Functional and Histological Characterization of a Preterm Rabbit Model of Bronchopulmonary Dysplasia Exposed to Hyperoxia

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Background and Aim

Bronchopulmonary Dysplasia (BPD) is a chronic respiratory disease that affects mainly premature infants [1]. Its aetiology is multifactorial, with a complex interplay of pre- and post-natal risk factors [2] contributing to the arrest of normal lung development [3]. Due to the limited availability of human samples, animal models play an essential role for understanding disease mechanisms and testing novel therapeutic strategies. The preterm rabbit model represents a good compromise between small and large animal species [4]. At Chiesi's Research Centre, a preterm rabbit model exposed to 95% of oxygen for seven days has been set-up and validated. However, a comprehensive characterization of this animal model is still lacking in the scientific literature. Therefore, the present study aimed to assess the effect of hyperoxia exposure on lung structure, integrating histological data by both conventional morphometric analyses and an innovative Artificial Intelligence (AI)-based software (Visiopharm, Denmark) with lung function measurements.

Materials and Methods

Experimental design

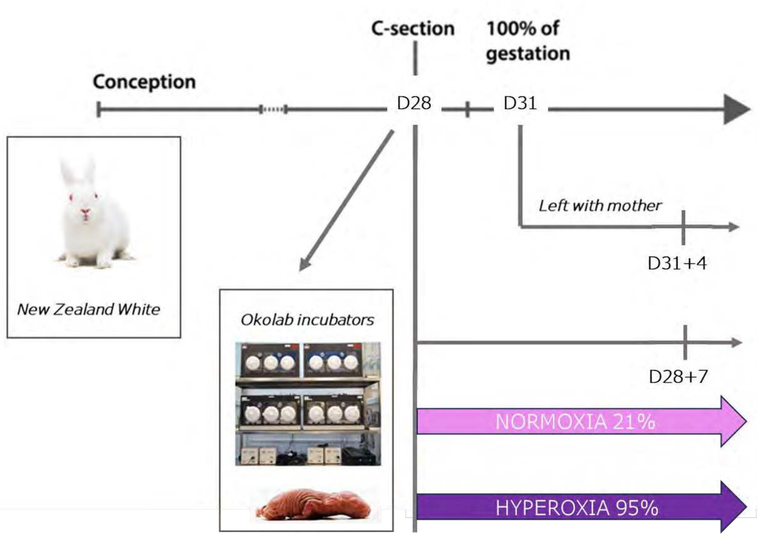

Image adapted from Salaets et al. 2017, Curr. Pharm. Des.

Term (T D31+4):
- NO prematurity
- NO hyperoxia
- NO milk formula (mother's milk)
- Equivalent age of preterm group

Preterm normoxia 21% (N21% D28+7):
- Prematurity
- Normoxia 21%
- Milk formula

Preterm hyperoxia 95% (H95% D28+7):
- Prematurity
- Hyperoxia 95%
- Milk formula

Outcomes

- Lung morphology
- Conventional lung morphometry (Radial Alveolar Count, Acute Lung Injury score and Mean Linear Intercept)
- Lung morphometry using an AI-based software (Secondary Crests count and Septal Thickness)
- Lung function measurements by FlexiVent ™ system
- Immunohistochemistry (IHC) for alpha-Smooth Muscle Actin (α-SMA)
- Automated quantification of pulmonary vascular medial thickness

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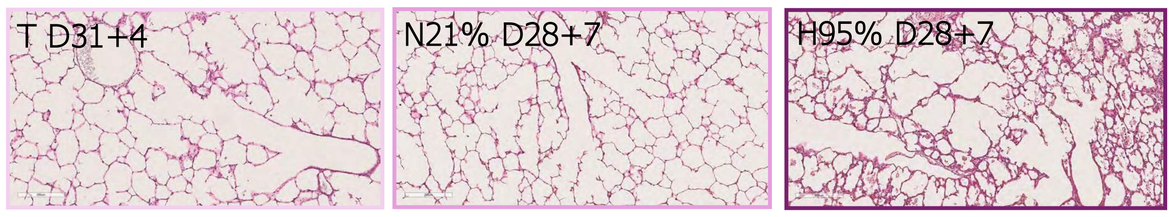
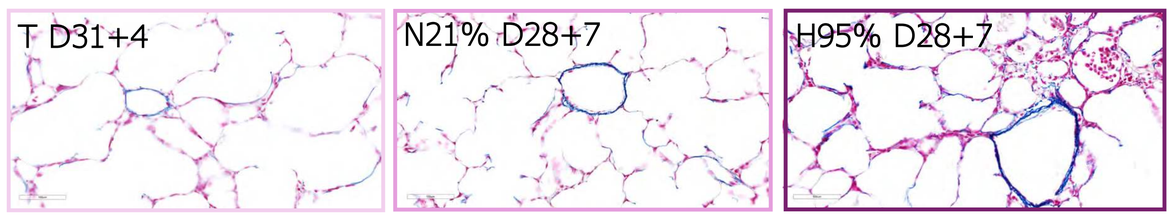
Results

Lung morphology

Hematoxylin and Eosin (H&E) staining



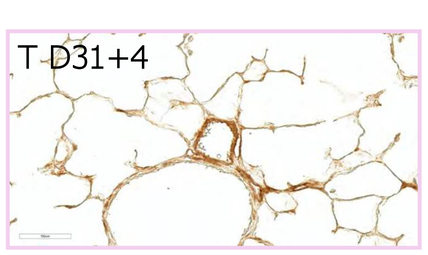
Miller staining (elastic fibers)

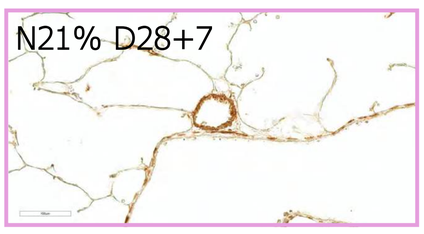


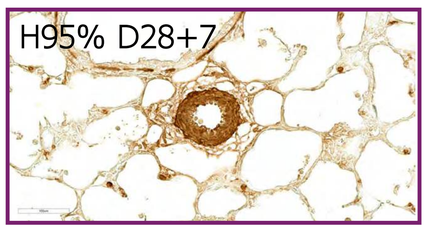
- Fewer alveoli
- Enlarged airspaces
- Thickened alveolar septa
- Infiltration of inflammatory cells
- Proteinaceous debris
- Disrupted elastic fiber architecture

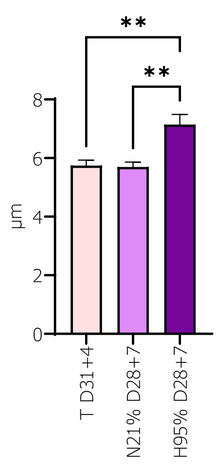
Automated quantification of pulmonary vascular medial thickness

IHC for α-SMA



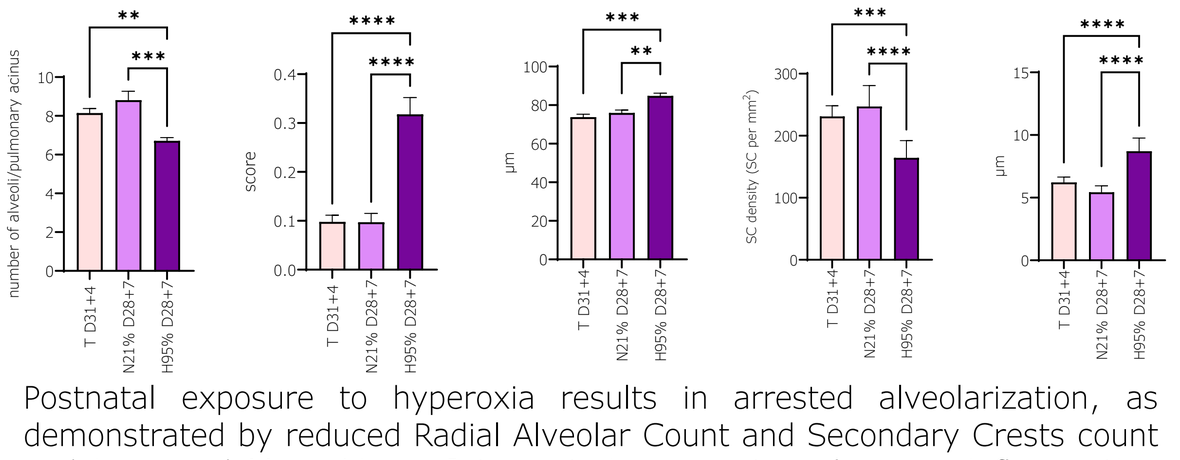






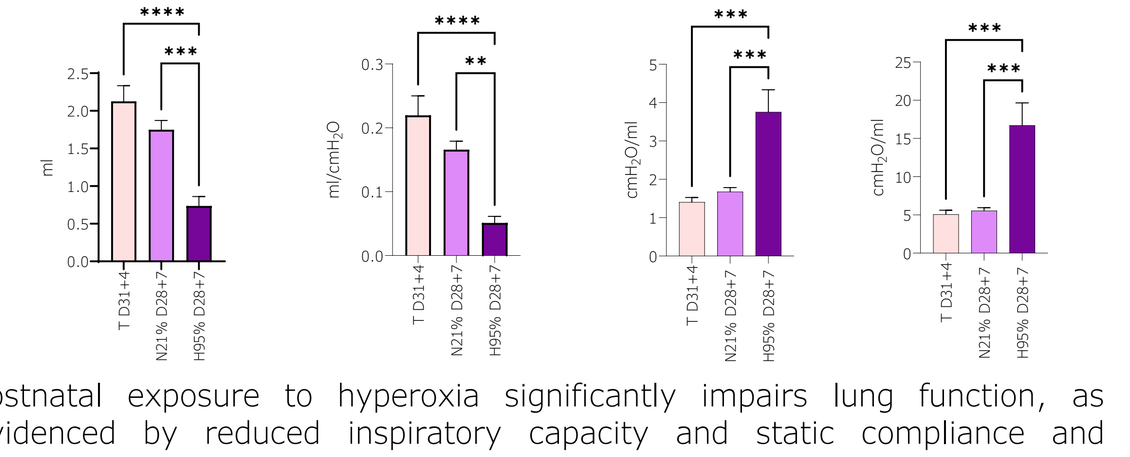
Postnatal exposure to hyperoxia results in a significant increase in thickness of media tunica of small pulmonary blood vessels (major axis length 30-100 µm).

Lung morphometry



Postnatal exposure to hyperoxia results in arrested alveolarization, as demonstrated by reduced Radial Alveolar Count and Secondary Crests count and increased Mean Linear Intercept, in an acute pulmonary inflammation and thickening of alveolar septa.

Lung function



Postnatal exposure to hyperoxia significantly impairs lung function, as evidenced by reduced inspiratory capacity and static compliance and increased tissue damping and tissue elastance.

Conclusions

- This study further confirmed the translational value of the preterm rabbit model exposed to hyperoxia, closely resembling key pathological features of human BPD.
- Morphometric results obtained by both conventional techniques and an innovative AI-based software supported the structural abnormalities observed in pulmonary sections stained with H&E.
- The decline in lung function measured in preterm pups exposed to hyperoxia is strongly correlated with structural changes.

References

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