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Title: A novel method for harvesting and culturing airway epithelial cells from neonates

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Body: Introduction:The airway epithelium is considered important in the pathogenesis of several respiratory conditions including asthma.While studies of airway epithelial cell(AEC) cultures obtained from adults and children have provided valuable insight into the role of these cells in airway disease, little is known about how neonatal AEC phenotype impacts on respiratory disease in later life.We describe a novel method for culturing nasal AEC within days of birth. Methods:AEC were sampled from healthy, unsedated infants within 72 hours of birth by gently brushing both nostrils with an interdental brush. Brushes were agitated in specialised AEC growth media and primary cell monolayers grown to confluence before sub-culture.IL-8 concentrations were measured in supernatants from tertiary passage AEC monolayers at rest and after exposure to IL-1β & TNF-α(both at 10ng/ml). Results:Sampling was acceptable to parents and there were no adverse effects. Primary cultures were successfully established in 74(82%) of 90 neonates sampled. Epithelial lineage of the cells was confirmed by morphological analysis and positive immunostaining for cytokeratin 19 and negative staining for potentially contaminating cell types.Constitutive IL-8 secretion was observed and was upregulated by cytokine stimuli. Conclusion:We describe a safe, minimally invasive and reproducible method of culturing AEC from neonates suitable for functional cell analysis and amenable to large population based studies.This novel technique offers a unique opportunity to study “naïve” AEC not yet exposed to the confounding effects of environmental pollutants and pathogens and may prove useful in elucidating the early origins of respiratory disease.