DEVELOPMENTAL ROLE OF MILK IN LUNG MATURATION OF MARSUPIAL POUCH YOUNG (TAMMAR WALLABY)

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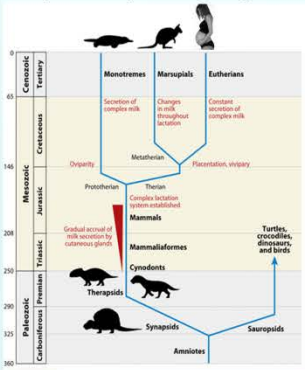
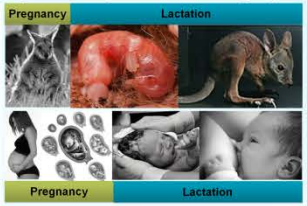
Background:

Respiratory complications are frequently seen in premature infants and the risk of preterm death is high due to incomplete development of the respiratory system. Identifying the components responsible for lung development may provide new intervention therapies to accelerate lung maturation and reduce preterm deaths. Our research is exploiting the unusual reproductive strategy of a marsupial, the tammar wallaby (M.Eugenii), as a unique model to better understand the factors involved in lung development. Marsupial tammar wallaby, have a short gestation and give birth to an altricial young.





The major development of the newborn occurs postnatally and depends entirely on the signalling factors provided through milk. The composition of the milk changes progressively during the Lactation cycle to provide appropriate nutrition and development signals to support this development.

AIM

In the present study we investigated the effect of milk factors in regulating postnatal development of the lung.

Postnatal Lung Development of Tammar wallaby Pouch young

Histological analysis showed that lungs of new born tammars were immature at the time of birth. The majority of development occurred during the early lactation period (day 20-80).

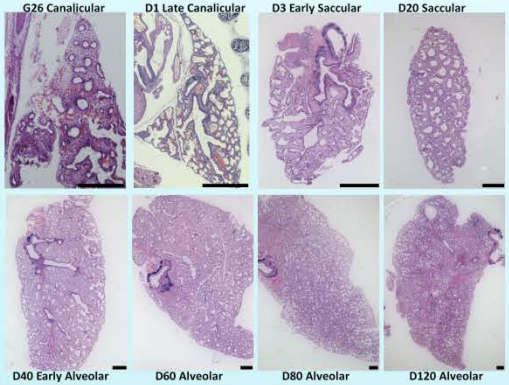
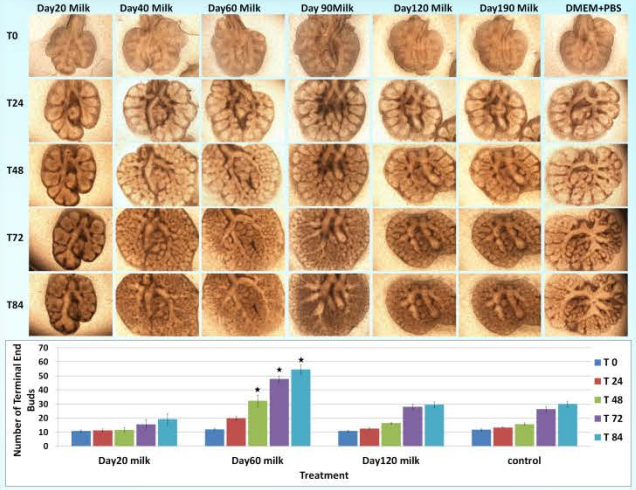


Figure: Histological examination of tammar pouch young lungs harvested during Prenatal gestation~day24 and postnatal day1, day 6, day 20, day40, day60, day80 & day120. Scale bar 1mm

The effect of tammar milk on development of cultured mouse E12 embryonic lung



To examine the role marsupial milk in lung development, embryonic lungs from mice at E12 were cultured in serum free media with 10% of tammar skim milk collected at day 20, 40, 60, 80, 90, 100 120 and 190 of lactation. As a control embryonic lungs were cultured in media with PBS. The embryonic lung cultured with day 20 milk showed no change in the number of terminal end buds. In contrast, embryonic lung cultured with milk from day 40, 60 and 90 of lactation commenced branching after 48h and increased the number of terminal end buds until 84h of culture. The embryonic lung cultured with day 120 and 190 milk underwent branching after 72 h of culture. The control culture of embryonic lungs showed delayed branching.

Expression of developmental marker genes in milk-treated cultures of mouse embryonic lung

Expression levels of SP-C, SP-B, Wnt-7b, BMP-4 and Id-2 marker genes was examined in lung explants treated with day 20, 60 and 120 milk, and a control. The embryonic lungs treated with day 60 milk showed a significant increase in the level of expression of all marker genes in comparison with the control embryonic lung.

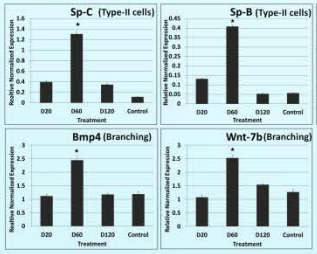


Figure: Expression of lung developmental marker genes in embryonic lung cultured with tammar milk. RNA was isolated from embryonic lungs cultured for 84h to observe the expression of Sp-c & Sp-B (type-II cells marker gene),wnt-7b, BMP4 and Id-2 marker genes. Statistically significant p values (<0.005) shown with an asterisk

Morphological analysis of cultured embryonic lung

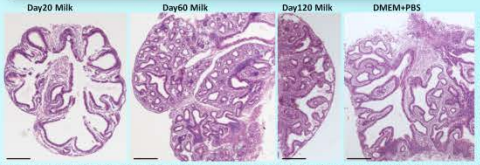


Figure: The lungs treated with day 20 milk had rudimentary branches. In contrast, the explants treated with day 60 milk showed a significant increase in branching. The explants treated with day 120 milk had small epithelial tubules with terminal end buds at peripheral regions of lung. In control cultures of lungs the majority of tissue was populated with long tubules with poor branching morphogenesis and the absence of epithelial sacs. Scale bar 250µm.

Expression of surfactant protein B, C and PCNA (cell proliferation)

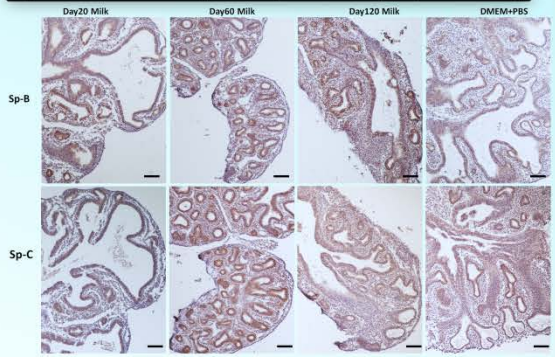


Figure: Immunohistochemical analysis of Sp-B and Sp-C in cultured mouse embryonic lung. The sections of lung treated with tammar milk from day 20, 60, 120 of lactation and PBS (control) were immunostained with type-II cell marker surfactant protein B and C. The Sp-B and Sp-C proteins were highly expressed in embryonic lungs treated with day 60 milk. Scale bar 100µm

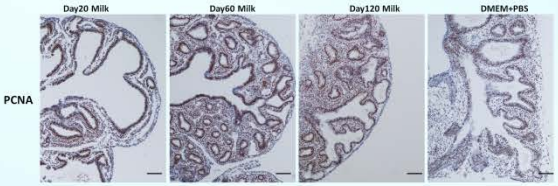


Figure: Cell proliferation in both mesenchyme and epithelium was measured by immunostaining with PCNA antibody and counterstained with haematoxylin. Lungs cultured with day 60 milk showed increased proliferation of the epithelial cells. Scale bar 100µm

Conclusion

The progressive change in the composition of milk and profound effect on marsupial pouch young development presents an excellent opportunity to investigate milk-derived developmental factors that support the maturation of the respiratory system. Tammar wallaby milk may have a significant role in lung maturation of pouch young, particularly between day 20 and 100 of lactation. Current studies are focused on identifying the bioactives responsible for these responses to determine their potential therapeutic applications in preterm human infants to improve short and long term health outcomes.