Rediscovering sheep as a viable animal model seen in BPD, Batten Disease, and Osteoporosis

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Animal Models in Biomedical Research

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Introduction

Animal models are a critical part of the process in understanding the characteristics, transmission, and treatments of human and animal diseases. Different animal models are used at various stages during the research of a disease. While some reviews may argue the use of sheep as an animal model have decreased and murine models have increased, one may argue the use of sheep are essential for specific diseases or moving from one phase of research to another which requires a more comparable model with the human system. The change in use of animal models can be seen through biomedical literature citing rates between the years 1966 and 2000. Although the number of publications citing animal models increased, there was a large increase in murine models and only a small increase in sheep models.¹

Even though mice as a model have shown a large increase, sheep are an important large animal model for the advancement of research after the use of murine models and for specific diseases where other models are not as compatible or appropriate. The available knowledge of the sheep’s anatomy and physiology along with its size allow for insertion of physiological monitoring devices and large blood and tissue sampling.¹ Various diseases will be discussed illustrating the importance of sheep in research and their contribution to the advancement of medicine.

Current Biomedical Research

Bronchiopulmonary dysplasia
Bronchiopulmonary dysplasia (BPD) is a common problem seen among preterm infants and occurs in half of preterm infants with low birth weights\(^2\). BPD, also known as neonatal chronic lung disease, is common to preterm infants with respiratory distress syndrome (RDS) requiring mechanical ventilation. The disease results in increased thickness of smooth muscle in arterioles and bronchioles leading to pulmonary hypertension and expiratory resistance\(^3\). Characteristic of BPD, hypertension becomes a major factor and results in severe morbidity and mortality\(^2\). Along with these symptoms is a strong correlation between inflammation and BPD. Suggesting the issue starts in utero, preterm infants have been noted as having elevated levels of pro-inflammatory cytokines as early as the first day after birth. The presence of chorioamnionitis, inflammation of fetal membranes due to bacterial infection, and aspirate evidence of inflammation can predict BPD\(^4\). These indicators were used in sheep models in order to better understand the disease and develop treatments.

Sheep are an ideal model for respiratory diseases. Sheep can model preterm birth, respiratory failure, and mechanical ventilation, which are critical for representing a clinical situation of an infant with BPD\(^3\). Also considering the knowledge of the sheep anatomy and physiology, the species is very well suited for surgeries and measuring respiratory functions that may not be possible in other smaller models\(^5\). Studies have shown promising comparisons between human conditions and those of preterm lambs. Infants who died with BPD have reductions seen in pulmonary microvascular beds, increases of elastin in arteriole walls, and thicker musculature
in pulmonary arterioles. All of these conditions are seen in preterm lambs exposed to prolonged ventilation and allow for the sheep to be an ideal model for bronchiopulmonary dysplasia\(^3\).

Studies show the antenatal inflammation alone may be a larger contributor to BPD than mechanical ventilation. When preterm lambs were given intra-amniotic endotoxin injections, there was impaired alveolarization. After only 1-4 days of receiving the injection of endotoxin, angiostatic chemokine IP-10 mRNA was induced and eNOS protein content in that lung was decreased by half of the control's value. At 4 to 7 days, there was increased collagen in resistance arterioles and smooth muscle hypertrophy. The endotoxin therefore may be decreasing endothelial cell function by affecting protein expression\(^2\). Considering prenatal inflammation can affect lung development, reducing inflammation prior to birth should reduce cases of BPD. Although a large decrease in RDS has been seen due to antenatal steroids that improve lung development, other postnatal factors including mechanical ventilation, high oxygen and inflammation still exist leading to BPD\(^5\). The use of non-invasive ventilation as opposed to mechanical ventilation proves to have its benefits. Preterm lambs using nasal LPHFV showed proper alveolar formation and capillary bed growth along with elastin and growth factors\(^3\). Since BPD is multifactorial, the use of preterm lambs to understand the antenatal and postnatal factors has been valuable for biomedical advancements.

\textit{Batten Disease}
Batten disease, also known as neuronal ceroid lipofuscinosis (NCL), is an inherited disease due to a mutation in one gene and results in blindness, seizures, loss of motor function, and possible death. The neurodegeneration seen is caused by accumulation of autofluorescent storage material in the lysosome of all tissues.

With 1 in every 12,500 live births resulting in Batten disease, it is the leading neurodegenerative disease in children and therefore it is important to find a cure and advance in treatments.

Since studies in humans with NCL are particularly strict, the use of animal models is essential to the understanding of the disease. Although significant development in understanding the genes involved with NCL has been accomplished with the use of murine models, they do not entirely represent the human disease pathology. Large animals are critical for translating advances to the clinic. Many large animals, including sheep, have naturally occurring NCL and are useful due to the similarities in the pathology of the disease as well as the anatomy of humans. Due to their longer lifespan than murine models, batten disease can be studied over a long period of time. Long-term research allows for advancements in understanding the progression of diseases and the results of treatments. Another benefit of using sheep as an animal model is their large brain, which can be used for more technical injections and surgical techniques that cannot be done in small animal models. Due to the naturally occurring nature, various genes causing disease have been identified among sheep and have aided in better understanding the pathology of Batten disease.
Two naturally occurring forms of batten disease, CLN5 and CLN6, have been found in sheep. These forms are both a subunit c of mitochondrial ATP synthase storing forms of batten disease. Mutations in CLN5 causes a loss of function of mannose-6-phosphate- tagged lysosomal protein, where CLN6 is characterized by a mutation in a nonsecreted endoplasmic reticulum membrane protein. Those affected with the disease in sheep and humans are both depicted by cortical atrophy and neurogeneration. These similarities allow us to associate our findings in sheep to humans.

Sheep have contributed to the research of the disease through the studies of gene therapy trials. Cultured sheep neural cells can be used to determine the transduction and tropism of lentiviral vectors to neural cells and their ability to target affected and non-affected NCL cells. Gene transfer can then be tested in vivo by the precision of stereotactic injection into the cerebrum of cerebral spinal fluid of sheep with form CNL6 NCL. Due to success of the lentiviral mediated gene transfer and transgenic protein production within the sheep, gene therapy trials of humans with Batten disease using AAV vectors have been approved. Other results also show increasing injection volumes directly increased vector spread, which is beneficial for a wider distribution without the need of multiple injections. These findings together with the use of sheep can be used to target other neural type NCL cells and neurodegenerative disorders and aid in the continual development of gene therapy to optimize functional effects.
Osteoporosis in Menopause

The incidence of osteoporosis fractures has been increasing. With every second, one woman and every third male will experience this effect in a lifetime. Post-menopausal osteoporosis is especially a major health problem among older women. Due to the large prevalence among populations and slow progression of the disease, long term research and the use of animal models is critical in finding treatments and prevention of the disease.

Animal models can be used to understand the mechanism of fractures, healing techniques, and properties affecting bone mineral density (BMD). Although initial research can be done with rats, large animal models that have Haversian systems and remodeling patterns similar to humans are needed. Unlike humans, osteoporosis is rarely naturally occurring in animals and therefore has to be induced. Upon induction, sheep have frequently been used due to the consistent results of BMD loss compared to cats and dogs that do not show significant reductions. Pigs have a more dense bone structure that is not representative of humans and primates are expensive. Therefore, sheep are an ideal model for osteoporosis.

Sheep can be used in studies to determine what actions can be taken in order to prevent and treat osteoporosis in humans. In order to induce osteoporosis in sheep, they receive an ovariectomy, dietary restrictions of calcium and Vitamin D, and are
given steroids such as glucocorticoids. A combination of treatments is needed to induce osteoporosis since individually none suffice to resemble osteoporosis seen in humans. Many studies report that physical activity can help bone structure by improving BMD and reduce fractures. In order to use this as a prevention and treatment, sheep models can help us understand what levels of activity will enhance the bone and what levels will become detrimental. In order to study this, sheep are confined to a chute where they receive vertical ground-based vibrations to their hind limbs. Sheep are also ideal for these studies due to their docile nature, which allows them to be manipulated easily in the chute. After six months, vibration therapy showed osteogenic effects in experimental conditions compared to the control. This is only one of many studies contributing to osteoporosis research.

Another study has been conducted with hydroxylapatite (HA) coating over porous ingrowth surfaces. The peri-apatite covering effects were tested based on the push out strength of implants in both distal femurs of ovariectomized sheep. Six weeks after implantation, the animals were euthanized and the BMD of the femurs were measured. Peri-Apatite coating showed advances in osseointegration where the bone and implant made a firm attachment. This study may suggest a new, more effective coating that can be used in humans. Overall sheep have shown to be an important animal model for osteoporosis.

**Discussion**

Choosing the correct animal model for a disease is paramount to the research at
hand. Although murine and rat models have been used for large amounts of biomedical research due to their size, price, and availability, they can only be used to a degree. To advance in research, it is key to find models that closely resemble the clinical state. Sheep have shown promising advancements in various diseases including bronchiopulmonary dysplasia, Batten disease, and osteoporosis. Although the use of sheep may not be as prevalent as other smaller models, it is important to remember they are essential in specific diseases and without them much research would go undiscovered.


8. Lill, CA, Gerlach, UV, Eckhardt, C, Goldhahn, J, Schneider, E. Bone changes due to glucocorticoid application in an ovariectomized animal model for
