CBI

Histomorphometric Measurement of Hyper-oxygenation Model of Bronchopulmonary Dysplasia: Validation of Phase Contrast Methodology

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A TRANSLATIONAL APPROACH TO PRECLINICAL RESEARCH

Bronchopulmonary Dysplasia

- Hyper-oxygenation of neonatal rat pups is an established model of bronchopulmonary dysplasia

- Prematurity, toxic lung injury, smoke inhalation

- Assessments of treatment: Pulmonary function and MLI is the typical parameters assessed

- CBI has developed and validated a method of assessment of the alveolar changes using a method of histomorphometric phase analysis based upon Jacob, et al. 2009

- This models offers a superior and more sophisticated method to assess changes in morphology is comparison to MLI determinations

Hyper-oxygenation Model

- New born rat pups are placed in a high oxygen environment in a hyperoxic tank with their dams for 2 weeks

- CBI has developed special proprietary methods of management of pups and dams

- Following removal, over a period of 1-2 weeks, alveolar walls are damaged and become thickened and fibrotic with accompanying inflammation

- Alveolar walls appear thickened, and the alveolar spaces appear to be dilated.

- Lesions and MLI data are compatible with literature (vonHaaften, 2009)

Hyper-oxygenation Model
# Hyper oxygen chamber used a CBI

Hyper-oxygenation Model
Histologic Findings in Bronchopulmonary Dysplasia

- Histology is the main assessment in this model

- Multifocal areas of pulmonary fibrosis-with increases in alveolar thickness with accompanying inflammation

- Decreases in alveolar lumen size with increased alveolar wall thickness with inflammation is present.

- Immunohistochemical changes of increases in
  - alveolar wall collagen I
  - numbers of alveolar macrophages
  - new capillary growth
  - PCNA expression
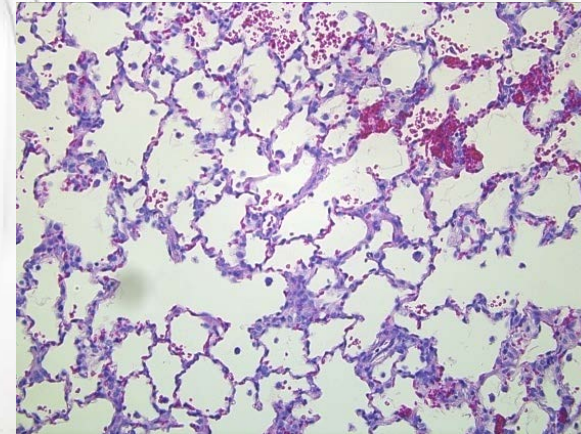
Histologic Findings

Normal lung
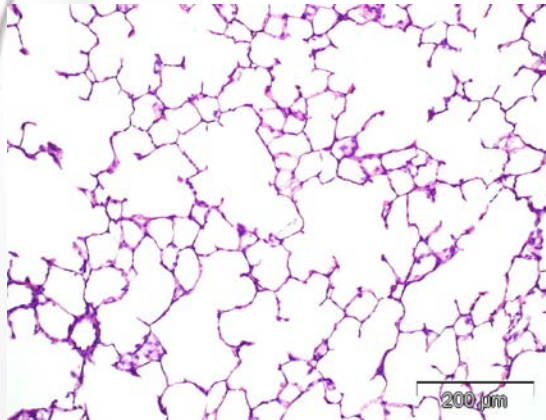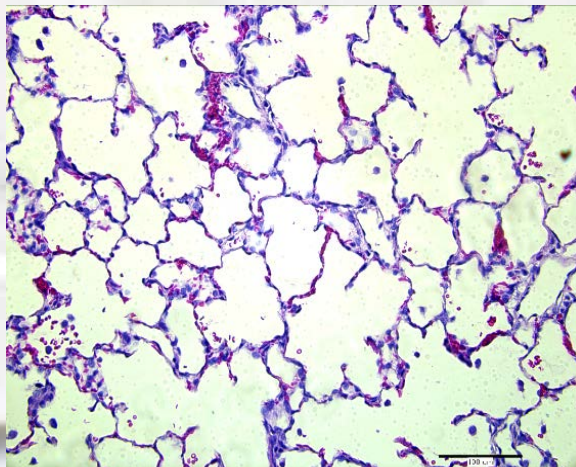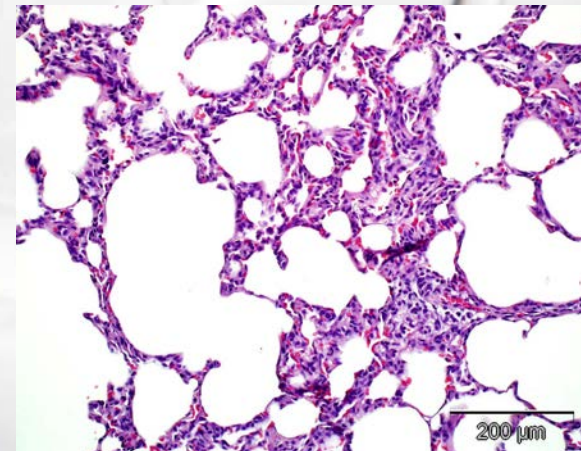
Hyperox-lung

Hyperox-lung   805

Hyperox-lung   904

Hyperox-lungs: There is thickening of the alveolar walls, with inflammation, edema, and hemorrhage and consequently reduced alveolar area

Immunohistochemistry

- IHC assessments may include
  - Collagen Type I*
  - SMA 1*
  - CD68*
  - vWF*
  - CD8
  - PCNA*
  - KI67

*clear differences between normal air and hyper-oxygen

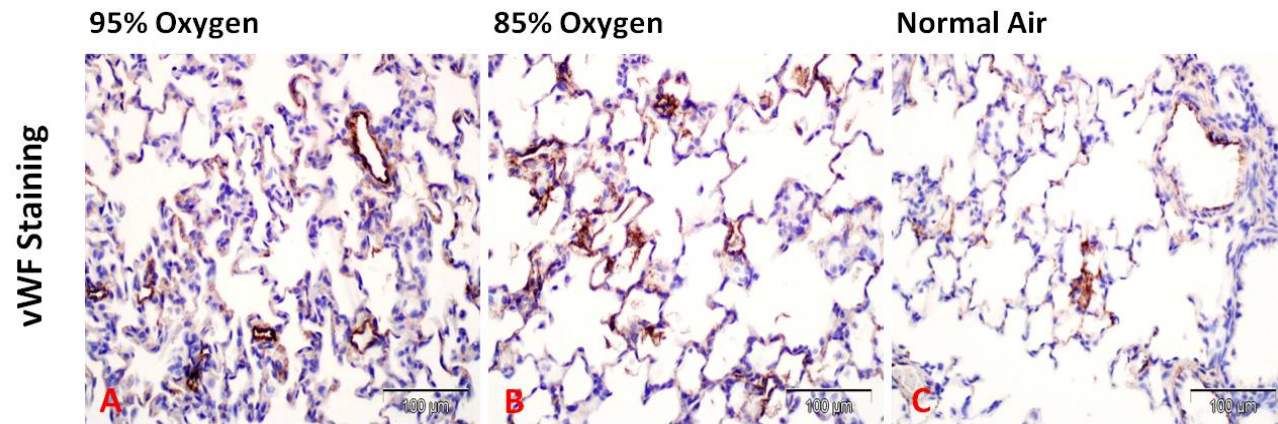# Hyper-oxygen-induced increases in vWF, PCNA.

Fig 5) Immunohistochemical analysis of lungs subjected to neonatal hyperoxygenation with vWF. There is intense vascular proliferation in case of 95% and 85% oxygen compared to normal air. Score: A: 3; B:3; C:2
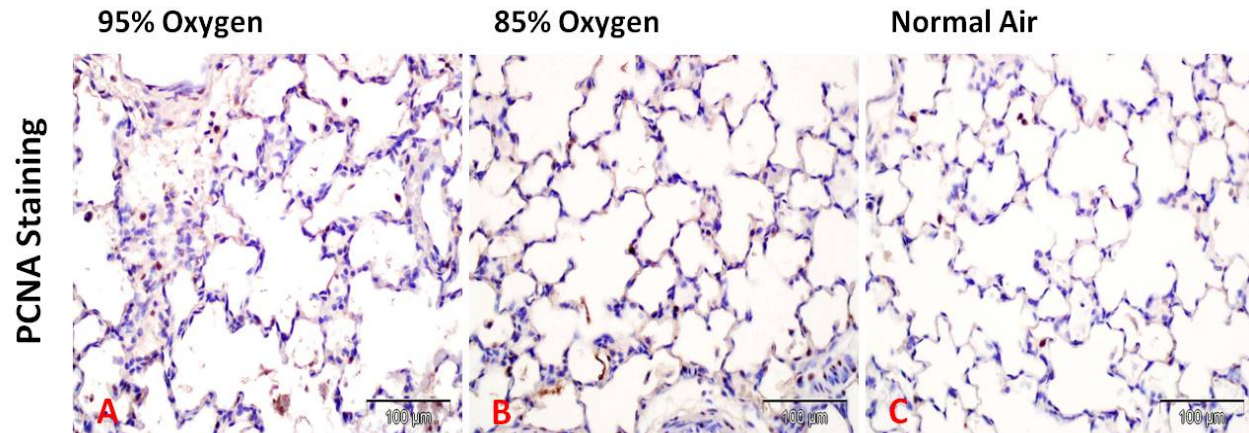

Fig 6) Immunohistochemical analysis of lungs subjected to neonatal hyperoxygenation with PCNA. There is increased PCNA expression in case of 95% and 85% oxygen compared to normal air. Score: A: 4; B:3; C:1

Hyper-oxygen-induced increases in Collagen I. Minimal changes in KI67

Collagen I Staining
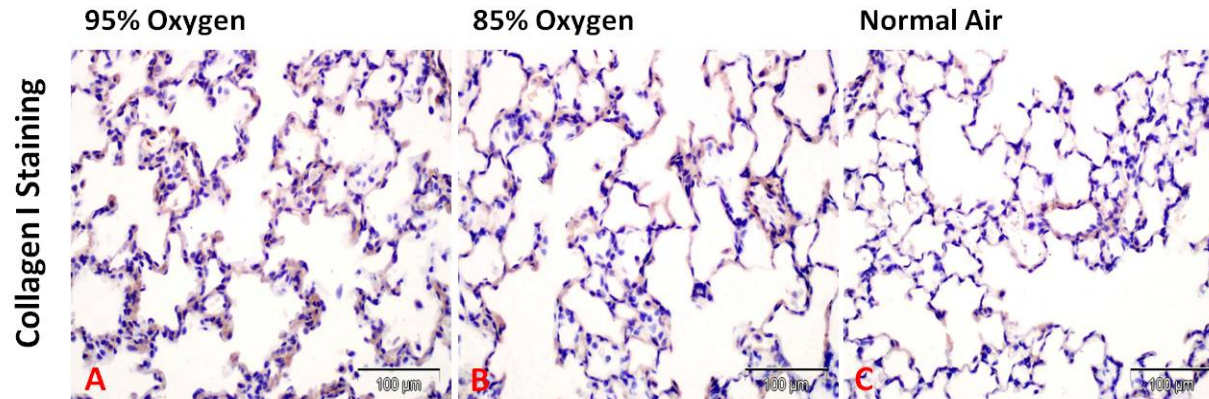
95% Oxygen | 85% Oxygen | Normal Air

Fig 3) Immunohistochemical analysis of lungs subjected to neonatal hyperoxygenation with Collagen I. There is aveolar wall thickening with increased collagen expression in case of 95% and 85% oxygen compared to normal air. Score: A: 3; B:2; C:1

Ki 67 Staining

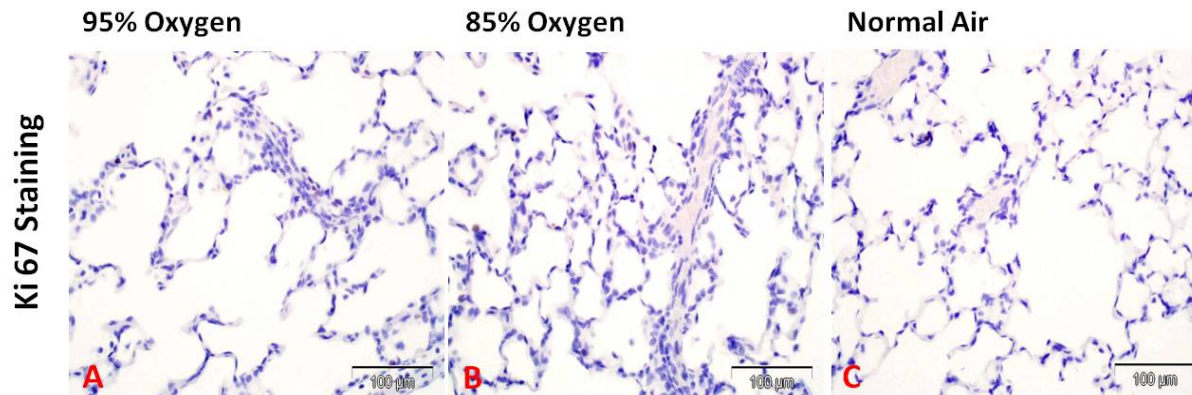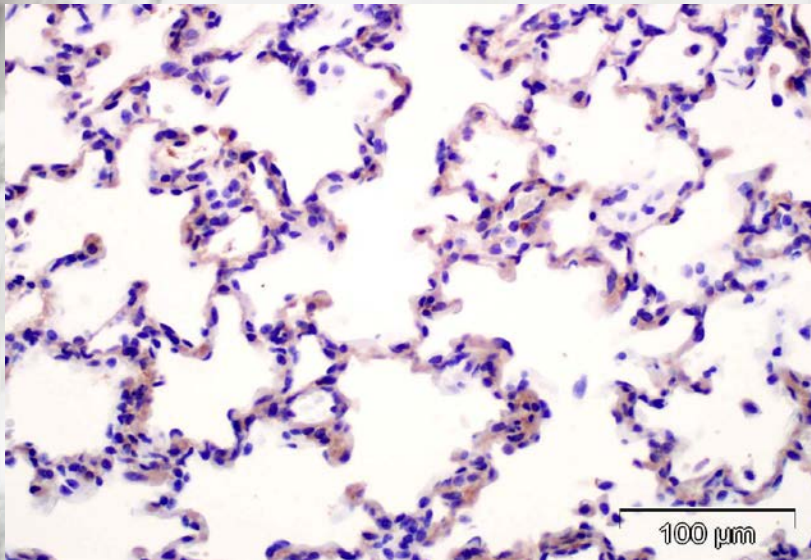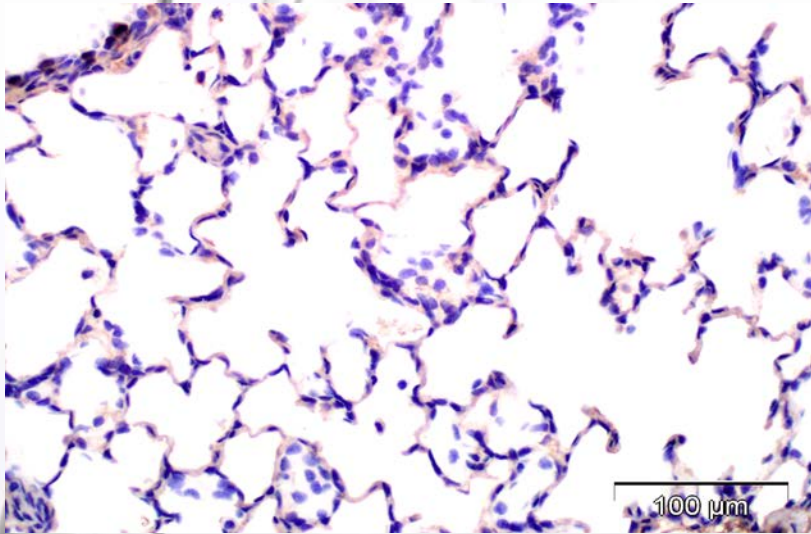95% Oxygen | 85% Oxygen | Normal Air

Fig 4) Immunohistochemical analysis of lungs subjected to neonatal hyperoxygenation with apoptosis marker Ki67. There is trace of Ki67 staining all cases. Score: A: 1; B:1; C:1
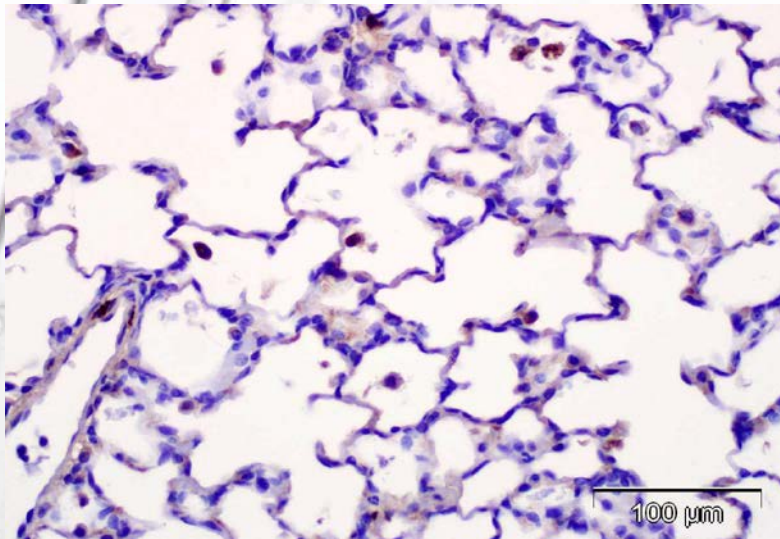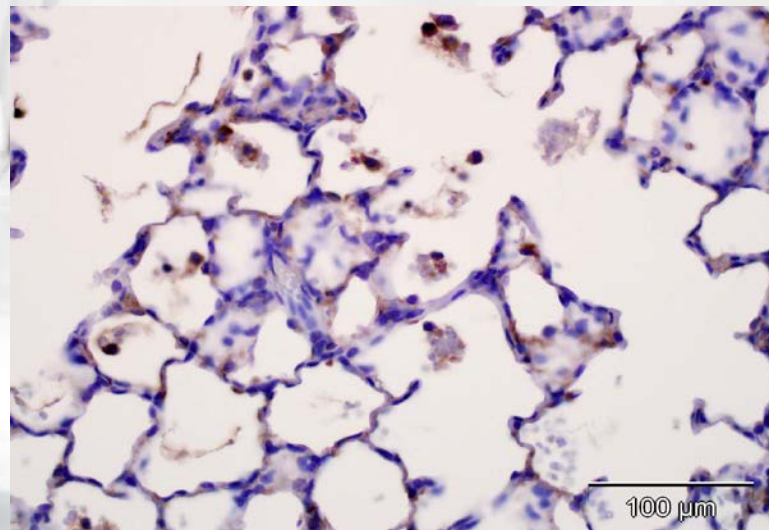
Immunohistochemistry

- **Collagen Type I**: Increased expression in alveolar walls of hyper-oxygenated lungs

Immunohistochemistry

- **CD68 Pan-macrophage:** Increased numbers of CD68 macrophages in alveolar walls of hyper-oxygenated lungs

Hyper-ox Model Validated at CBI
Mean Linear Intercept

- MLI is a common method to assess hyper-oxygen-induced changes in the alveolar walls

- CBI conducted several studies assessing normoxic vs hyperoxic lungs
  - MLI assessments at CBI were compatible with literature references
  - Histopathologic lesion were compatible with literature references

| Table 2A: Summary of MLI measurements		
	Normoxia (µm)	Hyperoxygenation (µm)
CBI MLI	35.5 ± 0.06	44.3 ± 5.3
Reference MLI Van Haaften, 2009	$\sim 35.5 \pm 3$	$\sim 50.3 \pm 2$

Mean Linear Intercept Assessments

- Older method using a stage microscope and a microscopic grid. The number of times an alveolar wall, or septa, intersects with a grid line are manually counted.

- Neonatal lungs respond to hyper-oxygenation by collagen deposition in the alveolar walls leading to thickening of the walls, and expansion of the alveolar space which leads to a reduction in the number of alveoli in a given area.

- MLI is reduced in hyper-oxygenated lungs by about 22% in this model in comparison to normal lung alveoli (Van Haaften, 2009)

MLI Assessment

- MLI (mean linear intercept) is a method to measure this change. This is an older method that is very labor intensive and does not take histopathologic changes in the lung into consideration
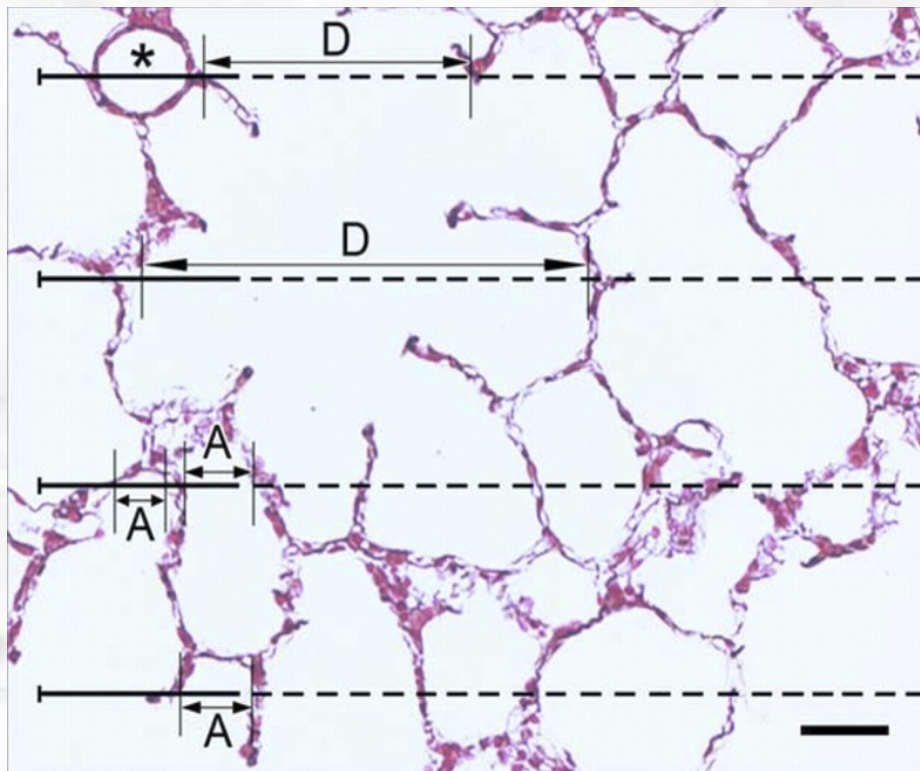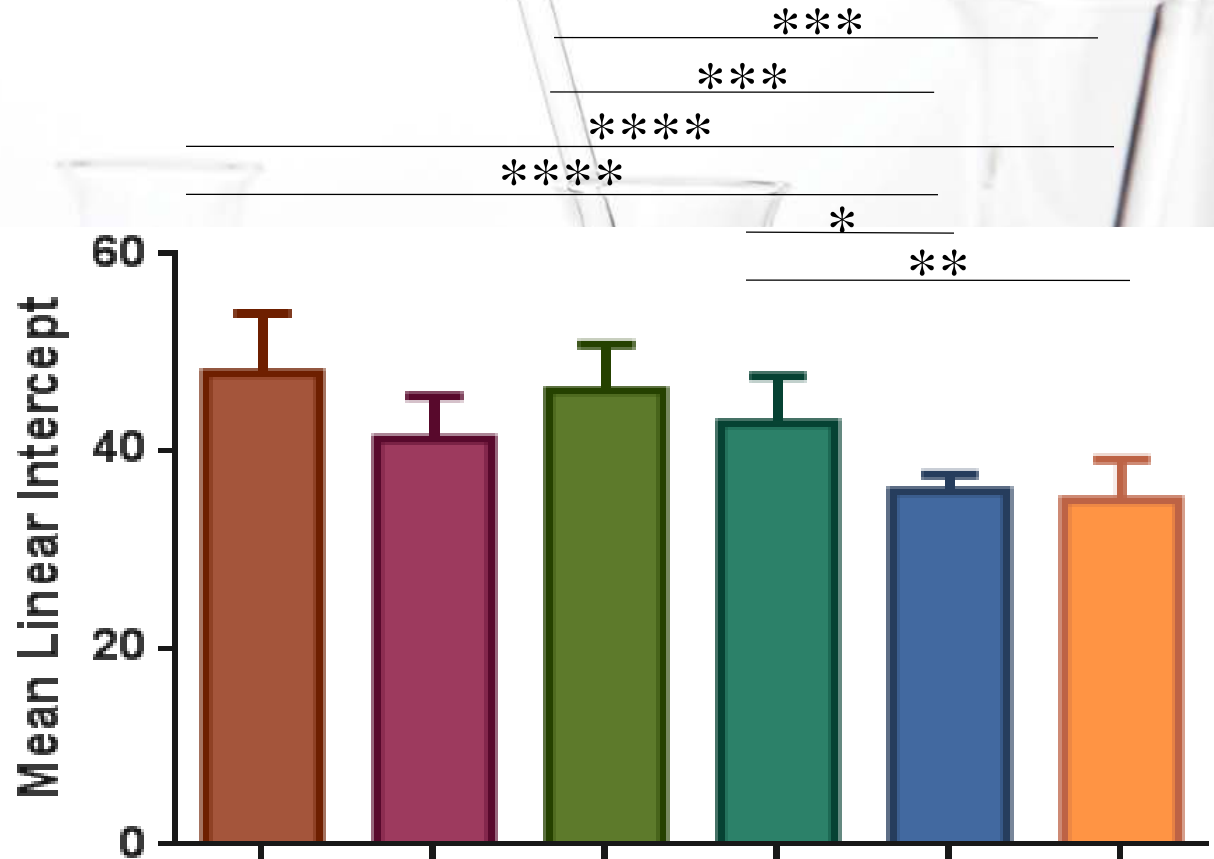
Group	1	3	7	8	9	10
Injection type	V	V	NA	NA	V	NA
Volume Injected	100ul	125ul	NA	NA	125ul	NA
Dams Swapped	Yes	Yes	Yes	No	No	No
			Hyperoxic		Normoxic	

MLI values in a study demonstrating increased MLI values in hyperoxygenated pups in comparison the normal air. There is about a 20% increase in size.

Mean Linear Intercept of rat lung images after fixing with formalin and staining with hematoxylin and eosin at P28 (n=6 for Group 1/3/7, n=10 for Group 8, n=9 for Group 9/10). ns (blank) P>0.05; *P≤0.05; **P≤0.01; **P≤0.001;

Phase Contrast Analysis

- CBI has developed a sophisticated histomorphometric phase contrast method
  - Method based in part upon Jacob, et al., 2009 and modified and enhanced and customized at CBI

  - Accurate and sophisticated

  - Reproducible

  - Reflects relevant changes in the alveolar wall thickness and changes in alveolar spaces

  - Validated and optimized for use in bronchopulmonary dysplasia

Example of PCA (Jacob, 2009 et al.)
Analysis shows differences in alveolar wall thickness and alveolar space area.
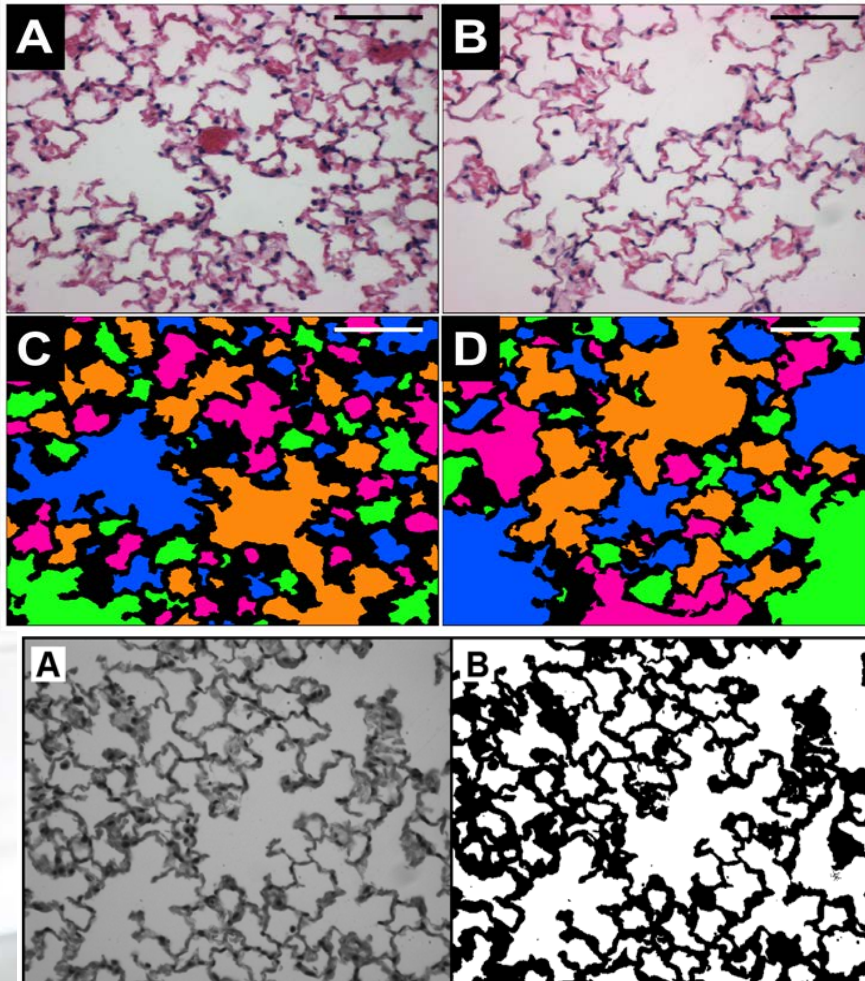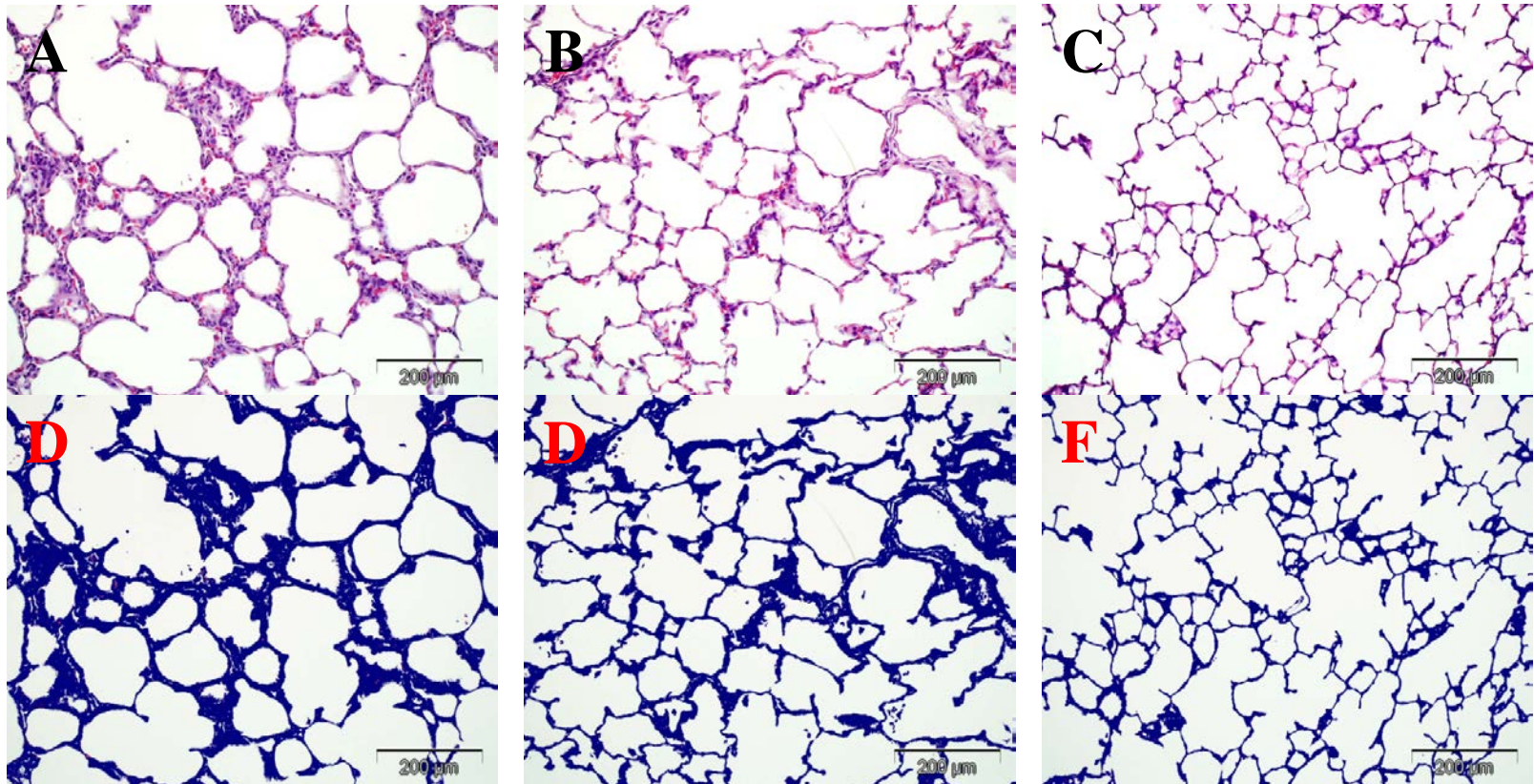
PCA at CBI: hyper-oxygen vs normal air. There are clear differences in alveolar wall thickness and alveolar space size
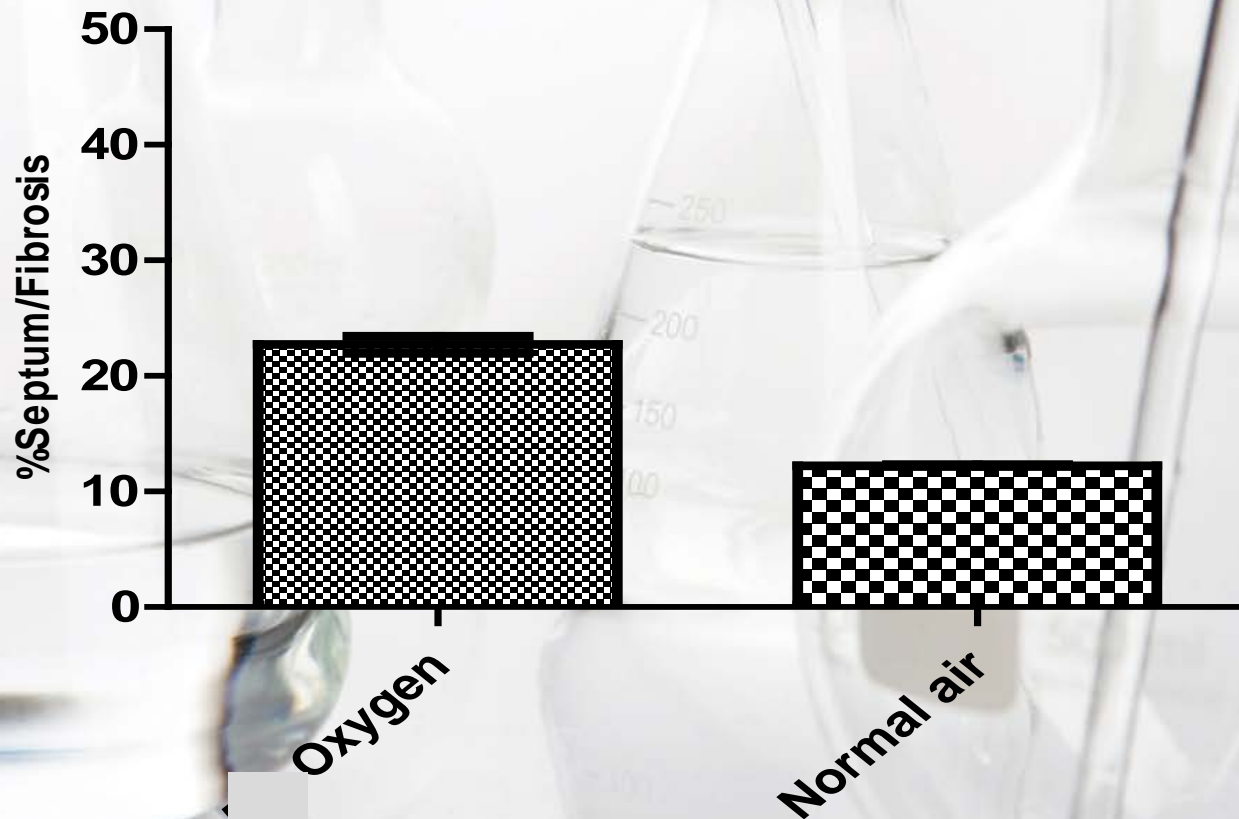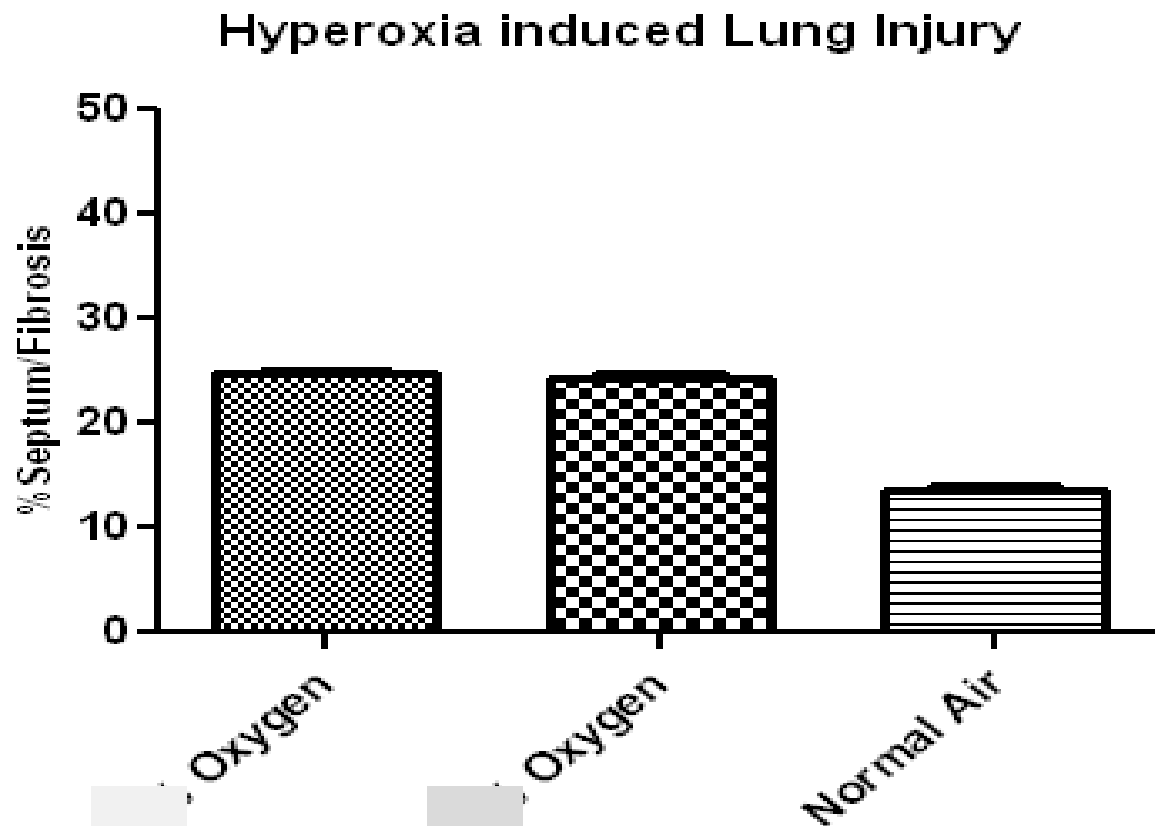
A&B Hyperoxic O2   C Normal Air

Top: H&E Images of lungs in a hyperoxic chamber at high Oxygen (A&B) and normal air (C).
Bottom: The corresponding pixel image analysis was shown in (D, E and F)

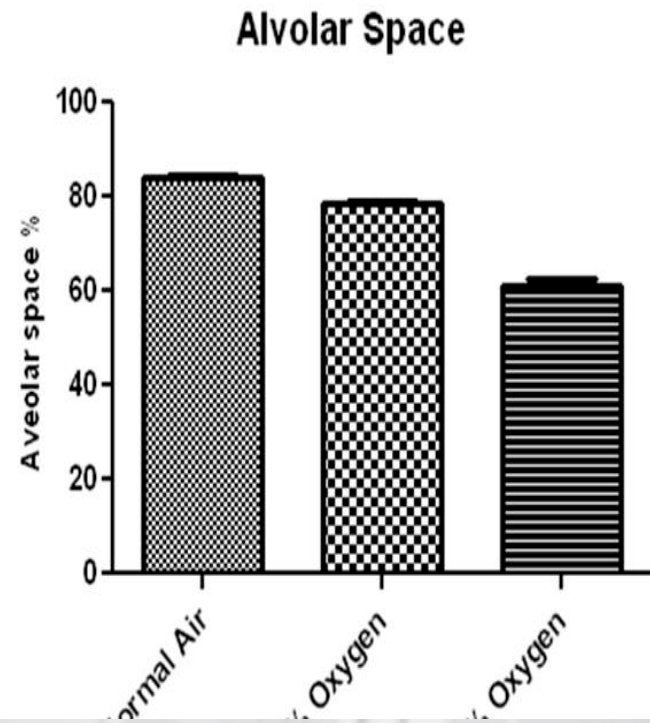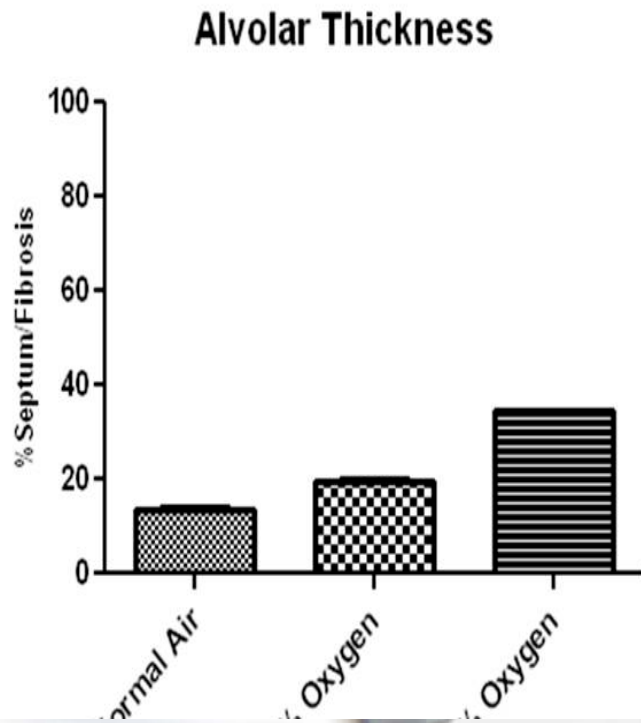# CBI PCA data showing clear significant increases in alveolar wall thickness

Oxygen vs. Normal Air

CBI PCA data showing clear significant increases in alveolar wall thickness

CBI PCA data showing clear significant increases in alveolar wall thickness and differences in the area of the alveolar spaces

Summary

- CBI offers a validated model of hyper-oxygen-induced bronchopulmonary dysplasia in neonatal rats

- Histopathologically there are changes in alveolar lumen size with increased alveolar wall thickness, decreases in alveolar area with inflammation present.

- Histomorphometric phase analysis as developed and validated by CBI is consistent with the histologic changes and offers a more robust and accurate assessment of alveolar wall changes than MLI.

- CBI PCA indicates a reliable increase in alveolar wall thickness and decrease in alveolar space