Distending Pressure Did Not Activate Acute Phase or Inflammatory Responses in the Airways and Lungs of Fetal, Preterm Lambs

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Abstract

Background
Mechanical ventilation at birth causes airway injury and lung inflammation in preterm sheep. Continuous positive airway pressure (CPAP) is being increasingly used clinically to transition preterm infants at birth.

Objective
To test if distending pressures will activate acute phase reactants and inflammatory changes in the airways of fetal, preterm lambs.

Methods
The head and chest of fetal lambs at 128±1 day GA were surgically exteriorized. With placental circulation intact, fetal lambs were then randomized to one of five 15 minute interventions: PEEP of 0, 4, 8, 12, or 16 cmH2O. Recruitment volumes were recorded. Fetal lambs remained on placental support for 30 min after the intervention. The twins of each 0 cmH2O animal served as controls. Fetal lung fluid (FLF), bronchoalveolar lavage fluid (BAL), right mainstem bronchi and peripheral lung tissue were evaluated for inflammation.

Results
Recruitment volume increased from 0.4±0.04 mL/kg at 4 cmH2O to 2.4±0.3 mL/kg at 16 cmH2O. The lambs were surfactant deficient, and all pressures were below the opening inflection pressure on pressure-volume curve. mRNA expression of early response genes and pro-inflammatory cytokines did not increase in airway tissue or lung tissue at any pressure compared to controls. FLF and BAL also did not have increases in early response proteins. No histologic changes or Egr-1 activation was present at the pressures used.
Conclusion
Distending pressures as high as 16 cmH$_2$O did not recruit lung volume at birth and did not increase markers of injury in the lung or airways in non-breathing preterm fetal sheep.

Introduction
The transition at birth from fluid filled airspaces to aeration of the lungs is fundamental to mammalian life [1]. This transition requires a coordinated clearance of fetal lung fluid, surfactant secretion and development of consistent breathing patterns to be successful [2]. The initial clearance of lung fluid from the airways requires the generation of large initial negative pressure breaths [1, 3]. Surfactant deficient preterm animals and humans may lack the muscle strength to generate sufficient negative pressures to overcome their compliant chest wall and high airway surface tension [2]. Consequently, many preterm infants require assistance with the transition from fetal life [4, 5]. The preterm lung can be easily injured by the assistance required, and the resultant lung inflammation may contribute to the progression towards bronchopulmonary dysplasia (BPD) [6, 7]. Mechanical can injure the preterm airways and contributes to the airway hyperactivity seen in infants with a history of BPD [8–10]. Despite the increasing survival of preterm infants, there have been no major changes in survival without morbidity over many years [4, 11]. New strategies to aid infants in the birth transition are needed.

Airway epithelial injury can begin with the initiation of ventilation at birth and is likely due to the rapid movement of lung fluid-air interface in the airways. The injury cascade takes only a few breaths to begin, as even a very brief large tidal volume ventilation can initiate an injury that is then perpetuated by continued ventilator support [12, 13]. The unequal distribution of tidal volumes within the lung and the repetitive opening and closing of airspaces also contribute to the lung injury from mechanical ventilation of the lungs of the preterm [10, 14, 15]. Stretching the preterm airways also leads to release of acute phase proteins (HSP70) that can activate inflammation [8, 16]. Efforts to recruit functional residual capacity and decrease fluid in airways with a prolonged sustained inspiration (SI) are being evaluated in preterm animals and infants. Unfortunately SI did not protect the lungs of preterm sheep from injury from mechanical ventilation; in fact SI alone caused a modest injury in surfactant deficient lambs [17–20]. The use of positive end-expiratory pressure (PEEP) to maintain alveolar patency is beneficial in the neonatal intensive care unit [21]. In preterm lambs, ventilation without PEEP causes airway injury and lung inflammation that can be in part mitigated with the addition of PEEP [22]. Recruitment of the lung with gradually increasing PEEP during ventilation also decreased lung injury in preterm sheep [23, 24]. In preterm, surfactant-treated lambs, a PEEP of 4 cmH$_2$O was protective against lung injury compared to 0 cmH$_2$O, but lungs of animals on PEEP 7 cmH$_2$O had neutrophil and protein injury responses similar to no PEEP [17, 25]. Ventilatory support via an endotracheal tube (invasive support) is injurious to the lungs of surfactant deficient preterm animals, but it is unclear if non-invasive support also follows this trend.

Several studies support the recommendation to use non-invasive strategies following birth to assist in the transition from fetal life [26, 27]. Even with the use of early CPAP in the delivery room, the decrease in the incidence of BPD is modest at about 10% [28, 29]. In clinical care non-invasive strategies are being used with more frequency to transition infants at birth and higher CPAP pressures are used to try to avoid intubation. Therefore, it is important to evaluate if higher airway pressures (PEEP) can injure the preterm airways or lung. In one report,
preterm lambs given PEEP alone had a modest increase in inflammatory markers, but the observation was not systematically explored [17]. The lack of a protective effect of PEEP 7 cmH2O compared to 4 cmH2O in ventilated newborn lambs also raises some concern [25]. Using a preterm fetal lamb model, which avoids ventilation and oxygen exposure, we tested the hypothesis that higher PEEP pressures cause airway injury and lung inflammation in non-breathing sheep.

**Methods**

**Fetal preparation for PEEP intervention**

This study was approved by the Animal Ethics Committees of the University of Western Australia, Saint Louis University, and Cincinnati Children’s Hospital Medical Center. Date mated Merino Ewes at 128 ±1 days gestational age (GA; term is about 150 days GA) were premedicated with 0.5 mg/kg of IM xylazine, 5 mg/kg of IV ketamine, and 0.25 mg/kg IV midazolam. The ewes were intubated and maintained during the procedure with anesthesia with isofluorane (0.5–2% in 100% O2). The isofluorane crosses the placenta, anesthetizes the fetus, and eliminates fetal breathing [17]. The fetal head and chest were exteriorized through a midline hysterotomy, with maintenance of placental blood flow [12]. The fetal lamb then received a tracheostomy to secure a 4 F endotracheal tube. Free-flowing fetal lung fluid (FLF) was gently removed with a 7 F catheter (average 13 ml/kg fetal weight) and an aliquot was snap frozen. The endotracheal tube was attached to a Neopuff (Fisher & Paykel, New Zealand) with a PEEP valve to adjust pressure. In line with the endotracheal tube, we placed a 1.3 mL pneumotach (RSS HR100 system, Hans Rudolph, Kansas City, MO) to continuously record the volume recruitment over time. Flow sensor was calibrated daily with manufacturer’s syringe and previously correlated well with respiratory inductance plethysmography [17].

**Fetal Interventions**

The fetal lambs were randomly assigned to one of five groups for a 15 minute intervention: 1) a PEEP of 0 cmH2O; 2) a PEEP of 4 cmH2O; 3) a PEEP of 8 cmH2O; 4) a PEEP of 12 cmH2O; or 5) a PEEP of 16 cmH2O with heated, humidified 100% N2 to avoid oxygen exposure. After the 15 minute intervention, the endotracheal tube was occluded and the fetus was returned to the uterus and maintained on placental circulation for an additional 30 minutes. The fetus was then euthanized with 100 mg/kg of pentobarbital, and fetal lung fluid (FLF) was collected and snap frozen. Cord blood gasses were measured using a Siemens Rapidlab 1265 (Siemens, Australia). The 45 minute experimental period was chosen to optimize the detection of mRNA for acute phase inflammatory response genes [30]. The twin lambs of the 0 cmH2O animals received maternal anesthesia but no intervention and were used as additional controls.

**Lung volumetrics, processing and BAL analysis**

Inflation and deflation pressure-volume curves were measured with a stepwise change in pressure to a maximum of 40 cmH2O [31]. Bronchioalveolar lavage fluid (BALF) of the left lung was collected by repetitive saline lavage. FLF and BALF were used for measurement of total protein [32] and sandwich ELISA assays for Heat shock protein (HSP) 70 and HSP60 in the airway fluids (R&D Systems, USA) [22]. Tissues from the peripheral regions of the right lower lobe near the pleural edge and the right mainstem bronchus at approximately 3rd branch point were snap frozen for RNA and protein isolation [33].
Histologic evaluation

Immunohistochemistry protocols used 5 μm paraffin sections of formalin inflation fixed lung tissues that were pre-treated with 3% hydrogen peroxide to inactivate endogenous peroxidases [34, 35]. The sections were incubated overnight with anti-human Egr-1 with 1:250 dilution (Santa Cruz, USA), in 4% normal goat serum, followed by biotin labeled secondary antibody. Immunostaining was visualized by Vectastain ABC Peroxidase Elite kit (Vector Laboratories Inc, USA). The antigen detection was enhanced with nickel-DAB, followed by TRIS-cobalt and the nuclei counterstained with nuclear fast red [34]. Hematoxylin and Eosin stained tissues were blinded and evaluated for airway injury and inflammation, as previously described [17].

Quantitative RT-PCR

Messenger RNA was extracted from lung tissue from the right mainstem bronchus and peripheral lung tissue with TRIzol (Invitrogen, Grand Island, NY). cDNA was generated from 1 mg mRNA using Verso cDNA kit (Thermoscientific, USA). We used custom Taqman gene primers (Life technologies, USA) for ovine sequences for Interleukin 1β (IL-1β), IL-6, MCP1, early growth response protein 1 (Egr-1), and connective tissue growth factor (CTGF) [22, 36]. Quantitative Real Time PCR was performed using iTaq Universal mix (BioRad, USA) in a 15 μl reaction on a CFX Connect machine and software (BioRad, USA). 18S primers (Life Technologies, USA) were used for the internal loading control. Results are reported as fold increase over mean for all of the intervention groups.

Western blot analysis

Protein concentrations from lung tissue were determined using Bio-Rad Protein Assays [37]. 40 μg of protein was denatured in BME at 95 degrees for 5 minutes, then run on Tris-glycine 10% gel and transferred to 0.45 μm nitrocellulose membrane (Bio-rad, USA). Membranes were blocked in 5% normal milk fat and then incubated with Egr-1 1:500 (Santa Cruz, USA) or β−Actin 1:2000 (Thermoscientific, USA) overnight at 4 degrees. Appropriate IgG-HRP secondary antibodies were applied at 1:10000 dilutions. Membranes were developed with ECL (Pierce/ThermoFisher) and then imaged on Syngene PXi multi-gel imaging system (Syngene, USA) and quantified with ImageJ 1.48V (N.I.H., USA). Egr-1 and β−Actin were analyzed on the same gels without membrane stripping.

Data analysis and statistics

Results are shown as mean (StDev) and reported as fold increase over the mean. Statistics were analyzed using Prism 6 (GraphPad, USA) using Student’s t-test, Mann-Whitney non-parametric, or ANOVA tests as appropriate. Significance was accepted as p<0.05.

Results

All fetal lambs survived the intervention and 30 minute recovery period on placental support. The gestational age (128±0.3 days), birth weight, gender and cord gases were similar between groups, with the exception of the 16 cmH2O group which was slightly heavier (Table 1). There were no differences in lung weights per kg (Table 1), suggesting no increase in lung edema or clearance with pressure exposure. In anesthetized fetal sheep without spontaneous breathing, the PEEP pressures recruited very small lung volumes over 15 min (less than 3 ml/kg), even at a pressure of 16 cmH2O (Fig 1A). All of the lambs were very surfactant deficient (Table 1). The post mortem lung gas volumes at 40 cmH2O were not different between the PEEP groups. The inflection point for the post-mortem lungs to inflate was over 30 cmH2O (Fig 1B). The
correlation between pressures and volumes recruited with 15 minutes of PEEP and the inflation arm of the pressure-volume curve with an open chest were high ($R^2 = .96$) (Fig 1C), demonstrating that chest wall constriction during PEEP did not limit lung expansion. There were no differences between surgical controls and 0 cmH2O animals, and values for markers of injury are reported as fold increase over the combined control groups.

Fetal lung fluid and bronchioalveolar lavage fluid

Increasing distending pressures did not increase total protein secretion in the fetal lung fluid collected at the end of the recovery period or the BALF compared to control animals (Table 1). The BALF also did not have increased surfactant levels following intervention. There were no differences between groups for release of acute phase proteins (HSP 60, HSP 70) in the FLF after the intervention or BALF (data not shown).

Cytokine and acute phase responses in peripheral lung and right mainstem bronchus

The intervention groups did not have significant differences in mRNA for IL-1β, IL-6 or MCP1 in the peripheral lung tissue or the right mainstem bronchus relative to controls (Table 2). There were also no significant differences in the acute phase response genes, Egr-1 or CTFG in either of these tissues. There were no differences on blinded evaluation of

Table 1. Animal characteristics, protein secretion, and surfactant levels.

| Group    | n  | Birth Weight (kg) | M:F | Cord pH | FLF Protein (mg/ml) | Surfactant (μmol/kg) | Left Lung Weight (g/kg) |
|----------|----|-------------------|-----|---------|---------------------|----------------------|-------------------------|
| Controls | 7  | 3.1 ± 0.5         | 2:5 | 7.29 ± 0.09 | 0.15 ± 0.03          | 1.09 ± 1.3               | 11.9 ± 1.1               |
| 0 cmH2O  | 6  | 3.1 ± 0.4         | 3:3 | 7.30 ± 0.03 | 0.25 ± 0.10          | 0.91 ± 0.94              | 11.8 ± 0.4               |
| 4 cmH2O  | 9  | 3.1 ± 0.4         | 5:4 | 7.29 ± 0.07 | 0.17 ± 0.08          | 1.00 ± 0.69              | 13.1 ± 0.5               |
| 8 cmH2O  | 8  | 3.2 ± 0.4         | 3:5 | 7.29 ± 0.03 | 0.16 ± 0.08          | 0.75 ± 0.67              | 12.2 ± 0.6               |
| 12 cmH2O | 8  | 3.2 ± 0.3         | 5:3 | 7.28 ± 0.10 | 0.22 ± 0.09          | 1.34 ± 1.30              | 13.6 ± 0.6               |
| 16 cmH2O | 8  | 3.8 ± 0.5*        | 4:4 | 7.27 ± 0.04 | 0.18 ± 0.07          | 0.26 ± 0.13              | 12.6 ± 0.5               |

Means±SD
*p<0.05 vs controls

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Fig 1. Lung Volume Recruitment and post-mortem pressure volume cures. (A) Average lung volume recruited at the end of intervention period plotted against PEEP pressure demonstrates a slight increase in ml/kg with increased pressures. (B) Post-mortem mean pressure-volume curves for study animals with chests open. (C) Pressure-volume relationship during recruitment maneuver (half circles) and from post-mortem pressure-volume curve (squares) show strong correlation demonstrating little effect of the closed chest wall and all PEEP pressures below the pressure at which the unrestricted lung expands.

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Hematoxylin and Eosin staining of lung sections. Relative to controls, no increased expression of Egr-1 was apparent on immunohistochemistry staining of airways (data not shown). Furthermore, Western Blot did not show increased expression of Egr-1 protein (data not shown).

**Discussion**

We found no effect of PEEP pressures from 0 to 16 cm H2O on markers of lung injury with a preterm fetal model of surfactant deficient lambs. These measurements should be sensitive indicators because the controls have no baseline inflammation and a maneuver such as a prolonged sustained inflation can elicit easily detected inflammation in this model [17]. Although contrary to our hypothesis, these results should be reassuring to clinicians choosing to use higher levels of CPAP to avoid intubation of preterm infants. In these poorly compliant lamb lungs, due to moderate prematurity and no antenatal steroid exposure, fifteen minutes of distending pressures up to 16 cm H2O did not recruit gas effectively to the lungs. There was a dose response curve noted as more pressure inflated more of the lung tissue, but at the highest pressure used, 16 cm H2O, only ~3 ml/kg of lung gas volume was achieved. As measured by the lung volume curves at necropsy (Fig 1B and 1C), this pressure did not effectively open the lungs. Although the animals in the 16 cmH2O group were slightly heavier, the lung to body weight ratio was similar and pressure should have been dispersed throughout lung thus not affecting markers of injury. The air that entered the lungs likely did not go beyond the larger airways, as we removed 13 ml/kg of fetal fluid prior to the application of PEEP. We thought that this airway inflation has the potential to initiate an inflammatory cascade because ventilation injury to the preterm lung is initiated in the airways [8]. Furthermore, HSP70, a marker of airway injury, was secreted from newborn lamb trachea when exposed to mechanical ventilation [16]. Also mechanical ventilation changes the tissue matrix with disruption of epithelial integrity in the lamb trachea [38]. It would follow that stretching of the lamb airways in this model could have initiated inflammation and airways injury. Contrary to this hypothesis, our results demonstrate no differences between airways containing 3 ml/kg of volume and 0 ml/kg of volume.

The lack of injury response in these lambs can be further put in context of the effects of mechanical ventilation or sustained inflation alone. Previously a 20 second SI at 50 cm H2O recruited a lung volume around 15 ml/kg in surfactant deficient fetal lambs, and this single large inflation alone initiated the process of lung inflammation and injury [17]. Other groups have found a SI to 7 ml/kg increased Egr-1 mRNA [39]. Fetal lambs treated with surfactant and smaller recruitment volumes (7 ml/kg) did not demonstrate the negative effects of SI [18, 39]. In previous studies of mechanical ventilation in the preterm lamb model, the normal lamb tidal volume of 7 ml/kg generated lung inflammation and Egr-1 activation surrounding the small airways and terminal bronchioles [36]. Mechanical ventilation with moderate tidal volumes also causes the release of heat shock proteins into the airway, which can worsen the

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**Table 2. Pro-inflammatory cytokine and acute phase gene mRNA expression.**

| Group  | Right Mainstem Bronchus | Peripheral Lung Tissue |
|--------|-------------------------|------------------------|
|        | IL-1β | IL-6 | Egr-1 | CTGF | IL-1β | IL-6 | Egr-1 | CTGF |
| Controls | 1.0±0.2 | 1.0±0.2 | 1.0±0.3 | 1.0±0.2 | 1.0±0.2 | 1.0±0.2 |
| 4 cmH2O | 0.8±0.1 | 0.9±0.1 | 0.8±0.1 | 0.7±0.1 | 1.2±0.2 | 1.7±0.4 |
| 8 cmH2O | 1.1±0.2 | 1.1±0.2 | 1.1±0.2 | 1.0±0.2 | 1.3±0.4 | 1.7±0.4 |
| 12 cmH2O | 0.9±0.3 | 1.0±0.3 | 0.9±0.2 | 1.1±0.4 | 1.0±0.2 | 1.3±0.3 |
| 16 cmH2O | 1.4±0.4 | 1.4±0.3 | 1.1±0.4 | 1.9±1.0 | 1.0±0.2 | 1.8±0.4 |

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inflammatory response through activation of toll-like receptors [36]. The higher levels of pro-inflammatory cytokines and Egr-1 activation seen in previous animals on PEEP 8 cmH2O, a PEEP pressure used in clinical studies, was not substantiated in this larger study [17, 40]. The largest difference between these studies was the amount of fetal lung fluid removed prior to intervention, with 13 ml/kg removed in current study compared to 8 ml/kg in study demonstrating injury effect [17]. The additional lung fluid in previous lambs, as evident by a higher left lung weight of 15 g/kg, may have increased the injury seen due to sheering effects on epithelium [8, 17]. Similar fetal models have removed 15 ml/kg of fetal fluid and used 8 cmH2O without an effect on inflammatory markers [23, 24]. The largest lung volumes recruited in this study did not even reach half of the moderate tidal volume previously shown to be injurious or the functional residual capacity recruited by a brief sustained inflation [17, 18, 36]. Although not tested, higher PEEP values above the lower inflection point of the lung expansion, 30 cmH2O, would likely have opened the lung and caused similar effects to a single sustained inflation. These sheep were not treated with antenatal steroids, thus there may have been different effects in a more compliant lung without as much lung fluid [41, 42]. Since the fetal animals are not breathing, spontaneously breathing in humans with higher PEEP could still cause injury.

Our results support the concept that lung injury is more likely caused by increased volume rather than barotrauma from high pressures. Several studies have addressed this fundamental question. In a rabbit model of high pressure lung injury, rabbits had chest casts placed to limit volume expansion of the chest and were exposed to high ventilator inspiratory pressures [43]. The casted animals, when compared to animals who did not have restriction to lung volume recruitment, did not have significant increases in signs of microvascular damage [43]. Similar studies in young lambs with their chest and abdomen bound to limit chest wall excursion during mechanical ventilation had decreased protein secretion and altered lymphatic changes compared to lambs with unrestricted tidal volumes [44]. Rats with abdominal and chest banding had similar lung protection when lung volume was limited [45]. These studies support the argument that increased volume causes the injury and inflammatory cascade in developing lungs, and not the effect of an absolute pressure. The distending pressures used in our current study did not result in significant lung expansion and thus the injury and the inflammatory cascade was not initiated.

We conclude that increasing distending pressure alone, when it does not result in significant lung expansion, does not trigger the injury response in the airways and lung. Further evaluation of the effects of CPAP or distending pressures in a breathing animal model would further strengthen these conclusions.

Supporting Information
S1 Dataset. 2014 Resuscitation data.xlsx contains primary data from study.
(XLSX)

Author Contributions
Conceived and designed the experiments: NHH AHJ MWK. Analyzed the data: NHH RP ER AHJ. Wrote the paper: RP NHH AHJ MWK. Animal procedures: NHH MWK YM AN.

References
1. Hooper SB, Kitchen MJ, Wallace MJ, Yagi N, Uesugi K, Morgan MJ, et al. Imaging lung aeration and lung liquid clearance at birth. Faseb J. 2007; 21(12):3329–37. PMID: 17536049.
2. Hillman NH, Kallapur SG, Jobe AH. Physiology of transition from intrauterine to extrauterine life. Clin Perinatol. 2012; 39(4):769–83. doi: 10.1016/j.clp.2012.09.009 PMID: 23164177; PubMed Central PMCID: PMC3504352.

3. Vyss H, Milner AD, Hopkins IE. Intrathoracic pressure and volume changes during the spontaneous onset of respiration in babies born by cesarean section and by vaginal delivery. J Pediatr. 1981; 99(5):787–91. Epub 1981/1/1/01. PMID: 7299559.

4. Fanaroff AA, Stoll BJ, Wright LL, Carlo WA, Ehrenkranz RA, Stark AR, et al. Trends in neonatal morbidity and mortality for very low birthweight infants. Am J Obstet Gynecol. 2007; 196(2):147 e1–8. doi: 10.1016/j.ajog.2006.09.014 PMID: 17306659.

5. Kattwinkel J, Perlman JM, Aziz K, Colby C, Fairchild K, Gallagher J, et al. Neonatal resuscitation: 2010 American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. Pediatrics. 2010; 126(5):e140–13. doi: 10.1542/peds.2010-2972E PMID: 20956432.

6. Mosca F, Colnaghi M. Respiratory management of the premature infant in the delivery room. J Matern Neonatal Med. 2004; 16 Suppl 2:17–9. doi: 10.1080/14767050410001727125 PMID: 15590428.

7. Lista G, Fontana P, Castoldi F, Cavigioli F, Dani C. Does sustained lung inflation at birth improve outcome of preterm infants at risk for respiratory distress syndrome? Neonatology. 2011; 99(1):45–50. doi: 10.1159/000298312 PMID: 20616570.

8. Hillman NH, Kallapur SG, Pillow JJ, Moss TJ, Polglase GR, Nitsos I, et al. Airway injury from initiating ventilation in preterm sheep. Pediatr Res. 2010; 67(1):60–5. Epub 2009/10/10. doi: 10.1203/PDR.0b013e3181c1b09e PMID: 19162339; PubMed Central PMCID: PMC2795027.

9. Fawke J, Lum S, Kirkby J, Hennessy E, Marlow N, Rowell V, et al. Lung function and respiratory symptoms at 11 years in children born extremely preterm: the EPICare study. Am J Respir Crit Care Med. 2010; 182(2):237–45. doi: 10.1164/rccm.200912-1806OC PMID: 20378229; PubMed Central PMCID: PMC2913237.

10. MacLean JE, DeHaan K, Fuhr D, Haritharan S, Kamstra B, Hendon L, et al. Altered breathing mechanics and ventilatory response during exercise in children born extremely preterm. Thorax. 2016. doi: 10.1136/thoraxjnl-2015-207736 PMID: 27259338.

11. Hillman NH, Kallapur SG, Pillow JJ, Moss TJ, Polglase GR, Nitsos I, et al. Airway injury from initiating ventilation in preterm sheep. Pediatr Res. 2010; 67(1):60–5. Epub 2009/10/10. doi: 10.1203/PDR.0b013e3181c1b09e PMID: 19162339; PubMed Central PMCID: PMC2795027.

12. Hillman NH, Moss TJ, Kallapur SG, Bachurski C, Pillow JJ, Polglase GR, et al. Brief, large tidal volume ventilation initiates lung injury and a systemic response in fetal sheep. Am J Respir Crit Care Med. 2007; 176(6):575–81. doi: 10.1164/rccm.200701-051OC PMID: 17604225; PubMed Central PMCID: PMC1994225.

13. Bjorklund LL, Ingimarsson J, Curstedt T, John J, Robertson B, Werner O, et al. Manual ventilation with a few large breaths at birth compromises the therapeutic effect of subsequent surfactant replacement in immature lambs. Pediatr Res. 1997; 42:348–55. PMID: 9284278.

14. Jobe AH, Hillman N, Polglase G, Kramer BW, Kallapur S, Pillow J. Injury and inflammation from resuscitation of the preterm infant. Neonatology. 2008; 94(3):190–6. PMID: 18832854 doi: 10.1159/000143721.

15. Tingay DG, Rajapaksa A, Zonneveld CE, Black D, Perkins EJ, Adler A, et al. Spatio-temporal Aeration and Lung Injury Patterns are Influenced by the First Inflation Strategy at Birth. Am J Respir Cell Mol Biol. 2015. doi: 10.1165/rcmb.2015-0127OC PMID: 26186685.

16. Chong E, Dysart KC, Chidekel A, Locke R, Shaffer TH, Miller TL. Heat shock protein 70 secretion by neonatal tracheal tissue during mechanical ventilation: association with indices of tissue function and modeling. Pediatr Res. 2009; 65(4):387–91. Epub 2009/01/08. doi: 10.1203/PDR.0b013e31819913f5 PMID: 19127221; PubMed Central PMCID: PMC2676716.

17. Hillman NH, Kemp MW, Noble PB, Kallapur SG, Jobe AH. Sustained inflation at birth did not protect preterm fetal sheep from lung injury. Am J Physiol Lung Cell Mol Physiol. 2013; 305(6):L446–53. doi: 10.1152/ajplung.00162.2013 PMID: 23873843; PubMed Central PMCID: PMC3763040.

18. Hillman NH, Kemp MW, Miura Y, Kallapur SG, Jobe AH. Sustained inflation at birth did not alter lung injury from mechanical ventilation in surfactant-treated fetal lambs. PLoS One. 2014; 9(1):e13473. doi: 10.1371/journal.pone.0113473 PMID: 25419969; PubMed Central PMCID: PMC4242618.

19. Polglase GR, Tingay DG, Bhatia R, Berry CA, Kopotic RJ, Kopotic CP, et al. Pressure- versus volume-limited sustained inflations at resuscitation of premature newborn lambs. BMC Pediatr. 2014; 14:43. doi: 10.1186/1471-2431-14-43 PMID: 24529326; PubMed Central PMCID: PMC43937019.

20. Tingay DG, Lavizzari A, Zonneveld CE, Rajapaksa A, Zannin E, Perkins E, et al. An individualized approach to sustained inflation duration at birth improves outcomes in newborn preterm lambs. Am J Physiol Lung Cell Mol Physiol. 2015; 309(10):L1138–49. doi: 10.1152/ajplung.00277.2015 PMID: 26408555.
21. Monkman S, Kirpalani H. PEEP—a "cheap" and effective lung protection. Paediatr Respir Rev. 2003; 4 (1):15–20. Epub 2003/03/05. PMID: 12615028.

22. Hillman NH, Nitoso I, Berry C, Pillow JJ, Kallapur SG, Jobe AH. Positive end-expiratory pressure and surfactant decrease lung injury during initiation of ventilation in fetal sheep. Am J Physiol Lung Cell Mol Physiol. 2011; 301(5):L712–20. doi: 10.1152/ajplung.00157.2011 PMID: 21856815; PubMed Central PMCID: PMC3290453.

23. Tingay DG, Polglase GR, Bhatia R, Berry CA, Kopotic RJ, Kopotic CP, et al. Pressure-limited sustained inflation vs. gradual tidal inflations for resuscitation in preterm lambs. Journal of applied physiology. 2015; 118(7):890–7. doi: 10.1152/japplphysiol.00985.2014 PMID: 25635005; PubMed Central PMCID: PMC459928.

24. Tingay DG, Rajapaksa A, Zonneveld CE, Black D, Perkins EJ, Adler A, et al. Spatiotemporal Aeration Patterns Are Influenced by the First Inflation Strategy at Birth. Am J Respir Crit Care Med. 2001; 164:494–8. PMID: 11500356

25. Kramer BW, Jobe AH, Newnham JP, Willet KE, Moss TJ, Kallapur SG, et al. Dose and time response meta-analysis of ventilation in preterm lambs. PLoS One. 2012; 7(6):e39535. Epub 2012/07/05. doi:10.1371/journal.pone.0039535

26. Lista G, Fontana P, Castoldi F, Cavigioli F, Bianchi S, Bastrenta P. ELBW infants: to intubate or not to intubate in the delivery room? J Matern Fetal Neonatal Med. 2012; 25 Suppl 4:63–5. doi: 10.3109/14776058.2012.715008 PMID: 22958020.

27. Network SSGotEKSNNR, Finer NN, Carlo WA, Walsh MC, Gantz MG, et al. Early CPAP versus surfactant in extremely preterm infants. N Engl J Med. 2010; 362(21):1970–9. doi: 10.1056/NEJMoa0911783 PMID: 20472939; PubMed Central PMCID: PMCPMC301534.

28. Schmolzer GM, Kumar M, Pichler G, Aziz K, O'Reilly M, Cheung PY. Non-invasive versus invasive respiratory support in preterm infants at birth: systematic review and meta-analysis. BMJ; 2013; 347; f5980. doi: 10.1136/bmj.f5980 PMID: 24144716.

29. Fischer HS, Buhrer C. Avoiding endotracheal ventilation to prevent bronchopulmonary dysplasia: a meta-analysis. Pediatrics. 2013; 132(5):e1351–60. doi:10.1542/peds.2013-1880 PMID: 24144716.

30. Polglase GR, Miller SL, Barton SK, Baburamani AA, Wong FY, Aridas JD, et al. Initiation of resuscitation with high tidal volumes causes cerebral hemodynamic disturbance, brain inflammation and injury in preterm lambs. PLoS One. 2012; 7(6):e39535. Epub 2012/07/05. doi:10.1371/journal.pone.0039535

31. Jobe AH, Newnham JP, Willet KE, Moss TJ, Ervin MG, Padbury JF, et al. Endotoxin induced lung maturation in preterm lambs is not mediated by cortisol. Am J Respirt Crit Care Med. 2000; 162:1656–61.

32. Hillman NH, Moss TJ, Nitoso I, Newnham JP, Jobe AH. Moderate tidal volumes and oxygen exposure during initiation of ventilation in preterm fetal sheep. Pediatr Res. 2012; 72(6):593–8. doi:10.1038/pr.2012.135 PMID: 23037872; PubMed Central PMCID: PMC4073615.

33. Cheah FC, Pillow JJ, Kramer BW, Polglase GR, Nitoso I, Newnham JP, et al. Airway inflammatory cell responses to intra-amniotic lipopolysaccharide in a sheep model of chorioamnionitis. Am J Physiol Lung Cell Mol Physiol. 2009; 296(3):L384–93. PMID: 19118089 doi: 10.1152/ajplung.90547.2008

34. Cullen AB, Cooke PH, Driska SP, Wolfson MR, Shaffer TH. The impact of mechanical ventilation on immature airway smooth muscle: functional, structural, histological, and molecular correlates. Biol Neonate. 2006; 90(1):17–27. PMID: 16534184.

35. Tingay DG, Wallace MJ, Bhatia R, Schmolzer GM, Zahra VA, Dolan MJ, et al. Surfactant before the first inflation at birth improves spatial distribution of ventilation and reduces lung injury in preterm lambs. Journal of applied physiology. 2014; 116(3):251–8. doi: 10.1152/japplphysiol.01142.2013 PMID: 24356523.
40. Morley CJ, Davis PG, Doyle LW, Brion LP, Hascoet JM, Carlin JB. Nasal CPAP or intubation at birth for very preterm infants. N Engl J Med. 2008; 358(7):700–8. PMID: 18272893 doi: 10.1056/NEJMoa072788

41. Tingay DG, Rajapaksa A, McCall K, Zonneveld CE, Black D, Perkins E, et al. The interrelationship of recruitment maneuver at birth, antenatal steroids, and exogenous surfactant on compliance and oxygenation in preterm lambs. Pediatr Res. 2016; 79(6):916–21. doi: 10.1038/pr.2016.25 PMID: 26866905.

42. Hillman NH, Pillow JJ, Ball MK, Polglase GR, Kallapur SG, Jobe AH. Antenatal and postnatal corticosteroid and resuscitation induced lung injury in preterm sheep. Respir Res. 2009; 10:124. doi: 10.1186/1465-9921-10-124 PMID: 20003512; PubMed Central PMCID: PMCPMC2802354.

43. Hernandez LA, Peevy KJ, Moise AA, Parker JC. Chest wall restriction limits high airway pressure-induced lung injury in young rabbits. J Appl Physiol (1985). 1989; 66(5):2364–8. PMID: 2745302.

44. Carlton DP, Cummings JJ, Scheerer RG, Poulain FR, Bland RD. Lung overexpansion increases pulmonary microvascular protein permeability in young lambs. J Appl Physiol (1985). 1990; 69(2):577–83. PMID: 2228668.

45. Dreyfuss D, Soler P, Basset G, Saumon G. High inflation pressure pulmonary edema. Respective effects of high airway pressure, high tidal volume, and positive end-expiratory pressure. Am Rev Respir Dis. 1988; 137(5):1159–64. doi: 10.1164/ajrccm/137.5.1159 PMID: 3057957.