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***Livestock Models in Translational Science***

**Roth and Tuggle. Livestock Models in Translational Medicine, pp. 1-6**

Domain 3: Research

TT3.3: Animal models (spontaneous and induced) including normative biology relevant to the research

SUMMARY: As the title suggest, this article focuses on livestock models in translational medicine.

When compared to rodent models, livestock as models for human disease offer 3 distinct advantages:

1.  Livestock species are more genetically diverse than rodents and reflect the complexity of applying medical advancements to an outbred species.

2.  Many livestock diseases are more similar to human diseases than are rodent diseases.

3.  Livestock models provide the advantage of similar organ size and function and the ability to serially sample an animal throughout the study period.

Livestock translational models of human disease were divided into two broad categories in this article.

Microbial and Infectious Disease

* Transmissible Spongiform Encephalopathies (TSE): Scrapie in small ruminants and bovine spongiform encephalopathy (BSE) are important models for studying prion diseases in humans.
* Mycobacterial infections: *Mycobacterium bovis* in cattle is a model for studying tuberculosis in humans. Advances in diagnostic testing and vaccine for the prevention of tuberculosis in people have been tested with *Mycobacterium bovis* infection in cattle.
* Influenza A Virus (IAV) Infection: IAV infection in pigs is an important model for studying influenza in humans mainly because the same subtypes are endemic in both species and are commonly exchanged between species. The swine model has been useful in understanding vaccine-associated enhanced respiratory disease (VAERD), which occurs when inactivated vaccines are not closely matched to the hemagglutinin protein of the challenge strain of virus.
* Vaccine Development: Large animals are often better than rodents for predicting vaccine outcomes in humans.
* Human Microbiota: Pigs are a great model for studying human microbiota.  Human Microbiota Associated (HMA) piglets have been established using inocula from human infants.

Metabolic, Neoplastic and Genetic Disorders

* Stem Cell biology: Pigs are used to study the safety, efficacy and stability of induced pluripotent stem cells (iPSC) therapy.
* Male Germ line cell biology: Unmatched donor and recipient of gene cell transfer are successful only in large animal species.
* Pulmonary adenocarcinoma: Ovine pulmonary adenocarcinoma in sheep is a model for studying non-small cell lung cancer in humans.
* Muscular dystrophy:  Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are caused by inadequate dystrophin function. Current animal models used to study DMD and BMD are the mouse mdx model and X linked muscular dystrophy in golden retrievers.  However, a porcine dystrophy model is advantageous because of the similarity of pig and human hearts, as cardiomyopathy is a growing cause of death in muscular dystrophy patients.
* Wound healing: The pig is commonly used to study skin wound healing. Diabetes is a chronic disease that adversely affects wound healing. Pigs are administered streptozotocin to induce diabetes and thus creating a diabetic wound healing model. Also, Duroc pigs are used as a model for studying hypertrophic scarring.

QUESTIONS

1.  What are 2 TSEs in livestock that have been used to understand prion disease in humans?

2.  True or False: Does subtypes of influenza A H3N1 occur in pigs?

3. What is the etiologic agent for ovine pulmonary adenocarcinoma?

4.  What drug is used to induce diabetes in a pig?

5.  What breed of pigs are used to study hypertrophic scaring?

ANSWERS

1.   Scrapie in small ruminants and bovine spongiform encephalopathy

2.  True, all influenza subtypes that occur in humans also occur in pigs

3.   Jaagsiekte Sheep Retrovirus (JSRV)

4.  Streptozotocin

5. Duroc

**Greenlee and West Greenlee. The Transmissible Spongiform Encephalopathies of Livestock, pp. 7-25**

Domain 1: Management of Spontaneous and Experimentally Induced Diseases and Conditions

SUMMARY

Prion Disease: A family of infectious protein misfolding disorders resulting from aberrant folding and accumulation of the prion protein that leads to neurodegeneration.  These commonly accumulate in lymphoid and nervous tissues (particularly CNS).  Prion diseases share similar mechanism with other protein misfolding disorders (ALS, Parkinsons, etc.), however are distinct due to the fact that they are readily transmissible.

Strains of Prion Disease

Strains can be differentiated by:

   Differing clinical signs

  Incubation periods and lesion profiles in mouse models

  Cellular and neuroanatomical deposition patterns of PRP

   Molecular profile on Western Blot

o   Electrophoretic activity

o   Reactivity to antibodies near the PK cleavage point

o   Glycoform ratios

Strains of BSE and scrapie can be classified as either classical (characteristics consistent with the disease endemic in a population) or atypical (can be differentiated from classical due to molecular or epidemiological characteristics which differ). Transmissibility and pathogenesis are dependent upon genotype of host animal, strain of TSE, and route of exposure.

Prion Diseases and Livestock

Fatal consequences for individual animals

Economic importance

Potential public health concern

   Scrapie

o   Can cost $10-12 million annually in production loss, export loss, and increased costs

o   Eradication program began in 1952, revisited in 2001, resulted 90% decrease in scrapie positive culls at slaughter

  BSE

o   Single case in 2003 in Canadian imported animal

o   $4.7 billion loss to beef industry in 2004

o   UK 180,000 cattle diagnosed

Prion Disease and Human Health

TSEs in humans classified as:

   Sporadic

  Genetic

  Acquired – iatrogenic Creutzfeldt – Jakob Disease (iCJD), kuru, vCJD (zoonotic)

TSEs of Livestock

Scrapie

   Genetics of susceptibility

o   136, 154, and 171 codons of PRNP significant in determining susceptibility or resistance

  171 most important

  171QQ – susceptible

   171RR – resistant

o   Allelic variation affects incubation times and susceptibility

o   K222 may make a good candidate for selective breeding programs to enhance scrapie resistance in goats

   Classical scrapie in sheep

o   First described (300 years ago) and most studied

o   First U.S. case 1947

o   Now has low incidence and is rare due to eradication programs

o   Vertical transmission and horizontal at lambing

o   2-7 yr asymptomatic period

o   Passes through enterocytes into bloodlymphreplication in lymphoreticular system

o   Nictitating membrane biopsies useful in ante mortem IHC diagnosis

   Atypical Scrapie

o   Nor98 – Norway

o   Western Blot band at 12 or lower kDa vs. classical at 19-21 kDa lower band

o   Does not appear to be very contagious

o   Also described in goats

  Classical scrapie in goats

o   First experimentally transmitted from sheep in 1939\

o   Lymphoid tissue – retropharyngeal and palatine tonsils important

o   Genotype at codon 142 determines susceptibility

o   Lower accumulation in placenta vs. sheep

  Atypical scrapie in goats

o   Histidine substitution at codon 154 risk factor

o   Not demonstrated in lymphoreticular system

   Bovine Spongiform Encephalopathy (BSE)

o   First diagnosed in U.K. in 1985

o   Origin unknown however spread via utilizing MBM (ruminant derived meat and bone meal) to cattle

o   Ataxia, aggression, and weight loss common

o   SRM – specified risk materials – defined as: brain, skull, spinal cord, trigeminal and dorsal root ganglia, eyes, vertebral columns in cows over 30 mos old and tonsils and ileum of all cattle

o   Three types

  Classical

    MBM foodstuffs, however no vertical or horizontal transmission demonstrated

  Highest susceptibility in first six months of life

  Atypical H-Type & L-Type differ in Western Blot markers and lesion distribution with shorter incubation times

o   All types do not accumulate in lymphoreticular as much (vs. scrapie in sheep)

  Experimental Interspecies Transmission

o   Oral and intracranial exposures most frequent

o   Primary passage often not efficient between species

o   Species barrier theory formed due to lack of susceptibility, incomplete attack rates, or prolonged incubation times

o   Scrapiewhite tailed deer orally and cattle IC (but very different pathogenesis and lesion distribution than both BSE and scrapie in sheep)

o   CWD – cervid prion disease not readily transmissible to sheep and cattle, however not orally

o   TME – minks, can be transmitted IC to sheep, goats, (orally and IC) and cattle (IC)

  Diagnosis of TSEs in Livestock

o   Scrapie – rectal mucosa, tonsil, third eyelid IHC shows promise for ante mortem diagnosis

o   BSE – no valid ante mortem test yet, behavioral observation key

QUESTIONS

1. The misfolded form of the prion protein is commonly denoted as what?

2. True or False: Normal cellular protein is in a predominantly alpha-helical structure.

3. True or False: IHC of rectal mucosa can be used as a preclinical ante mortem diagnostic test for identification of BSE in cattle.

ANSWERS

1. PRPSC

2. True

3. False

**Waters and Palmer. *Mycobacterium bovis* Infection of Cattle and White-tailed Deer, pp. 26-43**

Domain 1: Management of spontaneous and experimentally induced diseases and conditions

Tertiary Species: Other Livestock

SUMMARY: Bovine tuberculosis (TB) results primarily from infection with *Mycobacterium bovis,* a member of the *M. tuberculosis* complex. Comparative analyses of *M. tb* and *M. bovis* genomes indicate that *M. bovis* evolved from an ancestral *M. tb* strain, since the *M. bovis* genome is smaller than that of *M. tb. M. bovis* has a wide host range, is infectious to humans, and causes significant economic hardship for livestock farmers. It is estimated that more than 50 million cattle are infected worldwide, costing $3 billion annually. Zoonotic transmission of *M. bovis* occurs primarily via ingestion of unpasteurized dairy products or close contact with infected cattle. Increasingly, infection of cattle also results from direct or indirect exposure to *M. bovis*-infected wildlife. Tuberculosis due to *M. bovis* has been reported in a large number of wild ruminant species. As the prevalence of TB in cattle decreases, the relative importance of *M. bovis-*infected wildlife increases, and disease-control measures are required for both livestock and wildlife. The most recognized wildlife reservoir hosts include the Eurasian badger in the UK, brushtail possum in New Zealand, wild boar in Spain, and the white-tailed deer in the US. In humans and most species of animals, *M. bovis* infection is acquired through exposure to infectious aerosols. However, lesion distribution in naturally infected white-tailed deer suggests that oral exposure through ingestion is an important route of exposure. Bovine TB manifests as a chronic, caseonecrotic, granulomatous, inflammatory response primarily affecting lungs and lymph nodes. These characteristics can be applied to *M. bovis* infection in other ruminants such as sheep, goats, and deer. Tb lesions tend to be more liquefied or abscess-like in deer in contrast to the caseous nature of lesions in cattle.

The first effective vaccine used in humans was tested in cattle prior to its first use in a human infant in 1921. Field vaccination with BCG results in variable efficacy (0-80%) in cattle herds, and revaccination does not improve efficacy. The first known reservoir of *M. bovis* in white-tailed deer was identified in 1975 in northeastern Michigan. Two management strategies have been implemented to control *M. bovis:* 1) decrease deer population densities via hunting to biological carrying capacity. 2) restrict supplemental feeding of deer. A number of TB vaccines have been tested in cattle for efficacy against experimental infection with virulent *M. bovis* over the past 10 years. Oral immunization provides a level of protection similar to that of parenteral routes yet requires a higher dose of BCG. Oral BCG vaccines have been tested in deer as well, and have been shown to protect against disease and infection in certain situations. Methods have been developed and standardized for the assessment of the immunopathogenesis of *M. bovis* infection, particularly in cattle but also in other livestock and wildlife species.

QUESTIONS

1. What is a main risk factor for zoonotic transmission of *M. bovis?*

a. Consumption of milk from *M. bovis-*infected cows

b. Inhalation of aerosolized particles

c. Consumption of meat products from *M. bovis-*infected cows

2. Which route of exposure is considered more important in white-tailed deer than humans?

a. Inhalation of an aerosol

b. Contact

c. Ingestion

3. The most common site for tuberculous lesions to appear in deer are the \_\_\_\_, while in cattle it is \_\_\_\_.

a. Tracheobronchial lymph nodes; lung

b. Retropharyngeal lymph nodes; lung

c. Lung; retropharyngeal lymph nodes

ANSWERS

1. a
2. c
3. b

**Rajao and Vincent. Swine as a Model for Influenza A Virus Infection and Immunity, pp. 44-52**

Domain 3: Research

Primary Species: Pig (Sus scrofa)

SUMMARY: Influenza A viruses (IAVs) belong to the Orthomyxoviridae family and are classified according to the subtypes of their major surface glycoproteins hemagglutinin (HA) and neuraminidase (NA). Influenza virus can be spread by direct contact, contaminated fomites, and inhalation of virus-containing aerosols. Many species are used to study IAV with various limitations. Mice are easily acquired and maintained, but they do not shed virus and adaptation of the human virus is required. Ferrets are susceptible to many human IAVs without adaptation and they shed virus once infected, but they are more expensive with limited availability, and fewer species-specific immune reagents exist. Guinea pigs are easily acquired, readily infected with human viruses, and their respiratory tracts have similar traits to humans, but clinical disease is reduced and strain-dependent. Swine are natural hosts of IAVs, susceptible to both avian and human strains, their genome has a similar size and complexity to humans, their tracheobronchial structure and immune parameters closely resemble those of humans, and the same IAV subtypes are endemic in pigs and humans. These factors make pigs valuable for studying virus-specific and host-specific factors that determine the pathogenesis of human IAVs.

Virus-Specific Factors: HA plays a major role in determining the host range by binding to oligosaccharide receptors that contain terminal sialic acid (SA). Pig SA receptor expression patterns and binding specificity are similar to those of humans. Ecology and transmission of IAVs partially depends on pH and endogenous proteases. Human and swine IAVs share similar optimal pH variation and usually contain an arginine at the HA0 cleavage site, which determines infectivity, and they are activated by trypsin in vitro.

Host-Specific Factors: Protection from influenza infection involves the innate immune response in the early stages of infection via controlled replication by natural killer cells, alveolar macrophages, and dendritic cells, as well as the induction of adaptive immune responses. In adaptive immunity, a virus-specific humoral response to HA and NA correlates with protection in human IAV infection. Mucosal or secretory IgAs are important for local protection and neutralization and are elevated in both pigs and humans after vaccination with live attenuated virus. Cell-mediated immunity plays a critical role for resolution and clearance via CD4+ T cell activation of B cells and antibody production or through CD8+ T cell-mediated lysis of infected cells.

QUESTIONS

1. What virus family does influenza belong to?

a. Paramyxoviridae

b. Orthomyxoviridae

c. Orthomyxovirinae

d. Paramyxovirinae

2. Influenza virus can be transmitted by direct contact with infected individuals, contact with contaminated fomites, and inhalation of aerosols containing virus. What is the LOWEST biosafety level that should be used to study the virus?

a. BSL-1

b. BSL-2

c. BSL-3

d. BSL-4

3. Which of the following immune system features are involved in the regulation and clearance of influenza viruses?

a. Macrophages

b. Natural killer cells

c. CD4+ T cells

d. CD8+ T cells

e. None of the above

f. All of the above

ANSWERS

1. b

2. c (aerosol transmission = must be above BSL-2)

3. f

**Gerdts et al. Large Animal Models for Vaccine Development and Testing, pp. 53-62**

Domain: 1: Management of Spontaneous and Experimentally Induced Diseases and Conditions

T2: Control spontaneous or unintended disease or condition

SUMMARY: The ultimate goal of any vaccine is to elicit a protective immune response and memory to the pathogen of interest.

Animal models are typically used to assess:

1. Vaccine safety

2. Protection against challenge infection from the pathogen of interest

3. Dose and formulation of the vaccine (i.e., enhancement of the immune responses through adjuvants)

4. Optimal route of delivery

5. Onset, magnitude, and duration of the immune response

6. Type of immunity

7. Correlates of protection

8. The induction of specific immune compartments

9. Fate of the vaccine components following immunization

10. Potency of the vaccine after long duration of storage.

Two types of large-animal models are used to evaluate vaccine efficacy: natural and surrogate.

Natural Models

1. Animal pathogens are similar to the human infectious agent
2. Both large animals and humans can develop disease if infected by related pathogens.

Surrogate Models

1. The human pathogen is used to challenge a permissive heterologous animal species
2. The pathogen is able to replicate, but does not necessarily cause clinical disease
3. The surrogate model should have a similar route of entry and infection, replication and disease in the target organs.
4. If disease does develop, the symptoms are usually milder.

Criteria for a Good Animal Model in Vaccine Research

1. Needs to closely resemble the disease in humans
2. Access to various immune compartments
3. Similar response to the same vaccine as humans
4. Needs to have multiple readouts including protection, specific immune compartments, etc.

The Advantages of Using Large Animal as Vaccine Models

1. The outbred nature of large animals (low and high responders)
2. The neonatal period more closely resembles that of a human infant than does a rodent model
3. More accurately predict the minimal effective dose of the vaccine and adjuvant or delivery vehicle in the formulation.
4. More suitable for determining the transfer of both maternal antibodies, cytokines, and lymphocytes across the placenta and via milk and colostrum.
5. It provide access to various immunological compartments and allow serial collection of large quantities of cells over a long period of time
6. It allows testing various delivery routes
7. Comparable anatomical size to humans especially for testing the mucosal delivery
8. The afferent and efferent lymphatic vessels from both mucosal and peripheral tissues of sheep and cattle can be cannulated so that innate and adaptive immune responses to vaccination
9. Surgical models such as fetal immunization and gut loop for mucosal immunity studies
10. The distribution of the pattern recognition receptors such as toll-like receptors (TLRs) in rabbits and pigs resembles to humans more that rodent models
11. Duration of gestation
12. Fetal placentation, and the development
13. Fetal mmunocompetency similar to humans, In mice the peripheral lymphoid tissues are populated only after birth by B and T cells
14. Omnivorous like humans (pig)
15. Placentation does not allow the transfer of passive immunity during pregnancy, which is an advantage for investigating developmental immunology (cattle, goat, horse, pig, sheep)
16. Lifespan allowing the study of degenerative diseases and vaccine development in elders (cattle, goat, horse, pig, sheep)
17. High genome and protein sequence homologies with humans (pig)
18. Cheaper and ethically more acceptable than primates (cattle, goat, pig, sheep)
19. Breeding conditions are very standardized (cattle and pig)
20. IL8 has been identified in large-animal species (cattle, goat, horse, pig, sheep), not in mice
21. Large animals have economic value for themselves such that vaccines can be applied to livestock industries
22. Several zoonotic diseases are naturally present in large-animal species (influenza, tuberculosis, Johne’s disease)
23. Use of large-animal models offers a means of reducing the risk of a failure during human clinical trials, therefore could be cost effective to use large animal
24. May prevent discarding technologies that are not efficacious in mice but might be in humans

Disadvantages of Large Animal Models

1. More expensive than mice (cattle, goat, horse, pig, sheep)
2. Fewer tools than mice (cattle, goat, horse, pig, sheep)
3. Require bigger and more expensive facilities than mice (cattle, goat, horse, pig, sheep)
4. Genome still not fully annotated (cattle, goat, horse, pig, sheep)
5. Less close to humans than monkeys (cattle, goat, horse, pig, sheep)
6. Ethically less accepted than mice (cattle, goat, horse, pig, sheep)
7. Still limited access to inbred animals for basic research (cattle, goat, horse, sheep)
8. Large-animal research community smaller than its mouse counterpart
9. Clear differences with humans in terms of anatomy, immunology, and physiology (i.e., several stomachs in ruminants, extrathymic CD4+/CD8+ double positive T cells in pigs
10. No alphadefensins in pigs,
11. Large proportions of γδ T cells in pigs and ruminants

Pigs

1. One of the most appropriate and reliable models for human vaccines due similarity of immunity and physiology
2. The porcine immune system resembles humans for >80% of analyzed parameters, however mouse only 10%
3. Organs such as kidney and liver that are similar in size and function to those in humans
4. Porcine have inverted structure of peripheral and mucosal-associated lymph nodes. The majority of the lymphocytes exit directly into the bloodstream.
5. The upper and lower respiratory tracts of the pig are similar to those of humans; good animal model for intranasal vaccine studies
6. Pigs have tonsils, but they are absent in mice
7. The skin of pigs displays an epidermis-to-dermis ratio close to that found in humans
8. The epitheliochorial placenta
9. of pigs prevents transfer of antibodies throughout pregnancy
10. Pigs have a right-sided dominant circulatory system similar to that of humans
11. Relatively large litter sizes of genetically similar offspring is useful maternal antibody protection, as well as reducing the numbers of mothers required in large trials
12. The cost of pigs is not prohibitive as an animal model
13. Short generation interval (12 months) and all season breeding
14. Pigs grow quickly, and handling older animals requires experience and appropriate infrastructure
15. Porcine are used as animal model for *Bordetella pertussis, rotavirus, Chlamydia trachomatis*, *Mycobacteria* and influenza

Calves and Cattle

1. Gestation time similar to humans (9 months, cattle)
2. The closely related human respiratory syncytial virus (HRSV) and bovine RSV (BRSV) cause respiratory tract infections in young children and calves. Cattle are not susceptible to HRSV. Chimpanzees show similar permissiveness to HRSV
3. The neutralizing monoclonal antibodies to the fusion (F) protein of HRSV, which is the major protective antigen, recognize the F protein of BRSV, and vice versa
4. Bovine and human CD8(+) T-cells recognize similar proteins that are conserved between HRSV and BRSV
5. Vaccine candidates containing HRSV components can be tested against BRSV challenge
6. Mycobacterium tuberculosis in cattle is another natural model for human vaccine testing
7. M. tuberculosis and M. bovis share >99% genetic identity and induce similar host responses and disease profiles upon infection
8. Neonatal vaccination with the attenuated M. bovis Bacille Calmette-Guerin (BCG) vaccine confers partial protection in cattle

Sheep

1. Relatively low cost
2. Sheep are used extensively for studies on mucosal immunization
3. Challenge of lambs with BRSV or HRSV results in viral replication and lung pathology
4. Sheep as a natural model for testing a vaccine against a zoonotic pathogen is Rift Valley fever virus (RVFV)

Horses

1. Due to its longevity, good animal model for vaccine development for elderly
2. Horses used for development new generation of equine vaccines such as for modified live, temperature-sensitive, intranasal influenza vaccine, chimeric, live attenuated West Nile virus vaccine
3. An equine DNA West Nile virus vaccine, the first registered DNA vaccine in any species, subunit Hendra virus vaccine

QUESTIONS

1. Which of the following large animal model provide the opportunity to evaluate West Nile virus vaccine efficacy in outbred populations?
   1. Pigs
   2. Horses
   3. Sheep
   4. Cattle
2. Which of the following animal model IS NOT considered a natural model?
   1. Influenza in ferrets
   2. Rift Valley fever virus (RVFV) in sheep
   3. Neisseria gonorrhea in mice
   4. Rotavirus in calves
3. Which of the following animal has inverted structure of peripheral and mucosal-associated lymph nodes?
   1. Pig
   2. Sheep
   3. Rat
   4. Goat
4. All of the following are advantages of using large animals in vaccine research EXCEPT:
   1. Allow serial collection of large quantities of cells over a long period of time
   2. Fetal immunocompetency is similar to humans
   3. Genome fully annotated
   4. Use of large-animal models offers a means of reducing the risk of a failure during human clinical trials
5. Mice models are suitable for vaccine research EXCEPT:
   1. IL8 has been identified in mice
   2. In mice the percentage of γδ T cells in peripheral blood is similar to humans
   3. Cheaper and ethically more acceptable
   4. More reagents and tools are available for research
6. Which is the following is UNTRUE about human respiratory syncytial virus (HRSV) in bovine model (BRSV)?
   1. Both cause respiratory infection in young ( children and calves)
   2. The neutralizing monoclonal antibodies to the fusion (F) protein of HRSV, which is the major protective antigen, recognize the F protein of BRSV, and vice versa
   3. Bovine and human CD8(+) T-cells recognize similar proteins that are conserved between HRSV and BRSV
   4. Cattle are susceptible to HRSV infection
7. Which of the following statement is INCORRECT about surrogate animal models:
   1. Surrogate models have provided a useful tool for studying specific aspects of either disease pathogenesis or the host's immune response
   2. If disease does develop, the symptoms are usually milder.
   3. The pathogen is able to replicate, but does not necessarily cause clinical disease
   4. Both animals and humans can develop disease if infected by related pathogens.
8. T/F. The mouse immune system resembles humans for >80% of analyzed parameters, however porcine only 10%.
9. T/F. Porcine is used as animal model for *Bordetella pertussis*, rotavirus, *Chlamydia trachomatis,* *Mycobacteria spp*. and influenza.
10. T/F. An equine DNA West Nile virus vaccine, the first registered DNA vaccine in any species.

ANSWERS

1. b
2. c
3. a
4. c
5. a
6. d
7. d
8. F. The opposite is true
9. T
10. T

**Wang and Donovan. Human Microbiota-Associated Swine: Current Progress and Future Opportunities, pp. 63-73**

Domain 3

Primary Species: Pig (Sus scrofus)

SUMMARY

Introduction: The gut microbiota and the host have a mutualistic relationship, as the microbiota is important for digestion, vitamin synthesis, immunity, pathogen resistance, and brain development.  Fun fact: there are 3-10 more time more microbial organisms in the human gut than there are cells that comprise the human body!  The role of gut flora on health has been extensively studied in rodents, but since there are many physiological and metabolic differences between rodents and humans, and because many rodent models don’t develop the same clinical presentation as humans, there is a need for a more clinically relevant model such as the pig.  Three pig models are discussed in this article: the conventional, gnotobiotic, and human microbiota-associated.

The Conventional Piglet: The advantage of the use of pigs is the many physiologic and anatomic similarities between pigs and humans. The main limitation of this model is that the natural gut flora of the conventional piglet is quite different from that of human infants.

Germ Free and Gnotobiotic Pigs: Gnotobiotic animals are those that are colonized with known strains of bacteria or microbiota.  Germ free animals are completely free of all microorganisms.  Gnotobiotic pigs have been fairly extensively studied, and there is a table (Table 2) demonstrating the many differences between conventional and gnotobiotic pigs.  Some examples include: organ size, small intestinal length, small intestinal weight, epithelial cell turnover rate, crypt depth and villus height.  Despite these differences, germ free and gnotobiotic pig models have been valuable to study enteric diseases that affect both pigs and humans.

Human Microbiota-Associated (HMA) Pig: For a number of reasons, the HMA pig is proving to be a superior model to the HMA rodent and the conventional pig.  Thus far, transfer of human infant microbiota appears to be the most successful.  Specific areas of focus include testing the effects of pre- and pro-biotics and vaccinations on microflora.  Future directions for this model include further pursuing the study of the microbiome in general (most studies thus far have shown associations rather than true cause and effect), understanding the role of microbiota on development, and studying microbiome-related diseases such as dysbiosis, and the potential for microbiota-targeted therapies.

QUESTIONS

1.  What is the difference between germ free and gnotobiotic?

2.  Of the models described in this article, which one appears to be the best model to study the human microbiome?

3.  What is the predominant bacterial organism in human infants, vs. pig infants?

ANSWERS

1.  Gnotobiotic animals are those that are colonized with known strains of bacteria or microbiota.  Germ free animals are completely free of all microorganisms.

2. Human microbiota-associated (HMA) pig

3. Human: Actinobacteria; pig: Bacteroidetes and Firmicutes

**Roberts et al. Livestock Models for Exploiting the Promise of Pluripotent Stem Cells, pp. 74-82**

Domain 3; T3

SUMMARY: Pigs have been gaining a significant role in regenerative medicine.  Rodents have limitations in this field due to size, longevity and organ physiology in which pigs have fewer limitations. Induced pluripotent stem cells (iPS) have been created by genetic reprogramming of somatic cells in the pig.  There has been little success with cloning of iPSC which may be attributed to factors such that continued expression of reprogramming genes dooms success or that these cells spend little time in the G1 phase and too long synthesizing DNA to be a good donor.  Human iPSC have been introduced into pig cardiomyocytes post infarction leading to short term improvements.  Similar experiments have been done in treating spinal cord injuries.  Porcine iPSC have been used in limited number.  Porcine iPSC have been used to generate retinal rod cell precursors for injection into pigs with damaged retinas.  Porcine iPSC have been used for restoration of function of cardiac cells.

Porcine models of SCID-like phenotypes have been described.  Two groups with disruption of the IL2RG gene have been found and more recently RAG1 and RAG2 mutated pigs have been described.  Genetically engineering these mutations may become a valuable resource.  Pancreas-deficient phenotype in pigs has been created using cell lines for overexpressed HES1 under the control of PDX1 promotor.  This could eventually lead to production of human organs.  Stem cell production has also been used for cultivation of edible tissue in vitro.  Further development of culture systems would be required to establish a commercially feasible source.

QUESTIONS

1. What limitations in pluripotent stem cell research do murine species have that porcine do not suffer from?
2. What studies have been done introducing human iPSC into pigs?
3. What SCID-like phenotype gene mutations have been described?

ANSWERS

1. Rodents have limitations in this field due to size, longevity and organ physiology
2. Human iPSC have been introduced into pig cardiomyocytes post infarction leading to short term improvements.  Similar experiments have been done in treating spinal cord injuries.
3. Disruption in IL2RG gene, RAG1 and RAG2 mutated pigs

**Gonzalez and Dobrinski. Beyond the Mouse Monopoly: Studying the Male Germ Line in Domestic Animal Models, pp. 83-98**

Domain 3: Research

SUMMARY: Spermatogonial stem cells (SSCs) are the foundation of spermatogenesis and essential to maintain the continuous production of spermatozoa after the onset of puberty in the male. The study of the male germ line is important for understanding the process of spermatogenesis, unravelling mechanisms of stemness maintenance, cell differentiation, and cell-to-cell interactions. The transplantation of SSCs can contribute to the preservation of the genome of valuable individuals in assisted reproduction programs. In addition to the importance of SSCs for male fertility, their study has recently stimulated interest in the generation of genetically modified animals because manipulations of the male germ line at the SSC stage will be maintained in the long term and transmitted to the offspring. Studies performed mainly in the mouse model have laid the groundwork for facilitating advancements in the field of male germ line biology, but more progress is needed in nonrodent species in order to translate the technology to the agricultural and biomedical fields. The lack of reliable markers for isolating germ cells from testicular somatic cells and the lack of knowledge of the requirements for germ cell maintenance have precluded their long-term maintenance in domestic animals. Nevertheless, some progress has been made. In this review, we will focus on the state of the art in the isolation, characterization, culture, and manipulation of SSCs and the use of germ cell transplantation in domestic animals.

One Line Summary: The use of the mouse model to study the male germ line has led to many advances, but they have not been directly translatable to large/domestic species. The use of large/domestic species as research models to study the male germ line would have direct benefits both to the research field as well as agricultural production.

Key Points

   The study of the male germ line in domestic animals:

o   Spermatogenesis is a highly complex and coordinated process

  Establishes and maintains the daily production of fully differentiated spermatozoa throughout the reproductive lifespan

  Divided into three phases

   Spermatocytogenesis

o   Spermatogonia become primary spermatocytes via mitosis

   Meiosis

o   Spermatocytes become haploid round spermatids via meiosis

   Spermiogenesis

o   Round spermatids go through the seminiferous epithelium cycle to become mature spermatozoa

o   Length of the seminiferous epithelium cycle determines the duration of spermatogenesis

  Varies among species

o   Anywhere from millions to billions of spermatozoa are produced daily

  Requires a stem cell pool – *spermatogonial stem cells* (SSC)

   Foundation of spermatogenesis

   Unique ability to self-renew or commit to differentiation

  SSC difficult to study as they are very rare and lack specific markers to identify them in cell populations

o   Applications for the study of the male germ line

  Transplantation of SSCs as a complementary tool in assisted reproduction programs

  Harvest of spermatogonia from both immature and adult animals – preservation of reproductive material

  Propagation of certain traits

  Generation of genetically modified animals from SSCs

    SSC manipulations are maintained in the long term

   Available methods for studying spermatogenesis

o   In vitro assays – culture of SSCs, tissue culture, and three-dimensional culture

o   In vivo assays – testicular tissue xenografting, *de novo* formation assay of testicular tissue

  Xenografting:

   Grafting small fragments of testicular tissue from a donor under the back skin of an immunocompromised recipient mouse

   Grafted testicular tissue develops in the recipient and undergoes spermatogenesis

  Formation assay

   Ability of isolated testicular cells to reorganize into seminiferous tubules

o   Create a microenvironment able to support spermatogenesis when transplanted into the back skin of immunodeficient mice

  Allows assessment of loss or gain of function of certain genes involved in spermatogenesis

   Especially important in nonrodent species where KO or knock-in animals are not yet available

   The status of domestic animal research and the use of rodents vs. large/domestic animals

o   Mouse the model of choice in basic biological and medical sciences

o   Funding for research involving domestic/large animals decreased in the previous few decades

  European Union report states that domestic animals (pigs, goats, sheep, other farm mammals) account for only 1.2% of the total number of animals used in research

o   Discoveries in rodent species cannot often be easily extrapolated to other species or to clinics

    Germ cell isolation, characterization, culture, and cryopreservation

o   Germ cell isolation requires a series of enzymatic digestions of testicular tissue

  Digestion with collagenase and hyaluronidase – interstitial tissue removed from seminiferous tubules

  Incubation of seminiferous tubules with trypsin-EDTA – produces a single-cell suspension

   Suspension composed of somatic cells and germ cells – must be separated from the rest of the testicular cells

o   Differential adhesion to tissue culture plates

o   Velocity sedimentation at unit gravity

o   Percoll gradients

o   Magnetic activated cell sorting (MACS)

o   Fluorescence-activated cell sorting (FACS)

o   Markers of undifferentiated spermatogonia in domestic species

  Promyelocytic leukemia zinc finger protein (PLZF) highly conserved in mammals

  Widely accepted as a marker of undifferentiated spermatogonia

  Gold standard markers in mice are:

   *c-kit*

   Stimulated by retinoic acid gene 8 (*Stra8*)

   Spermatogenesis and oogenesis-specific basic helix-loop-helix ½ (*Sohl1/2*)

  Identification of surface markers to sort undifferentiated spermatogonia from other testicular cells would produce pure populations of spermatogonia for research use

  Markers used for identification and isolation of undifferentiated spermatogonia in rodents not useful for other species

  Use of flow cytometry has been successful in differentiating spermatogonia in fish

o   Long-term culture of SSCs has not been successful in large/domestic animals to date

o   Cryopreservation useful for long-term preservation of germ cells

  SSCs retain the ability to proliferate and colonize seminiferous tubules after freezing/thawing

   Can also generate offspring after long-term storage

  Preservation of the male germ line either by freezing a single germ-cell suspension or freezing of testicular tissue

  Cryopreservation of testicular tissue more challenging

o   Differing cell types display different sensitivity to cold damage

o   Permeation of tissue requires longer exposure to cryoprotectants

  Might result in higher tissue toxicity prior to freezing

o   Spermatogenic differentiation potential limited after thawing

   Supplementation of freezing medium with trehalose allowed cryopreserved mouse SSCs to restore fertility after thawing and transplantation

  Competence of preserved germ cells to support development into normal offspring still unknown

  Germ cell transplantation (GCT)

o   Method to transplant germ cells into the testes of infertile mice

  Germ cells eventually able to generate offspring

  Demonstrated ability to reproduce the donor haplotype in host tissues

  Provided a functional assay for SSCs

   Only SSCs are able to self-renew and proliferate in the long term

o   Can reestablish spermatogenesis after transplantation

    The more differentiated spermatogonia will disappear after only one cycle of spermatogenesis

o   Has resulted in the generation of donor-generated offspring through laparoscopic artificial insemination in sheep and after natural mating in goats

o   A testicular single-cell suspension can be transplanted into the seminiferous tubules in mice via one of three routes:

  Direct injection of the seminiferous tubules

  Direct injection of the efferent ducts

   Most widely used method

  Direct injection of the rete testis

o   Methodology of injection used in the mouse is not feasible nor efficient in large animals

  Least invasive, easiest to perform, and most efficient route for injection of the seminiferous tubules utilized ultrasound-guided intra-rete testis transplantation

   Use of ultrasound to guide injection is a feasible approach in both NHPs and human primates

  Have also injected cells through the extratesticular rete testis in ram lambs

    Has also been used in the domestic cat

o   Necessary to detect and distinguish donor cells from recipient cells following germ cell transplantation

  Use of microsatellite marker-based PCR allows detection and quantification of spermatozoa from the donor

o   Two methods to improve the efficiency of GCT

  Increasing the purity of the undifferentiated spermatogonia after testis cell isolation from the donor

  Proliferating germ cells sensitive to the effects of chemotherapeutic drugs and irradiation

  Preparing the recipient’s testicular environment in order to increase the number of vacant stem cell niches

   Chemotherapeutic drugs and irradiation have been used to eliminate or reduce the presence of endogenous spermatogonia in the recipient testis

o   Busulfant is an alkylating antineoplastic agent

  Giving busulfant to pregnant females generates male offspring primed for receipt of donor tissue

o   Effectiveness of irradiation depends on the species, the age at time of irradiation, and the radiation dose

   May not need to eliminate the endogenous stem cells in the recipient testes

o   GCT of donor cells to untreated recipients was still successful, although lower when compared to treated recipients

o   Cross-species transplantation of germ cells successful between phylogenetically close species (e.g., rat, hamster, and most)

  Complete development of spermatogenesis

  Increasing the phylogenetic distance resulted only in colonization of the recipient testis

   No progression through spermatogenesis

o   Rodent donors and recipients have to be either genetically matched or immune compromised

  Germ cell transplantation in domestic species has been successful

   Unmatched donor and recipient

   Heterologous transplantation (*Bos taurus* into *Bos indicus*)

   No immune rejection observed

o   Sertoli cells can be transplanted into the testis and restore fertility in males with defects in the somatic compartment of the seminiferous tubules

  Practical application of the study of SSCs

o   Study of spermatogenesis

  Spermatogonia stem cells reside along the basement membrane of the seminiferous tubules within a very specific microenvironment (the ‘niche’)

  SSCs receive specific cutes from surrounding cells and endocrine signals

o   Regulate quiescence, self-renewal, and differentiation into haploid cells

   Preferentially situated in areas of the seminiferous tubules adjacent to the interstitial issue of the testis without Leydig cells

o   Differentiating spermatogonia prefer regions of the tubules in close proximity to Leydig cells

  Domestic cat model of teratospermia

o   Preservation of fertility

  Male fertility preserved through collection and cryopreservation of sperm

  Spermatozoa are terminally differentiated cells

   A finite resource

  Spermatogonia contain full set of genetic information of the individual

   Are a theoretically infinite source of self-renewing cells

o   Generation of genetically modified animals

  Pig a prominent biomedical model in the last decade

  Canine and feline models important models of human genetic disorders

  Manipulations of the male germ line at the SSC stage are maintained in the long term and transmitted to the offspring

   Avoids the use of embryo manipulations and nuclear reprogramming issues associated with somatic cell nuclear transfer

   Spermatogenesis in vivo gives a natural environment to remove defective germ cells and cells carrying undesired mutations

   Modification of the male germ line a much shorter process of genetic modification compared to current technologies

o   Pronuclear injection, somatic cell nuclear transfer, and embryonic stem cell-based germline transmission

  SSCs are unipotent

   Already committed to spermatogenesis

   Not tumorigenic

  Maintain stable karyotype in culture

* Retain normal DNA-methylation pattern even in long-term culture

  Preferred method of delivering exogenous genes into cells is through the use of viral vectors

    Have been used to transduce male germ cells in pig, goat, cattle, sheep, and dog

o   Detected the transgene in donor-derived spermatozoa

  Other tools for gene targeting:

   Zinc-finger nucleases (ZFNs)

   Transcription activator-like effector nucleases (TALENs)

   Clustered regularly interspaced short palindromic repeats (CRISPRs)

QUESTIONS

1.  Name the three phases of the spermatogenesis cycle.

2.   Briefly describe the process of xenografting testicular tissue.

3.  True or False: the number of domestic/farm animals being utilized in research in the EU has been steadily increasing for the past few decades.

ANSWERS

1.   Spermatocytogenesis, meiosis, and spermiogenesis

2. Grafting of small fragments of testicular tissue from a donor under the back skin of an immunocompromised recipient mouse. Grafted testicular tissue develops in the recipient and undergoes spermatogenesis.

3.  False: it has been decreasing

**Youssef et al. Ovine Pulmonary Adenocarcinoma: A Large Animal Model for Human Lung Cancer, pp. 99-115**

Domain 1

SUMMARY

* Lung cancer is the leading cause of cancer deaths worldwide. The high mortality rate of lung cancer is due predominantly to the advanced stage at which diagnosis is made, which restricts the range and effectiveness of treatments that can be used.
* Animal models are valuable tools for studying oncogenesis in lung cancer. Mice have traditionally used for studying lung cancer in vivo, and a variety of spontaneous and transgenic models are available.
* Ovine pulmonary adenocarcinoma (OPA) is a naturally occurring lung cancer of sheep caused by retrovirus infection and has several features in common with adenocarcinoma of humans, including a similar histological appearance and activation of common cell signaling pathways.
* Natural cases of OPA appear as a progressive debilitating respiratory disease in which affected sheep struggle to recover from exercise. Commonly described in adult sheep of 2-4 years of age.
* The lungs of sheep clinically affected with OPA commonly have one or more large tumor masses that occupy a significant proportion of the total lung volume. A striking clinical feature of OPA is the excessive production of fluid in the lung, which may be discharged from the nose when the head is lowered (crude method of diagnosis in past).
* OPA tumors take many months or years to become clinically apparent. Many affected sheep do not develop clinical signs of respiratory disease during their commercial lifespan; however, they are still able to transmit the virus to other sheep.
* OPA is spread predominantly through the respiratory route via the inhalation of the virus. Lambs may become infected when suckling as the virus DNA has been detected in milk and colostrum of ewes.
* OPA tumors are thought to arise predominantly from type II alveolar pneumocytes and possibly also from bronchiolar club cells. Other cells can be infected, but at low levels.
* OPA more closely resembles the rare, multi-focal, noninvasive form of lung cancer and is less similar to the more common presentation of the disease.
* Association of macrophages with tumors is a common histological feature of OPA – making it a model for investigating the role of tumor-associated macrophages (TAMs) in lung adenocarcinoma.
* In Vivo Lamb Disease Model - important in establishing the link between the virus and the development of OPA. Subsequent studies showed that the development of tumors and clinical disease are reproduced much more consistently and with shorter incubation periods in young lambs compared with older lambs or adult sheep. Also able to study OPA tumors throughout the course of disease, from initial infection through to the development of tumors.
* Natural OPA as a Model of Lung Cancer – more advanced disease can be studied by examination of natural cases of OPA. Samples from such animals allow comparison of findings from in vitro studies and from experimentally infected lambs. They also offer the potential to study other events associated with advanced disease, such as metastasis and systemic immune responses.
* In Vivo Mouse Tumor Model – alternative in vivo system that does not require large animal facilities. However, the viruses used are not replicating forms of the virus.
* In Vitro Lung Slice Model of OPA – used to study virus replication and to model very early events in transformation in lung tissue in vitro.
* In Vitro Cell Culture Models – restricted by the lack of a cell line that can support efficient replication of the virus.

QUESTIONS

1. What are the reasons for why sheep have attracted attention as models for pulmonary function and disease?
2. What is the name of the virus that causes ovine pulmonary adenocarcinoma?

ANSWERS

1. The anatomy of the sheep lung is closer to that of humans than is the mouse lung and the similar size of ovine and human lung provides opportunities not available in mouse models.
2. Jaagsiekte sheep retrovirus

**Selsby et al. Porcine Models of Muscular Dystrophy, pp. 116-126**

Domain 3

Primary Species: Pig (Sus scrofus)

SUMMARY

Introduction: Duchenne Muscular Dystrophy (DMD) is a fatal X-linked disease resulting from a deficiency of dystrophin protein.  A majority of the cases are inherited, with only about 1/3 resulting from de novo mutations.  Dystrophin is part of the complex that participates in transmission of force generated during muscle contractions to the tendons and bones; without dystrophin the functional connection is lost and affected males experience progressive muscle deterioration and eventually death.  Becker Muscular Dystrophy (BMD) is a related disease also caused by dystrophin gene mutations, usually through a deletion that maintains an open reading frame, and symptom severity is highly variable.  There are many compounding factors that affect dystrophic muscle and lead to further cell death, including abnormal calcium homeostasis, increased superoxide and free radical production and injury, subsequent inflammation, and suppressed autophagy.  All of these factors affect disease severity in DMD and BMD patients, and they are all potential targets for therapy.  Animal models have been used to study these diseases and are essential for development and testing of new therapeutic approaches.

Conventional Animal Models of Dystrophinopathy: The mdx mouse is the most commonly used animal model of DMD and is very well-characterized.  A point mutation in exon 23 produces the dystrophic pathology, and new mutagenesis techniques have generated four novel mutations in the mouse dystrophin gene that are designated mdx2cv-5cv.  There are also two dystrophin knockout mice models.  Drawbacks of the mdx mouse model include a milder disease phenotype than that seen in human patients; higher expression of utrophin in mdx mice (utrophin is a protein with similar structure to dystrophin and may serve as an adequate substitute when dystrophin levels are low or non-existent); and poor correlation between effectiveness of therapies in mouse models to that seen in human trials.

The Golden Retriever Muscular Dystrophy (GRMD) model offers a large animal alternative to the mdx mouse model.  GRMD-affected dogs have a mutation in the 3’ end of intron 6 on the dystrophin gene, and the resultant phenotype displays similar severity and selective muscle injury to that seen in humans.  The dogs’ larger size also provides easier scaling up of drug interventions and gene and stem cell transfer-based approaches than is available with the mouse models.  There are two main deficiencies with the GRMD model.  One is that the affected dogs have a high degree of phenotypic variability, despite identical causative mutations.  This suggests underlying differences between the canine and human disease, and while understanding these differences may be important for developing therapeutic approaches, the increased phenotypic variability can confound data interpretation and endpoint determination.  The second drawback with the GRMD model is that the use of corticosteroids (the mainstay of treatment for human DMD patients) in dogs actually exacerbates the disease.  This severely diminishes the ability to evaluate how new therapies interact with corticosteroids in the dog model.

Other animal models include the hypertrophic feline muscular dystrophy HFMD) cat, rats with induced dystrophin gene mutations, and 2 strains of zebrafish dystrophinopathy models.  The feline model is rarely used due to complications from tongue and diaphragm hypertrophy that present significant animal welfare concerns.  The rat and zebrafish models, while useful in certain situations, still have limitations that make a novel large animal DMD model an attractive option for preclinical studies.

Porcine Models of Dystrophinopathy: Pigs are closer in size to humans than all other previous animal models, and their genome is three times more similar to humans than is the mouse.  Pig anatomy and physiology, particularly in regards to the cardiovascular system, is very similar to humans which is important as progressive cardiomyopathy is seen in DMD and BMD patients.  Porcine and human hearts share many similarities in terms of cardiac output, stroke volume, mean arterial pressure, heart rate, and perfusion.  Transgenic porcine DMD and BMD models have been developed and while some show promise, others produce disease severity that is so great as to impede its usefulness as a research tool.  A spontaneous model of porcine muscular dystrophy has recently been discovered and though it requires further characterization, it is a promising development in the search for new animal models to help further research of DMD and BMD.

QUESTIONS

1.  Which of the following is NOT a feature of DMD in humans?

a.  X-linked inheritance

b.  Development of cardiomyopathy

c.  Overabundance of dystrophin protein

d.  Progressive muscle deterioration

2.  What is the name of the most commonly used model for DMD?

a.   GRMD dog

b. mdx mouse

c.  pdx pig

d.  HFMD cat

3.  Which of the following characteristics makes pigs an attractive model for studying DMD in humans?

a.   Similar cardiovascular anatomy and physiology

b.  Genome is more closely related to human genome than is the mouse

c.  Closer in size to humans

d.  All of the above

ANSWERS

1.  c

2.  b

3.   d

**Seaton et al. Porcine Models of Cutaneous Wound Healing, pp. 127-138**

Domain 3: Research

Primary Species: Pig (Sus scrofa)

SUMMARY

4 Critical Phases of Wound Repair: Hemostasis, Inflammation, Proliferation, and Maturation

* Hemostasis- platelets form a fibrin plug and secrete growth factors and cytokines
* Inflammation- vasodilation and increased vascular permeability, chemokine release, inflammatory cells migrate into the wounds site (first neutrophils, then within 24-48 of wounding predominated by macrophages). Reactive oxygen species (ROS) are bactericidal but also cause additional tissue damage.
* Proliferative phase- TGF-β from macrophages is a potent stimulator of fibroproliferation and its accumulation leads to the proliferative phase. This phase occurs from 4-14 days after wounding. Epithelialization occurs during this phase.
* Maturation phase- Occurs from days 8-16 after wounding. Fibroblasts differentiate into myofibroblasts and mediate wound contraction. At this time the wound matrix is replaced with mature scar matrix.

Limitations to Rodent Models of Wound Healing

* Predominant contractile wound-healing phenotype related, in part, to the well-developed panniculus carnosus layer of striated muscle
* Much higher density of hair follicles
* Thin (only 50 um) making the creation of partial thickness wounds technically challenging
* Specifically in regard to burn models:
  + Can only tolerate 30% total body surface area burns which don’t provoke the hypermetabolic response seen in human patients with large burns
  + Immune system differences- human immune responses to a burn more like murine responses to sepsis than murine inflammatory responses to burn wounds!

Porcine Skin Anatomy and Physiology

* Advantages
  + Similar to humans, pigs have a relatively thick epidermis, distinct rete pegs, dermal papillae, and dense elastic fibers in the dermis
  + Histologic locations of epidermal keratins 10 and 16, dermal collagen IV, fibronectin and vimentin are also similar
  + Similar to human skin and unlike rodent, rabbit, and dogs skin; porcine skin is adherent to underlying structures and not loose
* However, there are important differences with humans
  + Porcine dermis and hair follicles are less vascular
  + Cutaneous endothelium doesn’t produce alkaline phosphatase
  + Pigs have only apocrine sweat glands with eccrine sweat glands isolated to the snout, lips, and carpal organ. In humans these eccrine sweat glands serve as essential sources of keratinocytes during wound epithelialization.

*Wound depth is a critical variable in wound healing models.*

Main Categories of Wound Models

* Incisional wounds- Created with a blade or electrocautery or laser surgical device. Appropriate for studying biomechanical properties of the wound and epithelial migration but leave little wound or scar tissue for analysis
* Partial thickness (PT) excisional wounds- Superficial wounds by repeated tape stripping or dermatome can be used for deeper PT wounds (still leave the base of sebaceous glands and hair follicles intact)
* Full thickness excisional wounds- Punch biopsy, scalpel, or multiple passes of a dermatome to remove the entire dermis down to the subcutaneous tissue or fascia.
* Burn wounds- Create more necrosis and generate greater inflammatory response. Can be created by application of heated metal bar or container filled with hot water, but consistent wound depth is more challenging to produce than with other wound types.

Pig Models of Wound-Healing Pathologies

* Ischemic chronic nonhealing wounds: Bipedicled skin flaps, length to breadth ratio optimized at 3:1 for flaps to survive 4-6 wks; resulting flap had a skin perfusion pressure consistent with chronic wounds in humans
* Pressure ulcers: Pressure sore development model by unilateral transection of nerve roots L1 through S2 resulting in monoplegic pigs then pressure over denervated skin to cause wounds
* Radiation injury: Cause of impaired wound healing in cancer patients undergoing treatment. Porcine model using 1500 Rad to skin of anesthetized pigs, focal radiation did not scatter to the opposite side of the body so pigs could serve as their own controls.
* Diabetic wounds: Streptozotocin injection to induce diabetes with subsequent wound creation. Poor wound healing noted even with short-term diabetes (14 days of hyperglycemia prior to wounding). Also streptozotocin treatment more consistently induced hyperglycemia in larger pigs (45-50 kg) than smaller pigs (25-30 kg).
* Burn wounds: “Burns cause an area of central tissue necrosis surrounded by an area of ischemia that may progress to necrosis and preventing this progression is a main goal of burn wounds care.” Pig model using contact burn with a brass comb with unburned tissue between the prongs to monitor burn healing/progression. Using this model it was discovered that 1 to 4 hours after a burn was vital timeframe for progression of burn necrosis
* Hypertrophic scarring: Hypertrophic scars (HS) are raised, red, and inflexible scars that are confined within the boundaries of the original wound and the incidence is high with burn wounds. Pathophysiology is poorly understood.
  + Duroc model for HS involves creating a deep dermal wound with residual dermis (can be achieved with electric dermatome). Red Duroc scar that is produced has similar gross appearance (thick, hairless, firm, and abnormally pigmented), disorganized collagen whorls, and abnormal expression of wound-healing mediators. Red Duroc model is commonly contrasted with Yorkshire pigs which have a more “normal” wound healing response. Disadvantages - Duroc is a farm pig that can grow quite large and there is genetic heterogeneity resulting in phenotypic differences between individual pigs (for example, coat color).
  + Juvenile Large White pigs with special wounding technique to cause HS. Create large burn wound (50 cm2) with tissue damage down to the base of hair follicles but sparing enough cells for healing over 3 to 4 weeks. Used 92ᵒC water in a Pyrex bottle applied for 15 seconds. Similar thickness, cell differentiation and proliferation markers, and TGF-β staining to human HS. Disadvantages: parallel collagen organization unlike HS, burn wounds result in unavoidable variability within the model.

Numerous studies have used pig models to assess wound treatments such as surgical and enzymatic debridement agents, negative pressure devices, silver dressings, collagen gel dressings, sprayed cell suspensions, and dermal substitutes

New Technologies to Study Wound Healing

* Laser capture microdissection to isolate specific cell types or histo structures
* Tissue profiling by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) allows analysis of protein content without losing localization
* Improvements in transgenic technologies, possible better transgenic swine models for biomedical research

QUESTIONS

1. Which of these is one of the 4 critical phases of wound repair?

a. Cytokine phase

b. Hyperplastic scarring phase

c. Proliferative phase

d. Vascular phase

2. Regarding wound models, rodents

a. Are the only animal model for the hypermetabolic response seen in humans with large surface area burn wounds

b. Have a predominate contractile wound-healing phenotype

c. Have thick skin that is well-adhered to underlying structures

d. Have very similar inflammatory responses to burn wounds compared to humans

3. Which is a characteristic of porcine skin?

a. Distinct rete pegs, dermal papillae, and dense elastic fibers in the dermis

b. The cutaneous endothelium produces alkaline phosphatase

c. Dermis and hair follicles have a similar degree of vascularization compared to human skin

d. Eccrine sweat glands are widely distributed

4. An investigator wishes to use a Duroc animal model of hypertrophic scar formation to study abnormal collagen deposition during scar formation. What is correct about this animal model?

a. As an albino breed phototoxic retinopathy should be prevented with low light levels in their housing area

b. As a mini-pig the pigs should be less than 50 kg once fully grown

c. Duroc models of HS is a poor choice because they have parallel collagen organization not the typical whorls and nodules seen with HS

d. The breed is at risk of porcine stress syndrome so anesthesia for wound formation should involve vigilant monitoring

ANSWERS

1. c. Proliferative phase

2. b. Have a predominate contractile wound-healing phenotype

3. a. Distinct rete pegs, dermal papillae, and dense elastic fibers in the dermis

4. d. The breed is at risk of porcine stress syndrome so anesthesia for wound formation should involve vigilant monitoring

**Thulin and Underwood. IACUC Considerations for the Use of Livestock in Translational Research, pp. 139-146**

Domain: 5: Regulatory Responsibilities

SUMMARY: This is a review article covering the concerns, challenges and issues an IACUC may encounter if livestock models are being used in translational research. This article is directed at IACUC’s where the livestock component of the program is small or the use of such species is infrequent. The article is broad is scope and general in description. The authors recommend reviewing the Guide for Care and Use of Agricultural Animals in Research and Teaching (Ag Guide) for more details and specifics on the issues raised.

Some of the highlights of the significant differences that may face the IACUC, are the severity and type of physical injury and zoonoses that may affect personnel working with livestock species. Examples of such injuries include butting, goring and trampling while examples of zoonoses include Q fever, cryptosporidiosis and influenza. The article also points out that growth rate and size of typical livestock species can present significant challenges for handling, housing and biocontainment and that the IACUC will want to verify that personnel are aware and prepared for these issues. Another highlight is that regardless of environment, proper aseptic technique should always be employed for surgery. However, an IACUC may have to adjust their relative tolerance and use professional judgement for pest management and control in the agricultural setting.

The bottom line is that the role and responsibilities of the IACUC are the same regardless of species involved but the differences between these species and the more commonly used and familiar laboratory animal species requires special attention.

QUESTIONS

1. Ruminants are extremely sensitive to \_\_\_\_\_\_ and are more likely to experience regurgitation, aspiration, myopathy and hypoxemia following general anesthesia.

a. Propofol

b.  Phenothiazine tranquilizers

c.   Alpha 2 agonists

d. Beta Blockers

1. True or False: Humane slaughter is an acceptable form of euthanasia for livestock used in translational research.
2. Lactating dairy cattle may need \_\_\_ to \_\_\_ weeks to acclimate and return to full production.
   1. 6 to 8
   2. 2 to 4
   3. 10 to 12
   4. 1 to 2
3. True or False: Health records do not need to be maintained for individual livestock animals used is translational research.

ANSWERS

1. c (p. 145)

2. True (p. 145)

3. b. (p. 144)

4. False (p. 144)