Supplementary material for:

**IMPACT OF BODY POSITION ON LUNG DEPOSITION OF NEBULIZED SURFACTANT IN NEWBORN PIGLETS ON NASAL CPAP**

***Preparation and anesthesia:***

Premedication with 0.4 mg midazolam, 3 mg ketamine, and 0.1 mg atropine was given im., and sedation was maintained with 1-3 µg·kg-1·h-1 dexmedetomidine and 1-3 mg·kg-1·h-1 ketamine. Sedation and analgesia were fine-tuned with iv. injections of propofol (0.5-1 mg·kg-1), dexmedetomidine (0.1-0.2 µg·kg-1) or remifentanil (0.5-1 µg·kg-1) as needed. Besides the parameters reported in the results section, transcutaneous carbon dioxide (TcCO2) and pulse oximetry (SaO2) were continuously monitored to check the adequacy of ventilation. Before prongs insertion, the piglets received 0.0125 mg of the nasal decongestant oxymetazoline chloride in each nostril, and adrenalin (0.5 mg) and lidocaine (2 mg) inhalation were given via a face mask and a jet nebulizer.

***Nasal prongs, nebulizer and the CPAP system***

The interface consisted of customized nasal prongs made with the 3.5 cm distal tips from two uncuffed tracheal tubes ID 3.0 mm (Rusch, Teleflex Medical GmbH, Kernen, Germany). These tube pieces were attached to the straight plastic connector from a demounted nasal cannula (Large Argyle™ CPAP Nasal Cannula, Covidien AB, Solna, Sweden). We inserted the modified prongs into the piglets’ nostrils with the bevels facing inwards. The prongs were then attached to a subject-bound eFlow-Neos investigational nebulizer system (PARI Pharma GmbH, Gräfelfing, Germany) placed in the Y-piece of a dual-limb infant Evaqua™ breathing circuit (Fisher&Paykel Healthcare, Auckland, New Zeeland). The nebulizer added approximately 8 mL to the circuit’s dead-space. An FP850 humidifier (Fisher&Paykel Healthcare, Auckland, New Zealand) was interposed in the breathing circuit. The CPAP source was a Servo-i ventilator (Maquet, Solna, Sweden). Respiratory rate, tidal volume, air leak in the respiratory circuit, and FiO2 were continuously displayed in the ventilator user interface. A Servo Duo Guard filter (Maquet Critical Care AB, Solna, Sweden) placed before the ventilator’s expiratory inlet, absorbed moisture and the exhaled radioactive mixture. After leaving the ventilator, expired gases went through an activated charcoal filter system before being evacuated by the laboratory’s gas scavenger system.

***Measurement of surfactant deposition***

The exact amount of radioactive tracer mixed with the surfactant was measured in a gamma counter chamber shortly before administration. After nebulization, we placed the piglets in a gamma camera (Philips Skylight, Philips AB, Stockholm, Sweden) in the prone position. An anterior and a posterior image of the head and chest were made before 25 MBq of a second radioactive substance, 99mTc-macro aggregated albumin (Tc-MAA, TechneScan LyoMAA, Covidien Sverige AB, Solna, Sweden) was given iv., and another two images were acquired. Since Tc-MAA is trapped in the lung capillaries, it helps delineate the lungs and allows for internal calibration. Finally, to define the limits between the upper airway and the trachea and better identify the regions of interest, the larynx was palpated, and a radioactive marker (60Co) coin was secured at this level when a third picture was taken. The percentage of the administered dose deposited in the lungs was calculated as: Droi = 100 × Croi/(Cwif(2) – Cwif(1) × (TcMAA dose)/(Tc-nanocolloid dose) [1]. Droi is the deposition in a region of interest, Croi the measured counts in the corresponding region before Tc-MAA is given. Cwif (x) is the recorded counts in the whole image field before (x=1) and after (x=2) Tc-MAA administration. Before the final calculations, all counts, including device residual radioactivity, were corrected for radioactivity decay according to the half-life of 99mTc. Residuals included the prongs and the nebulizer but excluded the respiratory circuit and filters.

1. Linner R, Perez-de-Sa V, Cunha-Goncalves D. Lung deposition of nebulized surfactant in newborn piglets. Neonatology. 2015;107(4):277-82.