

## Abstracts

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### Intravenous Lipopolysaccharide Induced Pulmonary Maturation and Structural Changes in Fetal Sheep via STAT-3

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**Rationale:** Antenatal pulmonary inflammation is associated with a reduced risk for respiratory distress syndrome but with an increased risk for aberrant lung development similar to bronchopulmonary dysplasia with reduced alveogenesis and microvasculature development. **Objective:** We hypothesized that systemic inflammation induced by intravenous lipopolysaccharide (LPS) injection would affect lung maturation and development in utero. We evaluated the signaling pathway in lung injury and recovery induced by signal transducer and activators of transcription (STAT)-3, a transcription factor activated by interleukin-6 which increases transcription of surfactant protein B (SP-B). **Methods:** Twenty-one fetal sheep were chronically instrumented at 107 days gestational age. Three days after surgery, control fetuses received saline (n = 12) and fetuses in the study group received 100 ng LPS intra-venously (I.V., n = 9). Animals were assessed for lung maturation and structure after 3 (n = 5) and 7 days (n = 4). **Results:** IL-6 concentration increased in the bronchoalveolar lavage fluid (BALF) more than 40-fold 3 days after LPS I.V. Phosphorylated STAT-3 detected by immunohistochemistry in the fetal lung was increased 3 days but not 7 days after LPS I.V. Western blot analysis of BALF showed processing of pro-SP-B to mature SP-B and increased SP-B concentrations 7 days after LPS I.V. Lung structure was matured with a 30 % decrease in alveolar wall thickness 7 days after LPS I.V. (p < 0.05 vs. control). Pressure volume curves showed increased lung gas volumes 7 days after LPS I.V. Deposition of elastin fibers at sites of septation was disturbed 3 days and

7 days after LPS I.V. **Conclusion:** Lung maturation and disturbed lung structure occurred following activation of STAT-3 after strictly intrauterine exposure to fetal inflammation, and this indicates possible new targets for therapy for BPD.

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### Bronchopulmonary Dysplasia: a Stem Cell Disease?

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**Background:** There is now increasing evidence for involvement of stem cells in a whole range of pathological conditions from arthritis to cancer. We sought to use an experimental mouse model of tissue-specific genetic ablation in mice to determine the role of stem cells in alveolar hypoplasia, which is a morphologic characteristic of bronchopulmonary dysplasia (BPD). In the mammalian genome, the *Pten* gene encodes a phosphatase and tensin homologue protein with reported function in maintenance of stem cell homeostasis. The role of *Pten* in lung development or pathogenesis of lung disease remains unknown. **Objective:** To generate lung-specific deletion mutations of *Pten* and to examine the consequent phenotype in the lung of newborn transgenic mice. **Methods:** Genetically engineered *Pten*(*LoxP/LoxP*); *Nkx2.1-Cre* mice were generated by appropriate mating. Morphological, molecular and biochemical characteristics of the mutant lungs were examined. **Results:** Mice carrying lung-specific deletion of *Pten* gene die at approximately 14 days of postnatal life due to respiratory insufficiency. Morphological assessment of *Pten*(*LoxP/LoxP*); *Nkx2.1-Cre* lungs showed a heterogeneous phenotype of epithelial and mesenchymal abnormalities. Importantly, there was clear evidence of alveolar enlargement and interstitial thickening resembling alveolar hypoplasia that is found in BPD lungs.

Molecular analysis showed alterations in expression of key genes with established roles in lung development. **Conclusion:** This is the first demonstration of the role of *Pten*, a gene with established function, in regulating stem cell homeostasis during the process of alveologenesis. Since interruption of alveologenesis is currently thought to underlie the pathogenesis of BPD, our results implicate a critical role for stem cells in the evolution of BPD.

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### 3

#### Effect of Surfactant Administration on Cytokine Concentrations in Bronchoalveolar Lavage Fluid of Human Premature Newborns

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**Background:** In contrast to animal studies showing increased pro-inflammatory cytokines in the lung, human studies suggest that surfactant therapy reduces the pro-inflammatory mediators, explained by reduced inflammatory phenomena. **Objective:** To analyze the effect of surfactant on bronchoalveolar lavage fluid (BALF) concentrations of IL-6, IL-8, IL-10 and MCP-1. **Methods:** Two groups of preterm newborns were studied: 10 surfactant-treated babies with respiratory distress syndrome (RDS) (27.2 ± 1.6 week; and 1,021 ± 236 g) and 10 non-treated newborns (26.9 ± 1.0 week; and 899 ± 228 g), needing mechanical ventilation for apnea of prematurity. Curosurf<sup>®</sup> was administered as soon as possible after birth, always in the neonatal unit, at a dose of 200 mg/kg. BALF samples were obtained at 24 and 72 hours for all 20 patients. **Results:**

Cytokine	Day	Surf no		Surf yes		p =
		n	mean ± SD	n	mean ± SD	
IL-6	1	10	5,470 ± 7,251	10	15,503 ± 17,028	0.10
IL-6	3	10	10,283 ± 19,464	10	9,848 ± 9,869	0.95
			p = 0.47		p = 0.53	
IL-8	1	10	49,552 ± 54,831	10	202,493 ± 348,845	0.19
IL-8	3	10	122,118 ± 72,405	10	165,488 ± 144,265	0.41
			p = 0.02		p = 0.76	
IL-10	1	10	314.3 ± 351.4	10	156.6 ± 188.8	0.20
IL-10	3	10	311.5 ± 447.3	10	65.9 ± 46.9	0.04
			p = 0.99		p = 0.07	
MCP-1	1	10	9,883 ± 6,929	10	19,038 ± 13,211	0.07
MCP-1	3	10	200,928 ± 196,717	10	43,801 ± 42,585	0.02
			p = 0.007		p = 0.10	

**Conclusion:** Surfactant reduces inflammatory phenomena. Our results disagree with previous data reporting an IL-10 increase after surfactant. It is possible that the different biological context may justify these disparities.

### 4

#### Differences in Mortality among Infants Treated with Three Different Natural Surfactants for Respiratory Distress Syndrome

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**Background:** Observations from a previous randomized, comparative surfactant clinical trial suggest an association between the type of surfactant used and neonatal mortality. **Objective:** To determine mortality differences in neonates with respiratory distress syndrome (RDS) treated with poractant alfa (PA) versus beractant (BE) and calfactant (CA), using a defined database which was independently collected and analyzed. **Methods:** Data were analyzed from 191 hospitals between January 2003 and June 2006 using Premier's Perspective<sup>™</sup> hospital-based, clinical database. Cohort selection included neonates ICD-9 coded for RDS and treated with PA, BE, or CA. Multivariate logistic regression analysis was used to compare in-hospital all-cause mortality while controlling for case mix and the following covariates: birth-weight, gestational age (GA), race, gender, and transfer status. Case-weighted models were evaluated for all infants plus a subset with complete patient data. The impact of missing data (primarily GA) was evaluated to test for selection bias. **Results:** Data were analyzed from 24,907 infants in the total cohort (TC) and a subset cohort (SC) of 10,237 with complete data. Mortality (table) in the PA cohort was lower vs. BE (22%, \* p < 0.001) and CA (34%, \*\* p < 0.001) for all covariates in both models (p < 0.001), except for missing GA data in the TC model (p = 0.94).

Surfactant type	PA	BE	CA
TC RDS treated infants	4,956	12,674	7,277
TC Unadjusted mortality, %	6.25	8.15	8.31
TC Adj OR (95% CI)	1.00	1.28 (1.20-1.36)*	1.47 (1.37-1.58)*
SC RDS treated infants	2,191	5,248	2,798
SC Adj OR (95% CI)	1.00	1.52 (1.32-1.70)**	1.60 (1.41-1.82)**

\* p < 0.001, mortality vs. BE was 22% lower, vs. CA 32% lower; \*\* p < 0.001, mortality vs. BE was 34% lower, vs. CA 37% lower.

**Conclusion:** Analysis of a large clinical database, using a diagnosis of RDS in premature infants, indicates that treatment with PA is associated with lower mortality than treatment with BE or CA.

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### Stable Microbubble Test in Gastric Aspirates to Predict the Need for an Additional Dose of Surfactant

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**Background:** Immediate extubation to continuous positive airway pressure after giving surfactant (INSURE technique) has been proposed. A significant proportion of preterm infants extubated need reintubation to receive further surfactant. We hypothesized that the need for a second dose could be predicted by the result of the stable microbubble test (SMT). **Objective:** To evaluate the usefulness of the SMT in gastric aspirates to predict the need for a second dose of surfactant in a group of preterm infants who received selective prophylaxis with surfactant based on low microbubble (MB) count. **Methods:** We retrospectively reviewed the charts of 100 preterm infants  $\leq 31$  weeks' gestation who had low MB count ( $< 25$  MB/mm<sup>2</sup>) and received selective surfactant prophylaxis. The association between the MB count and other neonatal variables with the need for a second dose of surfactant was also studied. **Results:** Twenty-eight of 30 babies who received a second dose of surfactant had an MB count  $\leq 10$  (sensitivity 93%; 95%CI 77–99%), predictive negative value 91% (95%CI 68–98%). However, specificity of the test was low. Patent ductus arteriosus and low gestational age were significantly associated with the need for a second dose of surfactant. Antenatal steroids were not associated with the need for retreatment. **Conclusion:** An MB count  $\leq 10$  indicates that the probability of needing an additional dose of surfactant is low. This may be of value in the decision to extubate after surfactant prophylaxis. **Ethical Approval:** approved by the Ethical Committee of PUCRS.

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### Recombinant Keratinocyte Growth Factor Increases Surfactant Protein-B but Does Not Change Surfactant Lipidomics in Neonatal Rats

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**Background:** Glucocorticoids stimulate Type-II-pneumocyte (PNII) maturation via fibroblasts, which secrete keratinocyte growth factor (KGF). While glucocorticoids exert systemic catabolic side effects, KGF acts directly via epithelial receptors on PNII. **Objective:** To compare the effect of recombinant human KGF (rhKGF) vs. glucocorticoid on (1) pool sizes of secreted surfactant and lung tissue, (2) the phospholipid profile and (3) surfactant protein-B (SP-B) concentration of secreted surfactant. **Methods:** Sprague Dawley rats (d1-d27) were treated for 48 h with rhKGF (2  $\times$  5 mg/kg, s.c.), betamethasone (BM; 2  $\times$  1 mg/kg,

i.p.) or rhKGF+BM. Lung lavage fluid (LLF) and lung tissue were analysed for phospholipid pools, phosphatidylcholine (PC) molecular species and SP-B using HPLC and Western blot. **Results:** RhKGF increased while BM decreased phospholipid pools of lung tissue. By contrast, both rhKGF and BM increased surfactant pools in LLF in immature lungs. However, in mature lungs only the combination was effective. In surfactant from immature lungs (d7) SP-B concentrations were increased from mean (SE) 4.6 (0.1) to 7.6 (0.2), 7.0 (0.5) and 7.8 (0.7)  $\mu\text{g}/\mu\text{mol}$  phospholipid by rhKGF, BM and rhKGF+BM, respectively. At d21 only rhKGF increased SP-B – Control: 11.4 (2.3); rhKGF: 17.9 (1.4); BM: 10.9 (1.3); rhKGF+BM: 22.0 (2.1)  $\mu\text{g}/\mu\text{mol}$  phospholipid. The profile of specific surfactant phospholipids was not influenced by either treatment: dipalmitoyl-PC (d7: 44–47%; d21: 40–42%); palmitoyl-myristoyl-PC (d7: 19–22%; d21: 26–27%); palmitoyl-palmitoleoyl-PC (d7: 12–14%; d21: 14–15%). **Conclusion:** For immature lungs rhKGF is a candidate to replace glucocorticoid treatment, since it (1) increases secreted surfactant with no changes in physiologic lipidomic profile, (2) increases the concentration of functionally important SP-B, and (3) exerts no catabolic action on lungs and other organs as glucocorticoids do. For treating surfactant deficiency/dysfunction of mature lungs, rhKGF may be a useful therapeutic option in combination with glucocorticoids.

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### Mixtures of Polymyxin B and Pulmonary Surfactant Prevent Proliferation of Gram Negative Bacteria in Ventilated Neonatal Rabbits

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**Background:** In neonatal pneumonia the surface activity of pulmonary surfactant is impaired and microorganisms may invade by-passing the air-liquid interface. Gram negative bacteria such as *E. coli* commonly cause neonatal pneumonia. Addition of the antimicrobial peptide polymyxin B (PxB) to modified porcine surfactant (Curosurf<sup>®</sup>) improves resistance to surfactant inactivation in vitro, while antimicrobial activity of PxB is maintained (Pediatr Res 2006; 59:407–411). **Objective:** To investigate whether antimicrobial activity of PxB in combination with Curosurf<sup>®</sup> is maintained in vivo. **Methods:** Curosurf  $\pm$  PxB were mixed at a ratio of 1:100 (w/w). After hysterotomy of New Zealand White does, neonatal near term rabbits were randomised to 4 (n = 4–6) groups, anaesthetised and prophylactically treated with intratracheal Curosurf<sup>®</sup> (200 mg/kg) and/or PxB (2 mg/kg). Rabbits treated with only saline served as controls. After 15 min of ventilation with standardised tidal volumes all animals received *E. coli*  $\sim 10^7$ /CFU (colony forming units) intratracheally. Ventilation was continued for 4h and pressure-volume curves were recorded in plethysmographs. At the end of the experiments, the animals were killed, the right lung was excised, homogenised and bacterial growth was determined after serial dilution of the homogenate.

**Results:** In animals receiving PxB  $\pm$  Curosurf<sup>®</sup> the growth of *E. coli* was significantly reduced compared to control animals or animals receiving only Curosurf<sup>®</sup> (median CFU/g lung: PxB group: 0; PxB+Curosurf<sup>®</sup> group: 3; control group: 10<sup>11</sup>; Curosurf<sup>®</sup> group: 10<sup>9</sup>). Animals receiving Curosurf<sup>®</sup>  $\pm$  PxB had slightly increased lung compliance (median: 0.4–0.47 ml/cm H<sub>2</sub>O\*kg) compared to controls or the PxB group (median: 0.28–0.3 ml/cm H<sub>2</sub>O\*kg). **Conclusion:** The antimicrobial function of PxB is maintained in vivo after intratracheal instillation in mixtures with pulmonary surfactant.

## 8

### Surfactant Proteins B and C Are Both Necessary for Alveolar Stability at End-Expiration

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**Background:** Synthetic surfactant preparations containing phospholipids and an SP-C analogue are as active as natural surfactants in a premature rabbit model of respiratory distress syndrome, provided that a small positive end-expiratory pressure (PEEP) is applied. Without PEEP, however, alveolar stability, measured as lung gas volume (LGV), is lower in animals treated with these synthetic surfactants than with Curosurf<sup>®</sup>. **Objective:** To investigate the effect of SP-B on alveolar stability in newborn premature rabbits treated with different synthetic surfactant preparations. **Methods:** Synthetic phospholipids were recombined with 2% native SP-C or 2% of the synthetic SP-C analogue, SP-C33, and/or 2% SP-B, and suspended in saline. Newborn premature (27 days gestational age) rabbits were tracheotomized at birth, treated with surfactant (80 mg/ml, 2.5 ml/kg) and ventilated for 30 min with a standardized sequence of insufflation pressures without PEEP. Curosurf<sup>®</sup> and non-treated animals were used as positive and negative controls, respectively. Lung-thorax compliance was calculated from tidal volume and pressure. LGV was measured at the end of the experiment. **Results:** Animals treated with Curosurf<sup>®</sup> or any of the synthetic surfactant preparations (8–12 animals in each group) had similar compliance values after 30 min of ventilation and these values were significantly higher than those obtained for non-treated controls. Median LGV was 4–6 ml/kg for the groups treated with synthetic phospholipids containing only SP-B or SP-C/SP-C33 but twice as high for the groups treated with surfactant containing both SP-B and SP-C/SP-C33. The LGV for Curosurf<sup>®</sup>-treated and non-treated animals were about 9–18 ml/kg and 1–2 ml/kg, respectively. **Conclusion:** The presence of both SP-B and SP-C/SP-C33 in synthetic surfactant is necessary for establishment of alveolar stability at end-expiration in newborn premature rabbits ventilated without PEEP.

## 9

### Variability of Surfactant Composition: a Challenge to Neonatology and Immunology

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**Background:** Composition of lung surfactant is highly complex and variable. Recently it was acknowledged that components other than dipalmitoylphosphatidylcholine (PC16:0/16:0), anionic phospholipids and surfactant proteins (SP) are specifically secreted into the alveolar space. Among these components are short chain PC species like palmitoyl-myristoyl-PC (PC16:0/14:0) and palmitoyl-palmitoleoyl-PC (PC16:0/16:1), which are present in natural mammalian therapeutic surfactants but absent from synthetic surfactants. **Objectives:** To correlate PC16:0/16:0, PC16:0/14:0 and PC16:0/16:1 concentrations of surfactant with pulmonary air:liquid interface dynamics and lung tissue morphology. To determine specific actions of PC16:0/16:0 and PC16:0/14:0 on macrophage (M $\Phi$ ) and T-cell function. **Methods:** Concentrations of surfactant PC species were plotted against diameters of gas exchange units and resting respiratory rates (RR) across various vertebrates. Human blood mononuclear cells were incubated with Curosurf<sup>®</sup> and individual PC species. Surface markers of M $\Phi$  differentiation were measured by FACS. The 5-carboxyfluoresceine diacetate succinimidyl ester assay was used to measure T-cell proliferation in the presence or absence of M $\Phi$ , Curosurf<sup>®</sup> or PC components. Palmitoyl-oleoyl-PC (PC16:0/18:1) was used as a negative control. **Results:** PC16:0/16:0, PC16:0/14:0 and PC16:0/16:1 correlated with RR (–0.6299, +0.6421 and +0.6052, respectively), while correlation with diameters was only +0.0141, –0.2480 and –0.11, respectively. Curosurf<sup>®</sup> and its physiological components PC16:0/16:0 and PC16:0/14:0 decreased CD14 expression of pre-incubated M $\Phi$ , while only PC16:0/14:0 and Curosurf<sup>®</sup> increased HLA-DR and CD80. Curosurf<sup>®</sup> and PC16:0/14:0, but no other PC species blocked the anti-CD3 mAb induced proliferation of T-cells, which afforded the presence of M $\Phi$ . **Conclusion:** PC16:0/14:0 as a physiological component of mammalian surfactant is important for surfactant function under dynamic conditions. This component of ‘natural’ therapeutic surfactants exerts specific actions on M $\Phi$  and T-cell proliferation that may be important to immune homeostasis of the terminal lung tissue.

### The Role of Complement and CD14 in Meconium-Induced Cytokine Formation

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**Objective:** Meconium aspiration syndrome (MAS) has a complex and poorly defined pathophysiology. Meconium is a potent activator of complement in vitro and in vivo, the latter associated with a systemic inflammatory response. The complement system and toll-like receptors are two important upstream components of the innate immune system which act partly independently in the inflammatory network. The aim of this study was to investigate the relative role of complement and CD14 in meconium-induced cytokine production. **Methods:** Human adult (n = 6) and cord (n = 6) whole blood samples anticoagulated with lepirudin were collected and distributed into tubes containing inhibitory antibodies (anti-CD14, anti-C2, anti-factor D, or combinations thereof). The tubes were preincubated for 5 min before adding meconium or buffer and then incubated for 4 h at 37°C. Complement activation was measured by quantification of the terminal sC5b-9 complement complex by ELISA. A panel of inflammatory mediators (cytokines, chemokines and growth factors) was measured using a Bio-Plex 27-plex panel on a Bioplex Array Reader<sup>®</sup>. **Results:** Fourteen of 27 mediators measured were induced by meconium both in cord and in adult blood. In cord blood two additional chemokines were induced and the inflammatory response was in general more potent. Blocking complement and CD14 differentially reduced the formation of most mediators, anti-CD14 being more effective. The combined inhibition of complement and CD14 almost completely abolished meconium-induced formation of the cytokines and the chemokines and markedly reduced the formation of growth factors (p < 0.05). The endogenous lipopolysaccharide content of meconium could not explain the CD14-mediated response. **Conclusion:** Meconium-induced triggering of the cytokine network is differentially mediated by complement and CD14. A combined inhibition of these effector mechanisms may be an alternative approach to reduce the inflammatory reaction in MAS.

## 11

### Effects of Low Oxygen Saturation Limits on Closure of the Ductus Arteriosus in Extremely Low Birth Weight Infants

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**Background:** Extremely low birth weight (ELBW, <1,000 g) infants are at increased risk for developing retinopathy of prematurity (ROP), and lower oxygen saturation limits have decreased

the incidence of severe ROP. While oxygen is known to constrict the ductus arteriosus (DA), a localized ductal wall tissue hypoxia is required for permanent closure of the DA. **Objective:** To compare the incidence of patent ductus arteriosus (PDA) and the need for ductal ligation in ELBW infants before and after implementation of lower oxygen saturation limits. **Methods:** Retrospective data analysis of prospectively collected data on ELBW infants admitted to two of our neonatal intensive care units 4 years before and after the implementation of a new oxygen saturation limit for prevention of ROP was performed in this study. Infants with a gestational age <23 weeks were excluded. During the period prior to implementation of the lower saturation limits (83–89%), ELBW infants requiring oxygen were maintained at oxygen saturations of 89–94%. **Results:** 362 ELBW infants comprised our study population. There were 192 infants of mean (SD) birth weight (BW) 756 (134) g and mean (SD) gestational age (GA) 25.9 (1.9) week before and 170 infants of BW 752 (131) g and GA 25.8 (1.7) week after the implementation of the lower oxygen saturation limit protocol. There was a significant reduction in incidence of Stage III or greater ROP (42.2 vs. 18.8%, p < 0.0001). However, the incidence of PDA (62 vs. 57.6%) and ductal ligation (22.9 vs. 23.5%) were unchanged. **Conclusion:** Use of the lower oxygen saturation limits in ELBW infants resulted in a significant reduction in severe ROP without significantly affecting the incidence of PDA or the need for DA ligation.

## 12

### Dexamethasone Therapy in Preterm Infants with Developing Bronchopulmonary Dysplasia: Effect on Pulmonary Surfactant Disaturated Phosphatidylcholine Kinetics

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**Background:** The role of corticosteroid treatment for severe bronchopulmonary dysplasia (BPD) is still debated. Scanty data are available on the effect of corticosteroids on surfactant metabolism in newborn infants. **Objective:** To compare surfactant kinetics in preterm infants with developing BPD, before and after dexamethasone (DEXA) treatment. **Methods:** Twenty-two studies were performed in 11 preterm infants (birth weight 718 ± 170 g, gestational age 25 ± 1 week) on high ventilatory setting before (age 24 ± 12 days) and after (age 34 ± 12 days) DEXA 13C-labeled dipalmitoyl-phosphatidylcholine was administered endotracheally to trace pulmonary surfactant. Surfactant-disaturated phosphatidylcholine (DSPC) kinetics, alveolar and tissue pool sizes were calculated from the DSPC 13C-enrichment curves from serial tracheal aspirates (TA) and bicompartamental analysis. Total protein and myeloperoxidase (MPO) activity from tracheal aspirates were measured and expressed per ml of epithelial lining fluid (ELF). Data are presented as mean ± SD or mean ± interquartiles, comparisons were performed by Wilcoxon test and the level of significance was p < 0.05. **Results:** After DEXA DSPC alveolar pool increased significantly from 6.6 ± 5.6 to 9.7 ± 9.7 mg/kg (p = 0.05), total protein and MPO were reduced

from  $9.3 \pm 9.0$  to  $3.9 \pm 2.5$  mg/mlELF ( $p = 0.03$ ) and from  $2,144 \pm 1,755$  to  $1,499 \pm 1,487$  mU/mlELF ( $p = 0.038$ ), respectively. **Conclusion:** DEXA treatment in mechanically ventilated preterm infants with severe respiratory failure and at high risk of developing BPD increases alveolar surfactant pool and reduces inflammatory markers. **Ethics:** Project was approved by local Ethics Committee and IRB and written parental consent was obtained.

### 13

#### Neutrophil Activity in Bronchopulmonary Dysplasia and the Effect of Chorioamnionitis

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**Background:** Oxidative stress is postulated as a major factor in the pathogenesis of bronchopulmonary dysplasia (BPD). The migration and oxidative capabilities of neutrophils may be contributory. **Objective:** 1. To evaluate migration potential and respiratory burst in cord blood neutrophils of infants <32 weeks' gestation with respiratory distress syndrome (RDS), 2. To examine differences in neonates who subsequently develop BPD, and 3. To investigate the effects of chorioamnionitis (CA) on neutrophil activity. **Methods:** Respiratory burst in neutrophils (heparinized cord blood) was assessed in response to *E. coli*, N-formyl-met-leu-phe (fMLP) and phorbol 12-myristate 13-acetate (PMA) and sur-

Group	n	Burst test % neutrophils positive, median (IQR)			p value	
		<i>E. coli</i>	fMLP	PMA		
No CA	18	14.7 (4.2–34.3)	1.5 (0.2–3.18)	62.5 (20.0–86.7)	NS	
CA	14	13.4 (3.3–31.6)	0.7 (0.3–1.4)	51.9 (22.5–64.0)		
NO BPD 28	15	13.4 (4.7–23.5)	1.1 (0.2–1.8)	62.1 (26.8–82.8)	NS	
BPD 28	15	12.5 (2.4–33.2)	1.0 (0.15–2.9)	36.9 (18.0–80.5)		
NO BPD 36	20	13.4 (4.1–23.3)	0.9 (0.2–2.1)	53.4 (20.4–81.7)	NS	
BPD 36	9	13.5 (4.3–34.3)	0.7 (0.2–1.4)	51.9 (22.5–64)		
		Surface marker % neutrophils positive, median (IQR)				
		CD18	CD14	CD11b	CD54	
No CA	18	84.9 (55.2–90.4)	1.7 (1.5–14.0)	6.2 (3.1–10.2)	14.6 (4.6–22.0)	NS
CA	14	62.2 (45.0–86.9)	5.2 (2.5–22.9)	5.4 (4.1–12.3)	10.8 (4.3–19.0)	
NO BPD 28	15	77.8 (54.6–90.3)	2.2 (1.5–4.2)	8.1 (4.4–13.2)	16.1 (5.8–19.9)	NS
BPD 28	15	62.7 (44.6–89.9)	5.9 (1.7–28.1)	4.8 (2.7–9.9)	11.2 (4.0–22.0)	
NO BPD 36	20	71.8 (38.0–90.4)	2.8 (1.7–12.6)	6.2 (4.2–11.3)	12.6 (4.1–19.7)	NS
BPD 36	9	79.1 (57.0–89.5)	8.0 (1.6–28.8)	3.4 (2.3–10.5)	12.9 (5.1–24.3)	

face markers by FACS analyses. Infants recovering from RDS were classified at discharge as non-BPD and either 1. BPD d 28 (oxygen at day 28) or 2. BPD 36 weeks, (dependence at 36 weeks corrected gestational age). CA was determined by placental histopathology. **Results:** 32 infants of median (range) gestational age 27 weeks (26–29) and median (range) birth weight 864 g (695–1,134) were studied. Results are shown in the table. **Conclusion:** Surprisingly there were no significant differences in early neutrophil respiratory burst activity or surface marker expression between infants exposed to CA or those developing BPD compared to unaffected comparison infants.

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#### Dose-Dependent Increase of Hydroxyl Radical Formation and DNA Oxidation by Newborn Resuscitation with 40, 60 or 100% Oxygen

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**Background:** Newborn resuscitation is one of the most common procedures carried out in perinatal medicine. Oxidative stress induced by reoxygenation may be responsible for short and long term pathological consequences. **Objective:** Our aim was to study dose-response correlation between different FiO<sub>2</sub> levels used for resuscitation and urinary markers of oxidative damage to DNA and amino acids. **Methods:** Newborn piglets (12–36 h) were asphyxiated following a standardized protocol and resuscitated for 15 min with either 21, 40, 60 or 100% oxygen and observed for 1 h. Urine samples were collected for measurement of 8-oxo-dG/2dG, ortho-tyrosine (o-Tyr) and phenylalanine (Phe) by mass spectrometry as indicators of DNA oxidation and hydroxyl radical formation. **Results:** The 8-oxo-dG/2dG and o-Tyr/Phe ratios were significantly higher in piglets resuscitated with high oxygen concentrations.

FiO <sub>2</sub>	8-oxo-dG/2dG	p vs. 21%	o-Tyr/Phe	p vs. 21%
21%	5.76		19.07	
40%	8.44	p < 0.01	56.9	p < 0.001
60%	10.82	p < 0.01	87.7	p < 0.001
100%	22.44	p < 0.01	148.7	p < 0.001

**Conclusion:** Hypoxia and subsequent resuscitation for 15 min with 21, 40, 60 or 100% O<sub>2</sub> cause increased oxidative stress and a dose-dependent oxidation of DNA and phenylalanine. These markers can be easily determined in urine and may be useful as clinical tools. The increase in the hydroxyl attack may lead to a pro-oxidative status and risk for genetic instability.

## DNA Damage and Repair Capacity in Newborns after Oxidative Stress

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**Background:** Oxidative stress (OS) might be an important contributor to complications in preterm infants and in asphyxiated newborns. Effects of OS on DNA damage and repair capacity are not well documented in this age group. **Aim:** To investigate in vitro DNA damage after OS in healthy newborns compared to adults taking into account genotypes relevant for DNA repair. **Methods:** Lymphocytes from cord blood and adult peripheral blood from seventeen mother-daughter couples were exposed in vitro to H<sub>2</sub>O<sub>2</sub>. Genotoxic effects and repair capacity were tested using the Comet assay and the cytokinesis-block Micronucleus assay. Genotypes relevant for DNA repair (*hOGG1*, *XRCC1*, *XRCC3*, *XPB*) and folate metabolism (*MTHFR*) polymorphisms

were assessed. The study was approved by the Ethical Committee of the hospital. **Results:** Initial DNA damage was similar for neonates compared to their mothers. After 2 h repair, residual DNA damage was lower in the newborns suggesting a more efficient DNA strand break repair capacity after OS. This was confirmed by significantly higher frequencies of MN observed in mothers versus newborn daughters for several genotypes. No genotype with a significant effect on DNA repair capacity in newborns was identified. Concerning MN frequencies, however, newborns carrying the variant *XRCC3*<sup>241</sup> genotype might be at higher risk for the induction of MN by oxidative stress. Multivariate analysis revealed a significant protective effect of maternal antioxidant supplementation during pregnancy against oxidative DNA damage in newborns. **Conclusion:** This preliminary and small study showed that term healthy newborns have a better DNA repair capacity after OS compared to adults. Further and larger studies are needed to investigate DNA damage induced by OS on cells of premature infants. We plan to evaluate the impact of gestational age, genotypes and maternal nutritional status. In vitro DNA damage will be compared with clinical outcome and OS related diseases.