratory rates with varying inspiratory:expiratory ratios, low functional residual capacity, small and varying tidal volumes, small airway calibers, and high resistance are some of the challenges encountered when trying to administer nebulized drugs to the premature neonate. We have developed a relevant experimental neonatal in vivo model to test the feasibility of administering undiluted nebulized surfactant during the noninvasive ventilatory support of spontaneous breathing.

Aware of the above challenges, we tried to improve and control the extrinsic factors that potentially affect the delivery of the drug to the lungs. In an earlier study of our group, using the same system with diluted poractant alfa surfactant, we observed a lung deposition of 14% of the administered dose [80](ref). To improve lung deposition, we placed the nebulizer between the interface (nasal prongs) and the y-piece of the ventilatory circuit, aiming to reduce drug spillage due to the ventilator bias flow, but this increases the apparatus dead-space by 8 mL. We have observed in all three studies an increase in $PaCO_2$ that could be partly due to the increased dead-space besides fluid accumulation in the large airways. To further reduce losses to the ambient, we used well-fitted customized prongs. We kept the animal's mouth closed, which might explain the slight decrease in carbon dioxide elimination during the procedure resulting in statistically significant hypercarbia, probably negligible from a physiological standpoint. The level of CPAP and NIV used was restricted by the highly active Hering-Bruer reflex in these healthy term piglets. The maximally tolerated level was around 3–4 cm H_2O of CPAP and a peak inspiratory pressure of 6 cm H_2O.

There is controversy on the effect of the tidal volume's size and its effect on the achieved lung dose in neonates. Higher tidal volumes and minute volumes are, however, usually associated with improved delivery of the nebulized drug to the lungs. Our findings in study III partly support this observation. The minute volume was 60% higher in the NIPPV group than in the CPAP group, corresponding to a 35% higher amount of surfactant delivered to the lungs.

Study V

The presence of an integrated catheter channel did not increase the success rate of blindly placing the catheter tip below the vocal cords (Insertion attempt study). The ability to visualize the laryngeal entrance and the vocal cords increased the success rate of accurately placing the catheter tip from 23% to 89 % in the LMA-camera group. Contrary to our primary hypothesis, visual guidance did not ensure an equivalent lung dose to the one obtained with endotracheal instillation. The total

lung deposition in the LMA groups was significantly lower than in the InSurE group. However, the median amount of surfactant lost in the stomach and nasopharynx was less in the LMA-camera than the LMA standard group. Mimicking the LISA technique and based on clinical data suggesting that a slow injection over 1–3 min is preferable to bolus instillation[94], we chose to administer the surfactant in the LMA-camera group as an injection over one to two minutes with the animals spontaneously breathing on nasal CPAP. In the standard LMA group and the InSurE groups, the bolus instillation was followed by pressure-controlled ventilation to enhance the surfactant's spreading. It is possible that a brief period of ventilation in the LMA camera group would have enhanced the delivered dose and minimized the amount of reflux. The little or absence of reflux seen in our intubated piglet group contrasts with studies in preterm babies by Pinheiro *et al.* [64](2016) and Sadeghnia *et al.* [63](2014), using uncuffed endotracheal tubes instead of cuffed ETT. The adequate insertion distance below the vocal cords is dependent on the piglet's size and is also discussed when performing LISA/MIST in humans. The advantage of LISA compared to InSurE does not exclude the necessity of skills to perform laryngoscopy and to visualize the glottic entrance to have success in administering surfactant via a catheter. Current clinical guidelines for neonatal care [48] recommend stabilization with noninvasive ventilatory support instead of intubation and mechanical ventilation. This new approach has limited the opportunities for new generations of neonatologists to perform laryngoscopy [95]. Foglia et al. [96], reporting data from an international registry study, showed a first attempt success rate for endotracheal intubation of 49% in the NICU and 46% in the delivery room. In contrast, in a case series from Smee *et al.* [97], the success rate for correct placement of an LMA was 78% in the first attempt and 98% in the second attempt, respectively. The LMA is supposed to be an option in babies > 2000 gram if face-mask ventilation is ineffective or intubation is not feasible, according to ILCOR (The International Liaison Committee on Resuscitation) guidelines from 2015. The laryngeal mask is considered a noninvasive, safe alternative to tracheal intubation during anesthesia and sedation and is used since the late 1980s [98]. The desire to avoid laryngoscopy, intubation, and mechanical ventilation together with the more frequent use of LMA's, associated with the development of smaller sizes of LMAs, has raised the interest in using the LMA as a conduit for administering surfactant to newborns. One of the first reports on using an LMA for surfactant replacement therapy in preterm babies dates from 1992[99]. Since then, randomized controlled trials, including 154 infants receiving surfactant therapy via an LMA, show a positive effect on reducing oxygen requirement after treatment [62-66]. Venozzi *et al.* [100] combined the LMA with a catheter technique and called the method CALMEST (Catheter And Laryngeal Mask Endotracheal Surfactant Therapy). This modified MIST method aims to deliver the surfactant directly into the trachea with a catheter using the LMA as a guide. They first performed a simulated study on a mannequin and obtained a success rate of 93%. The catheter was positioned blindly

and afterward checked for correct placement by video laryngoscopy. This first part was followed by an in vivo study. Four spontaneously breathing children supported with CPAP, with a BW ranging from 1.9 kg to 3.6 kg, were treated for RDS with 150–200 mg poractant alfa via a catheter. The catheter positioning was checked by connecting the catheter, via a 3.5 mm tube connector, to an end-tidal CO_2 monitor and registered the end-tidal CO_2 wave. Oxygenation improved in all four children, and there were no major adverse effects. The very high success rate of Vanuzzi, in the first part of their study, could not be reproduced by others [101], and the success rate of catheter placement in vivo is questionable. The proper placement of an LMA is monitored by bilateral auscultation of the thorax, observations of proper chest expansion, and the presence of CO_2 elimination by capnography. Observations by others describe a low rate of proper alignment of the LMA despite unobstructed breathing and normal end-tidal capnography tracing [102]. We reproduced the Calmest study in our animal model allied to the possibility to evaluate the positioning of the catheter by assessing video recordings afterward. When introduced blindly, only 3 out of 25 animals the catheter was below the vocal cords and would have successfully delivered surfactant under the glottis. We demonstrate that even an experienced operator has a failure rate of 50% if the placement of an LMA is done blindly, which is in line with Bonadies *et al.*'s observations [101]. Measurements of surfactant deposition after LISA treatment in spontaneously breathing preterm lambs are reported by Niemarkt *et al.* [103]. They used samarium oxide labeled surfactant and found a deposition of only 18 % of the lung deposition obtained by endotracheal administration. This contrasts with our findings, where the LMA camera group reaches approximately 75% of the deposition achieved with InSurE. In recent times, Ricci *et al.*[104] use a desaturated-phosphatidylcholine quantification in bronchoalveolar lavage samples as a proxy method for surfactant deposition in a rabbit model. They found no difference between LISA and the InSurE techniques. We use scintigraphy, the gold standard method for assessing lung deposition, and found a significant difference between Insure and the two groups of LMA's. Even though there was no statistically significant differences between the two LMA groups, the median deposition in the LMA camera group was 65% higher than the LMA standard group. Even after excluding the two animals in the LMA camera group with the lowest deposition, the deposition is still 20% lower than in the InSurE group. However, the lung dose in the LMA groups is still within the range expected to elicit a physiological response [63,102]. The obligatory need for sedation and analgesia when performing laryngoscopy favors the use of the laryngeal mask airway. The LMA is definitively easier to place with less impact on comfort and hemodynamic stability. If the attending physician does not possess the necessary competence to perform laryngoscopy, the use of a standard LMA to administer surfactant as a bolus in the main lumen could be of benefit. This hypothesis will have to be tested in larger clinical trials.

Conclusions

Paper I:

In a model of sedated term newborn piglets spontaneously breathing on nasal CPAP, we showed the feasibility to attain a median lung deposition of 40% of the total administered dose of poractant alfa using a new device for supraglottic surfactant atomization.

The total amount of lipids, i.e., 80 mg/kg, deposited in the lungs is expected to elicit a physiological effect and was almost half of the amount achieved with endotracheal administration.

Paper II:

Regardless of the noninvasive ventilatory support mode used during nebulization, 16% to 22% of the nebulized surfactant was found in the newborn piglets' lungs. Our findings show that the eFlow-Neos investigational neonatal nebulizer system can deliver relatively large amounts of aerosolized poractant alfa to the lungs during noninvasive ventilation, an amount enough to elicit a pulmonary function improvement in the context of RDS of the newborn.

Paper III:

The lung deposition of poractant alfa obtained with the eFlow-nebulizer system ranged from 13% to 32% of the nominal dose.

The highest deposition was achieved with the animals in the prone position.

There was an influence of gravity in the lateral postures resulting in larger amounts of surfactant in the dependent lung.

In spontaneously breathing healthy piglets on nCPAP, the lung dose is affected by body posture during nebulization.

These findings need to be confirmed in a surfactant deficient model.

Paper IV:

We nebulized 600 mg/kg poractant alfa using the eFlow Investigational nebulizer system and obtained a mean lung dose of phospholipids of 138 ± 96 mg/kg. This amount of phospholipids is comparable to 200 mg/kg poractant alfa administered intratracheally.

These experimental findings suggest that it might be feasible to reach therapeutic lung doses of phospholipids by surfactant nebulization while on nCPAP.

Paper V:

The surfactant deposition obtained with the administration via an LMA was lower than that achieved with endotracheal instillation. Albeit not statistically significant, introducing the catheter below the vocal cords under visual control using an LMA with an integrated camera improved surfactant delivery by 65%.

Our findings show that an LMA can be a viable way to deliver surfactant to the lungs.

Populärvetenskaplig sammanfattning

Barnen som föds mer än en till två månader för tidigt har omogna lungor och kan lida brist på surfaktant. Surfaktant är ett kroppseget ämne, en blandning av protein och fett, som håller ytspänningen i lungan låg och motverkar att de små luftvägarna och lungblåsorna faller ihop vid utandning. Respiratory distress syndrome (RDS), kallas lungsjukdomen som surfaktant brist orsakar hos för tidigt födda barn. Nyfödda med RDS visar påverkad andning, nedsatt syresättning och behov av andningsstöd. Införandet av respiratorvård till för tidigt födda barn är redan i sig ett framsteg som lett till minskad dödlighet och minskad sjuklighet hos de sköra barnen. Surfactantbehandling etablerades på 1980-talet och den gängse rutinen sedan början på 1990-talet är att tillföra ämnet direkt ner i luftstrupen, en behandling som årligen räddar tusentals liv på för tidigt födda barn. Tillförsel av surfaktant sker via en plastkateter som förs ned i luftstrupen med hjälp av ett speciellt luftvägsinstrument, laryngoskop. Den här luftväggsmanipulationen är smärtsam och förenad med diverse komplikationer. Det är önskvärt att hitta mindre riskfyllda metoder att tillföra surfaktant på för att slippa laryngoskopi och behovet av intensiv mekanisk respiratorvård.

Min avhandling granskar olika tillvägagångssätt att med skonsammare metoder tillföra surfaktant till nyfödda under spontanandning. I vår försöksmodell med nyfödda grisar, har vi med hjälp av ett radioaktivt ämne (^{99m} Technetium) som blandas med surfaktant, uppskattat hur mycket av den tillförda mängden som återfinns i djuren och särskilt hur mycket som når lungorna genom att avbilda djuren med en gammakamera efter avslutad behandling.

I den första delen provades ett nytt sätt, ett sprutmunstycke som finfördelar surfaktant via en speciell kateter placerad framför luftstrupens öppning. Under behandlingen andades grisarna spontant med andningsstöd i form av näs-CPAP (kontinuerligt luftvägstryck, continuous positive airway pressure), en näsmask med en luftström som hjälper till att hålla luftvägen öppen. Vi mätte depositionen, det vill säga hur mycket surfaktant som kom till lungorna, sedan jämförde vi med den gängse referensmetoden som är att via plastkatetern spruta ned i luftstrupen. Resultatet är att cirka 40% av den givna dosen hamnade i lungorna, nästan hälften jämfört med den hos kontrollgruppen.

Med syfte att hindra lungkollaps är andningsstöd med näs-CPAP via en näsmask eller näskanyl, standardbehandling hos för tidigt födda barn om de inte behöver

omedelbar intubation och respiratorbehandling. Det är även möjligt att öka andningsstödet med hjälp av näs-IPPV, (intermittent positive pressure ventilation) som ger större andetag vid inandning. Metoden används alltmer på neonatalavdelning.

I det andra arbetet undersökte vi om det fanns någon skillnad i mängden surfaktant som uppmättes i lungorna vid inhalation av en fin aerosol-spray skapad av en speciell nebulisator under dessa två metoder av andningsstöd. Resultatet blev att båda metoderna ger relativt bra deposition, 16% i näs-CPAP gruppen och 22% i näs-IPPV gruppen.

I det tredje arbetet undersökte vi om kroppsläge under tiden grisen andades in surfaktant påverkade resultatet. Vi jämförde fyra olika kroppslägen, och fann bäst resultat när grisen låg på mage. Det är troligt att andningsmönstret förbättrades jämfört med om den låg på rygg, vänster respektive höger sida. Vi bekräftade ett fynd som vi noterat i tidigare studier, att tyngdkraften har stor betydelse för surfaktantdistribution i lungorna. Det kommer mer surfaktant till den lunga som ligger nederst. Men vi kan också visa att det finns en sidoskillnad där den större högra lungan får mer av surfaktant oavsett kroppsläge.

Den fjärde studien utgick från nutida rekommendationer vid RDS där barnen behandlas med 200 mg/kg kroppsvikt av surfaktant via kateter i luftstrupen. För att kunna uppnå motsvarande mängd med nebuliseringen antog vi att det skulle behövas 600 mg/kg kroppsvikt. Resultatet jämfördes mot kontrollgruppen som fick 200 mg/kg via kateter i luftstrupen. Vi fann det möjligt att nebulisera en så stor dos utan att upptaget till lungorna skilde sig signifikant mellan grupperna, 138 mg/kg via nebulisering respektive 172 mg/kg i den intuberade gruppen.

Avslutningsvis i den femte studien utvärderade vi en modifierad larynxmask (LMA) som är försedd med kamera och kateterkanal. Tanken med en kateterkanal är att det ska underlätta införandet av katetern nedanför stämbanden och att kameran ska medge insyn utan att behöva använda laryngoskopi. LMA kan användas vid återupplivning när ett barn föds om maskventilation inte är tillräckligt effektiv eller om det saknas kompetens att intubera barnet. LMA har i andras försök visat sig vara användbar vid surfaktant tillförsel. I jämförelse med surfaktantadministration via kateter i luftstrupen var LMA inte lika bra. Vi provade även en helt vanlig larynxmask, utan kamera eller kateterkanal, och fann då att tillgång till bild och kateterkanal förbättrar resultatet med 65%. Oavsett vilken LMA vi använder, är surfaktantdeposition tillräckligt bra för att förväntas ha en god effekt vid surfaktant brist när barn föds för tidigt.

I alla fem studierna har vi använt poractant alfa (Curosurf®), ett naturligt surfaktant med porcina lunglipider och proteiner tillverkat av Chiesi farmaceutici S.p.A., Parma, Italy. och i de tre nebuliserings studierna har vi använt en speciellt framtagen

nebulisator för surfaktant tillförsel (eFlow-Neos Investigational nebulizer system®, PARI Pharma GmbH,Germany)

Future directions and perspectives

We have shown that it is feasible to deliver surfactant via nebulization. However, even in the best scenario, the available nebulizers deliver at the most 20–30% of the nominal dose of aerosolized surfactant to the lungs under controlled experimental conditions. Surfactant is expensive, and the administration by inhalation needs further improvements.

Synchronized nebulization could be an attractive way to reduce surfactant loss during expiration. However, synchronization to the inspiratory phase is not easy during rapid and shallow breathing. An option could be to use a method able to capture the electrical signal from the diaphragmatic activation to trigger the nebulizer. One such possibility would be to try to adapt the signal obtained from the NAVA catheter (neurally adjusted ventilatory assist, Getinge AB) to trigger the nebulizer at inspiration. Such a technique might even help to decrease the need for sedation for infants on ventilatory support. A triggering signal could even be obtained via a pressure transducer in the esophagus. The development of these techniques could be tested in our animal model.

Another attractive way to deliver surfactant non-invasively and more efficiently could be to nebulize it through a laryngeal mask airway. The LMA-technique can reduce leakage during nebulization, and it might provide a safer and more comfortable airway even during prolonged nebulization.

When investigating the clinical effects of the delivered surfactant, it is essential to use a surfactant depleted model; we have performed a few successful pilot experiments showing the feasibility of keeping our animals in nCPAP after lung lavage and surfactant depletion. Such a model opens the possibility for studying both deposition and clinical efficiency at the same time. Besides, we are trying to find methods to increase the spatial resolution during the evaluation of the surfactant distribution in the different lung regions. In collaboration with image specialists, we are attempting to develop a suitable protocol to analyze single-photon emission tomography (SPECT) in small piglets. SPECT gives us a three-dimensional view of the deposition in the regions of interest. Another alternative is the use of electrical impedance tomography (EIT), a noninvasive and radiation-free imaging technique that we have tested in this piglet model. It allows monitoring the ventilation and gas distribution in the lungs, which could be used as a supplementary method to access the surfactant effects and distribution of ventilation during nebulization.

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