



COMPARATIVE BIOSCIENCES, INC

A TRANSLATIONAL APPROACH TO PRECLINICAL RESEARCH

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Postnatal Hyperoxia-induced Lung and Retinal Injury in Infant Rats: Qin Zhang, Alireza Ebrahimnejad, Felisha Paniagua, Robert Sukhu, Carol Meschter

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Hyperoxygenation: Pulmonary Dysplasia and Oxygen-induced retinopathy

Neonatal hyperoxygenation has been demonstrated to produce at least two significant syndromes, **Pulmonary Bronchodysplasia** and **Retinopathy of Prematurity** in children. High quality preclinical rodent models exist for both of these syndromes. Subjecting mouse or rat pups to 2 weeks of neonatal hyperoxygenation will induce both pulmonary bronchodysplasia or oxygen-induced retinopathy translationally that are useful and robust models of the human diseases.

We offer both of these models at CBI.

Pulmonary Bronchopulmonary Dysplasia

Exposure of neonatal infant rats to 2 weeks of hyper-oxygenation followed by return to room air leads to pulmonary alveolar wall fibrosis, enlargement of the alveolar space and inflammation. This is analogous to pulmonary fibrosis in premature human neonates due to bronchopulmonary dysplasia, the treatment of which represents a significant unmet medical need. CBI offers a sophisticated and validated model of oxygen-induced bronchopulmonary dysplasia in neonatal rats.

- Hyper-oxygenation of neonatal rat pups is an established model of bronchopulmonary dysplasia
- Models prematurity, toxic lung injury, smoke inhalation
- Assessments of treatment: Histology, immunohistochemistry, PCA are typical parameters assessed
- Phase Contract Analysis CBI has developed and validated a proprietary 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

Histopathology, immunohistochemistry and histomorphometry are the main methods of assessment in this model

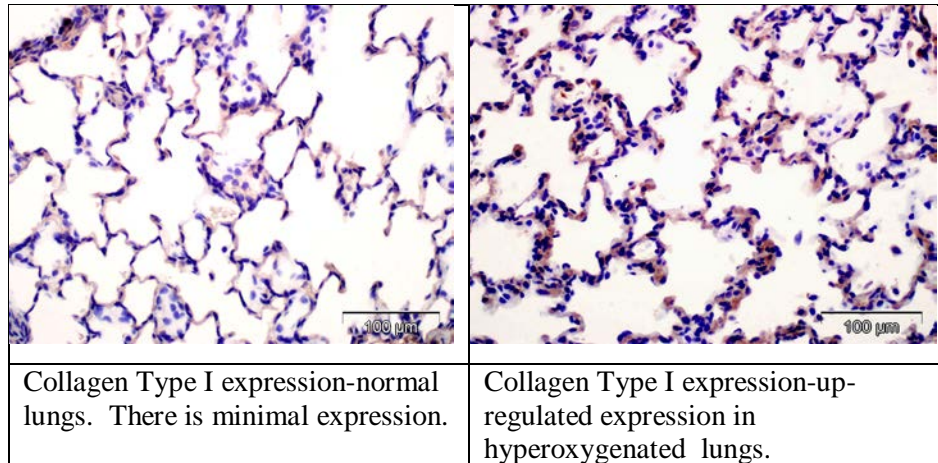
- **Histopathology**
 - Multifocal areas of pulmonary fibrosis-with increases in alveolar wall thickness with accompanying inflammation and hemorrhage.



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

- **Immunohistochemistry**

- Increased expression in hyperoxygenated lungs of
 - alveolar wall collagen I
 - smooth muscle actin
 - numbers of alveolar macrophages
 - new capillary growth
 - PCNA

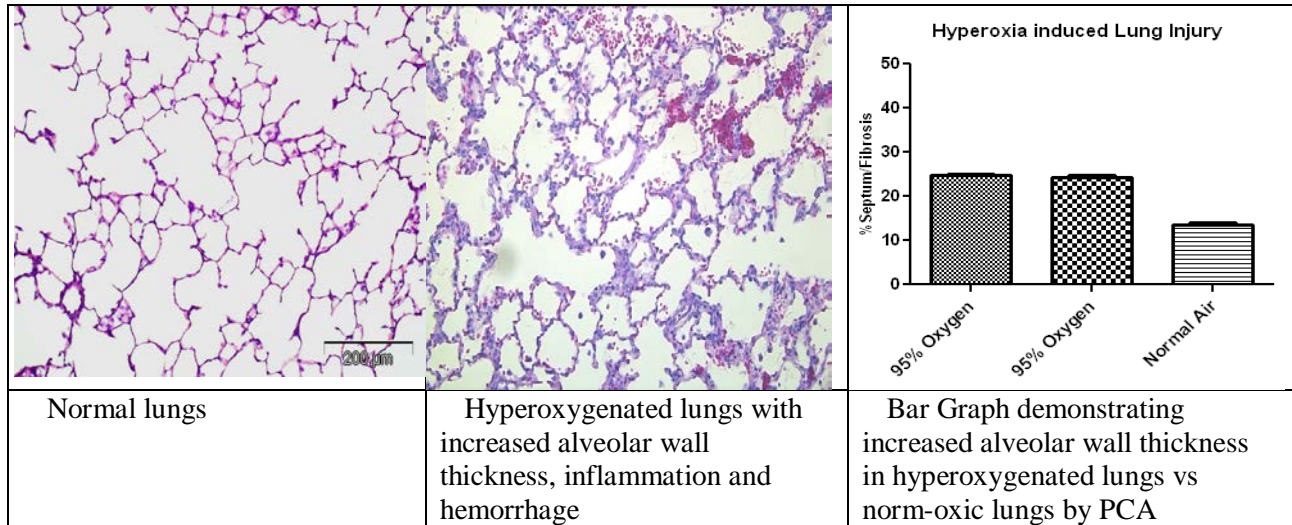


- **Proprietary Histomorphometric Phase Contrast Analysis**

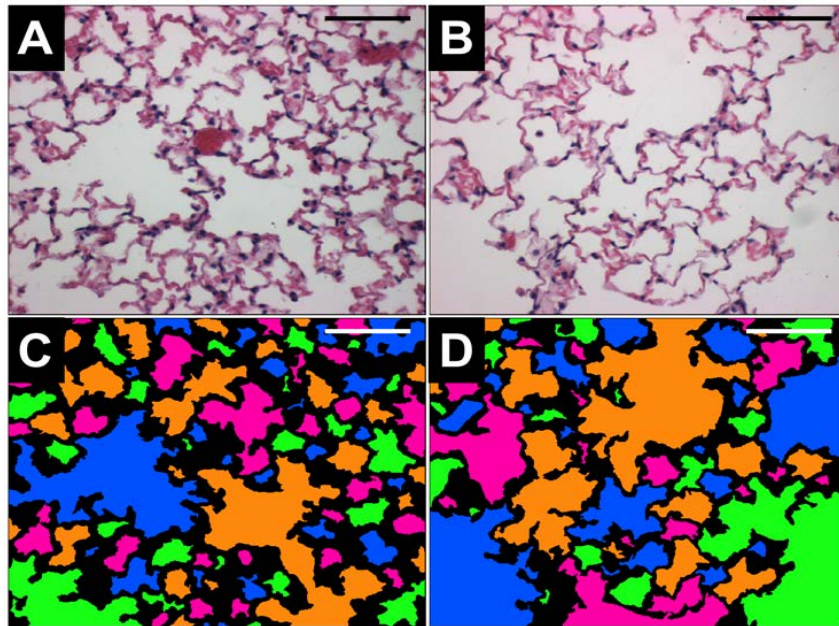
CBI has developed a histomorphometric phase contrast method that is superior to the historical Mean Linear Intercept Method

- Method based in part upon Jacob, et al., 2009 and modified and enhanced and customized at CBI
- More accurate, reproducible, and sophisticated than MLI
- Reflects relevant changes in the alveolar wall thickness and changes in alveolar spaces
- Validated and optimized for use in bronchopulmonary dysplasia

The figures below show normal lungs versus hyperoxygenation lungs and the corresponding Phase Contract Analysis which histomorphometrically compares the bronchioalveolar wall thickness between normal and hyperoxygenated lungs.



CBI has developed and validated a histomorphometric Phase Contrast Analysis method to measure changes in the alveolar wall thickness and size of the alveolar wall spaces. Below are figures demonstrating the comparison between normal and hyperoxygenated lungs.



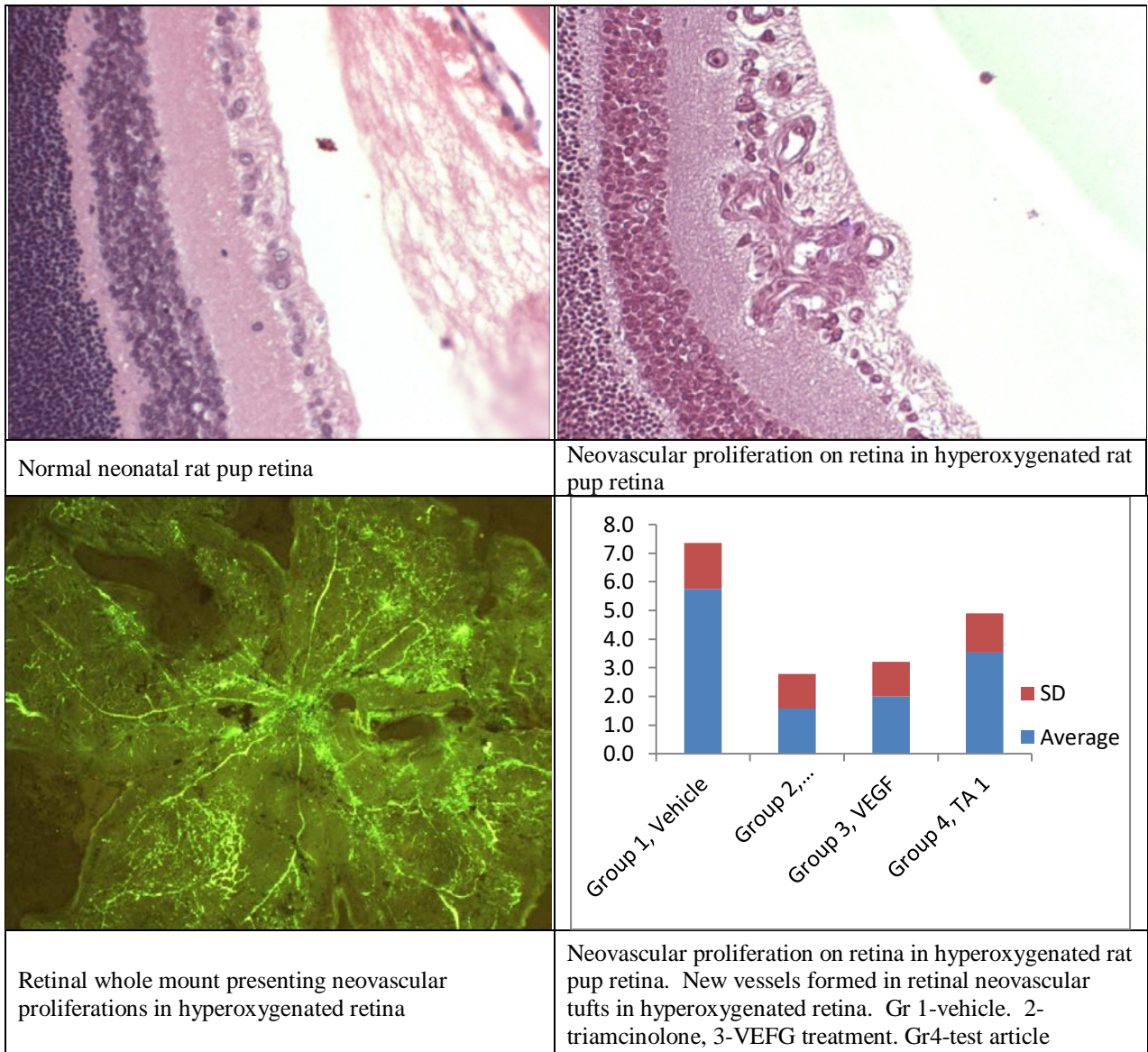
Example of PCA (Jacob, 2009 et al.): Analysis shows differences in alveolar wall thickness and alveolar space area.



Oxygen-induced retinopathy

Exposure of neonatal infant rat or mouse pups to 2 weeks of hyper-oxygenation followed by return to room air leads to a proliferative neovascularization of the retina that models the Retinopathy of Prematurity in premature infants. CBI offers a sophisticated and validated model of oxygen-induced retinopathy in neonatal rats and mice.

- Hyperoxygenation of neonatal rat pups is an established model of OIE
- Models prematurity primarily or provides mechanistic or therapeutic information on retinal neovascularization
- Assessments of treatment: Angiography, histology, vessel counts, immunohistochemistry, flat mounts.





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