Mature surfactant protein-B expression by immunohistochemistry as a marker for surfactant system development in the fetal sheep lung

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Introduction: Evaluation of type II alveolar epithelial cells (AECs) is an important measure of the lungs' ability to produce surfactant. The gold-standard for staining type II AECs in lung tissue utilizes antibodies against mature SP-C, which is regarded as the only specific surfactant protein (SP) marker in rodents. Despite sheep being routinely used to study lung development, no study has demonstrated an ideal marker of type II AECs *via* immunohistochemistry in the sheep. Here, we examine surfactant pro- and mature-protein staining in the fetal sheep lung across gestation in order to identify the most reliable marker of type II AECs.

Methods: All experiments were approved by the University of Adelaide and the University of South Australia/IMVS Animal Ethics Committees and performed according to the guidelines of the Australian code of practice for the care and use of animals for scientific purposes. Pregnant ewes were humanely killed at 55 (n=5), 90 (n=5), 120 (n=4), 130 (n=4) and 140 (n=5) d gestation (term, 150±3 d) with an overdose of sodium pentobarbitone (Virbac Pty Ltd, NSW, Australia). Lung tissue from fetal sheep was paraformaldehyde fixed and paraffin processed. Sectioned tissue at each gestational age was used to optimise a panel of primary antibodies directed against either the pro- or mature- proteins for SP-B and SP-C for use in immunohistochemistry. To test for specificity differences in staining of the fetal sheep lung, an anti-human and anti-bovine mature SP-B antibody was also used. Following optimization all antibodies were used on serial sections. Antibody staining patterns were examined at each gestational age by light microscopy and the numerical density of SP positive staining cells in the alveolar epithelium was determined at 120-140d gestation by point counting using Visiopharm NewCAST stereological software.

Results: Staining of all antibodies was restricted to the developing airways at 55 and 90d gestation. With advancing gestation (120-140 d gestation), there was a significant increase in the numerical density of positively stained cells identified in the alveolar epithelium with antibodies directed against SP-B but not SP-C. Moreover, in comparison to the numerical density of positive cells detected with the pro-SP-B antibody, the anti-mature SP-B antibody stained type II AECs more reliably from 120-140 d gestation. The specificity of the anti-mature SP-B antibody used (anti-human *vs* anti-bovine) did not impact on the numerical density of identified SP-B positive cells present in the alveolar epithelium.

Conclusion: The use of a mature SP-B antibody is ideal for identifying type II AECs by immunohistochemistry to evaluate surfactant development in the fetal sheep lung.