Pathology of Minipigs
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Why swine?
• Similar to human anatomy/physiology:
  • Cardiovascular
  • Integumentary
  • Urinary
  • Digestive
• True omnivores like humans
• Increasing market in safety pharmacology

Basic terms
• Swine/hog/pig: denotes the species *Sus scrofa*
• Sow: sexually mature F
• Gilt: sexually immature F
• Boar: sexually mature M
• Barrow: castrated M
• Piglet: juvenile swine

Resources
• McAnulty, PA et al. 2011
• Bollen et al, The Laboratory Swine
• Gad, Animal Models in Toxicology
• Hau & Van Hoosier, Handbook of Laboratory Animal Science, Vol. 3
• Svenson, The Minipig in Toxicology
• Swindle, Swine in the Laboratory
• Upcoming Tox Path supplemental issue focused on minipigs
  ▶ Minipig Research Forum on LinkedIn

Why swine?
• Alternative to dog or monkey (3Rs)
• Shorter time to sexual maturity, high reproductive rate
• Better NSAID tolerance
• No supply concern (dogs in EU, primates worldwide)
• Fewer zoonoses than in primates

Breeds
Common breeds

By increasing size:
- Gottingen (9 kg at 4 mo.)
- Sinclair
- Yucatan micro
- Yucatan
- Hanford (20 kg at 4 mo.)
- Farm pig (65 kg at 4 mos)

Minipig strain comparisons

<table>
<thead>
<tr>
<th>Breed</th>
<th>Göttingen</th>
<th>Sinclair</th>
<th>Yucatan</th>
<th>Hanford</th>
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</thead>
<tbody>
<tr>
<td>Age, diet</td>
<td></td>
<td></td>
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<tr>
<td>Male, female</td>
<td>6-8 kg</td>
<td>8-10 kg</td>
<td>8-10 kg</td>
<td>10-12 kg</td>
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<tr>
<td>White</td>
<td>White</td>
<td>Black, grey</td>
<td>Black, white</td>
<td>Black, white</td>
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<tr>
<td>Skin</td>
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<tr>
<td>6-mo mass</td>
<td>12 kg</td>
<td>15 kg</td>
<td>15 kg</td>
<td>20 kg</td>
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<tr>
<td>Notes</td>
<td>Well-defined and strictly managed genetic background</td>
<td>White-skinned, short snout, pot belly</td>
<td>Only naturally occurring miniature pig available in NA; native to southern Mexico &amp; Central America</td>
<td>Darkly pigmented skin with little or no hair</td>
</tr>
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</table>

Gottingen
- Supplied by Marshall (US), Ellegaard (EU), Oriental Yeast Co. (Japan), and Woojung BSC (Korea)
- White-skinned, short snout, pot belly
- 6-mo weight ~12 kg (if fed age-appropriate diet and not ad-lib)
- Many study types including juvenile and reproduction studies

Yucatan
- Supplied by Sinclair Bio-resources
- Only naturally occurring miniature pig available in NA; native to southern Mexico & Central America
- Minipig: 6-mo weight ~30 kg
- Micropig: 6-mo weight ~20 kg
- Darkly pigmented skin with little or no hair
- White skin line for dermal studies
- Spontaneous ventricular septal defect line
- Docile, easy to handle

Hanford
- Supplied by Sinclair Bio-resources
- Looks like a conventional pig
- White-skinned
- 6-mo weight ~40 kg
- Surgical and cardiovascular research (lack of fat, size)
- Develops adult human-sized organs and structures between 6-8 months of age

Bodyweight

Caution: Gottingens have been known to reach 50 kg at 24 mo. of age – depends on diet

![Bodyweight graph](image-url)
Sinclair

- Supplied by Sinclair Bio-resources
- First mini stock developed specifically for research purposes
- 6-mo weight ~25 kg
- Different colors; easy identification
- White strain now available
- Used for cardiovascular, osteoporosis, diabetes, and fetal alcohol syndrome research
- Melanoma model line (spontaneous regression)

Genetically engineered pig models for human diseases

Organ transplants

- α-gal problem:
  - alpha-1,3-galactose (α-gal) coats the surface of pig blood vessels but is absent from human tissues
  - Antibodies against this protein → complement → holes punched in membranes of the foreign cells on contact
  - genetically engineered pigs are available that lack gene that makes α-gal

Behaviour / husbandry

Husbandry issues

Claw overgrowth

Pen Construction

- Feeders
- Watering Systems
- Flooring
- Grid
- Solid
- Sturdy
Behavior: Strong, Smart & Destructive
- Can be destructive due to their strength, curiosity, and drive to forage for food/root
- Primary enclosures should be of solid construction
- Lixits and feeders need to be securely fastened
- Locks and closures should be secure and in good condition

Behaviorally Relevant Enrichment: Foraging & Rooting
- Hay or bedding make a good substrate to root in
- Teflon balls stand up well to wear and tear and are easily sanitized
- Feeders with hinged lids encourage foraging

Swine Enrichment
- Hay or bedding make a good substrate to root in
- Teflon balls stand up well to wear and tear and are easily sanitized
- Feeders with hinged lids encourage foraging

Behavior: Cleanliness
- Swine prefer a clean environment
- Given proper space, they will use a single corner for elimination
- May not play with enrichment objects or drink water once contaminated with feces

Restraint slings

Stereotypic behaviors
- Repetitive, nonfunctional, abnormal behaviors are called stereotypies:
  - Tail or ear biting
  - Snout rubbing
  - Bar biting
  - Pacing
  - Frequent vocalization
  - Jumping against barriers

Handling & restraint
- V-board
- Restraint slings
Quickly acclimated to laboratory procedures

- Daily human interaction during acclimation
- Pressure on the side of the body/belly rub will cause the animal to relax

Communicate with tails

- Curled tail = good health
- Straight tail = stressed (cold/sick/fearful)
  - (Note: Yucatanas have naturally straight tails)
- Wagging can indicate a variety of situations: greeting, aggression, or frustration

Behavior: Social structure

- Wild swine live in groups
- Complex social dominance order; several generations of animals
- Even newborns have a social order: more dominant piglets get the more anterior teats that produce more milk

Swine in the laboratory

- Should be housed in groups whenever possible
- Dominance fighting when new animals are introduced or at feeding times
- If individual housing is necessary, swine should be able to see, smell, hear or touch other pigs in order to maintain the sense of safety of the herd

Mirrors

- If only one animal, can use a mirror to “simulate” another animal.
Anatomical similarities and differences

The NALT in minipigs is a single organ, located at the roof of the nasopharyngeal duct, comparable to the adenoid or nasopharyngeal tonsil in humans and dogs.

Inhalation studies

Skin

Similarities
- Lightly pigmented, sparse hair
- Thick epidermis
- Pig: 70-140 um
- Human: 50-120 um
- Rat: 10-20 um
- Epidermal morphology and cell turnover
- Dermal penetration

Differences
- Thick stratum corneum
- Eccrine glands limited to snout and carpal areas
- Extensive subcuticular adipose
- Skin pH 6-7 (human = 5)

Ventral abdomen

Skin caudal to ear

Porcine

Human
Skin

- Standard model of wound healing/burns (a standard plastic surgery model)
- Heals primarily by reepithelialization rather than contraction
- High surface area available
- Göttingen melanocytes have c-kit mutation so don’t migrate correctly to skin (deliberate from crossing in Landrace)

Drug Administration
Dermal Studies

Continuous subcutaneous infusion (24 hours for a 10-day period) is possible

Folliculitis/furunculosis common with high viscosity ointments or vehicles
**Pulmonary intravascular macrophages (PIMs)**

- Primary site of removal of blood-borne particulates in pigs and ruminants
- Similar function to the hepatic Kupffer cells in dogs, rodents, primates
- PIMs are resident cells, junctionally adherent to pulmonary capillary endothelium

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**Cardiovascular**

- Hanford heart/vessel size more like humans vs. dog or NHP
- No collateral blood supply

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Abdomen—spiral colon

Fig 16

Fig 17

Cecal tonsil
CNS development: majority of CNS cell division before birth
- Myelination primarily post-natal
- BBB immature at birth
- Highly developed eye
- Teratology studies

Diseases / Pathology

Thrombocytopenic purpura-like syndrome
- Rare
- Associated with type III hypersensitivity
- Immune-complexes accumulated in smaller blood vessels → compromised mural integrity → vascular leakage.
- Subcutaneous hemorrhages
- Disseminated visceral hemorrhages
- No causal infectious agents identified
- Low platelet count (<20,000/μl, +/- anemia)
- Fatal
Thrombocytopenic purpura syndrome
Severe thrombocytopenia, Multiorgan hemorrhages

- Hemorrhages of:
  - Urinary bladder
  - Lymph nodes
  - Urothelium
- Ulceration of torus pyloricae
- Vascular lesions
  - Renal pelvis
  - Coronary arteries
  - Neointimal proliferation, thrombosis, medial deposits of myxoid matrix
  - Renal glomeruli have membranoproliferative lesions that are positive for C1q

Urinary bladder

Increased number of apoptotic megakaryocytes in bone marrow

Erythema Multiforme

- “Dippity pig” syndrome
- Similar to a hypersensitivity syndrome
- May be painful to touch
- Heal spontaneously in 1-2 weeks with no treatment (may use NSAIDs for pain). May recur
- All Gottie age groups affected.

“Dippity pig syndrome”

- Sudden onset, believed to be from stress, although unknown etiology.
- Signs are similar to those of sunburn (pigs dip their backs, vocalize, and show signs of extreme pain) but with no history of sun exposure.
- Serum-oozing lesions of various sizes are seen on lumbar skin surfaces.
- May develop secondary staph infections of the skin

Exudative epidermitis/eczema/dermatitis of Gotties

- Usually 3-4 mos of age
- Periocular hyper-parakeratosis may extend to the head and progress to become more generalized
- No infectious agent identified – *Candida albicans* occasionally associated with lesion (part of NF)
- *NOT* the same as disease caused by *Staphylococcus hyicus*
- General health not affected
- Self limiting, heal in few months
- Excluded from dermal toxicology and wound healing studies.
Porcine juvenile pustular psoriasiform dermatitis (Pityriasis rosea)

- Etiology unknown
- Young white breeds
- Macroscopic lesions
  - Circular to serpiginous, erythematous lesions with crusts
  - Ventral abdomen, inner thighs
- Microscopic lesions
  - Early: perivascular eosinophilic and lymphoplasmytic inflammation, spongiform pustules, & psoriasiform hyperplasia
  - Later: Hyperplastic plaques with little inflammation, resolve completely after 6-8 weeks

Rotavirus

- Diarrhea – profuse yellow watery
- Villus atrophy
- Younger than 2 wk resolved in 2-4 d
- If fever, stops eating, depressed, then suspect e. coli, lawsonia or coccidia

Cutaneous melanoma

- Specific line of Sinclair S-1 pigs is a model for malignant melanoma (also occur in Durocs and Libechov minipigs)
  - ~50% of piglets have tumors at birth, 85% by 6 weeks of age
  - Microscopic features, pattern of metastasis very similar to humans
  - However: spontaneous depigmentation and regression between 6-12 months of age

- Regression associated with infiltration of macrophages
- Two populations of tumors:
  - Macular
    - Cell types primarily melanocytes (Melan A, PNL2 (+))
  - Raised
    - Primary cell type is melanophage (Iba 1 (+))
- Pigmentation of regional lymph nodes due to melanophages
Respiratory diseases
- Minipigs susceptible to all porcine respiratory diseases
- Actual disease rare in lab setting
  - SPF/barrier-raised pigs
  - Vaccination
  - Husbandry

Respiratory
- Porcine Respiratory Disease Complex
  (Rare in SPF animals but possible)
  - Primary pathogens
    - M. hyopneumoniae
    - A. pleuropneumoniae
    - SIV
    - PRRSV
    - PRV
    - PRCV
    - PCV
  - Secondary pathogens
    - P. multocida
    - S. suis
    - H. parasuis
    - B. bronchiseptica
    - A. pyogenes
    - A. pleuropneumoniae

Mycoplasma hyorhinis

Swine flu
- Reverse zoonosis possible
- H1N1, H1N2, H2N3, H3N2
- Epithelial cells of respiratory tract → necrotizing bronchitis and broncholitis
Others (unlikely in the lab)

- *Staphylococcus hyicus* (greasy pig disease)
- *Erysipelothrix rhusiopathiae* (diamond skin disease)
- *Haemophilus parasuis*
- *Lawsonia intracellularis*
- PCV-2
- Parakeratosis (zinc-responsive dermatosis)
- *Ringworm*

Greasy pig – *Staph. hyicus*

*Erysipelothrix rhusiopathiae*

*Haemophilus parasuis: Glasser’s disease*

*Lawsonia intracellularis*
PCV-2

- PMWS
- PDMS
- PCVAD
- PRDC
- APE
- PAN
- Reproductive failure
- Granulomatous enteritis
- Necrotizing lymphadenitis
- Exudative epidermitis

- Infects endothelial cells, macrophages
- Co-infection with other agents – viruses, bacteria, etc.
- Genetic factors – some breeds (Landrace) more susceptible than others
- PCV-2b becoming more prevalent than PCV-2a
- Vaccine efficacy is variable
Porcine Stress Syndrome
- Historically a mutation in RYR
  - Unregulated release of calcium from sarcoplasmic reticulum → excessive myofiber contraction → increase in body temp
  - Triggered by anesthetic agents (halothane) or stress
  - Autosomal recessive
  - Nearly bred out of existence
- Recently associated with mutation in dystrophin gene
  - Especially seen with transport stress

Porcine stress syndrome/ Malignant hyperthermia

PSS
- Rapid rigor mortis
- Muscles appear pale and soft due to increased lactic acid present
- Animals with the dystrophin mutation have cardiomyofiber degeneration

Salt poisoning (Water deprivation syndrome)
- Hyperosmolarity (hypernatremia) in brain
- Excessive intake of Na salts, or dehydration followed by rehydration
- Inappetence, head pressing, incoordination, blindness, circling, paddling, convulsions
- Gross: congestion/edema of leptomeninges and cerebrum
- Histo: cerebrocortical laminar necrosis plus astrocytic swelling, +/- perivascular eosinophils

Salt poisoning
- Husbandry related?
Rickets and vitamin D metabolism

- If animals in controlled environment, vitamin D requirements usually supplied by diet.
- Emaciation, difficulty rising, bowed forelegs, joint swelling, painful movements.
- Folding and erosion of articular cartilage, enlarged long bone metaphyses
- Genetic form exists (PDDR Hannover) – progressive hypocalcemia, hypophosphatemia
- Associated with deletion in P450C1 coding region (frameshift with premature stop codon)

Hypervitaminosis D

- Heterotopic calcification
  - Left atrial endocardium, submucosa of gastric fundus, other sites
- Edema, deposition of calcium, granulomatous reaction and repair
- Calcium / magnesium WNL in serum and bone

Gastric ulcer syndrome

- Begins with hyperkeratosis pars esophagea
- Etiology unknown, but has been associated with Helicobacter pylori-like organisms, high carbohydrate diet
- Exacerbated by stress
- Stomach acid damages epithelium

Gastric Ulceration

Background pathology

Procedural trauma

- Long soft palate and laryngeal diverticulum make intubation or gavage difficult
- Edema / acute inflammation in the pharynx, larynx, and cervical region
- Blood collection may result in hemorrhage of cervical structures
- May see red frothy fluid following procedure; can bite tongue
Euthanasia solution artifacts

Melanosis
- Pigmentation in internal organs
- Normal in pigmented swine
- Meninges, lungs, spleen, heart valves, liver, lymph nodes

Mononuclear cell infiltrates
- Normal background finding
- Many organs
  - Liver
  - Adrenal
  - Kidney
  - GI tract
  - Heart
  - Brain
  - Pancreas
  - Salivary gland
  - Skin

Thymic granulomatous lesions in pigs
- Thymus most common, but also seen in mesenteric LN, spleen

Thymic granulomatous lesions

Y. Baba et al. Vet Pathol 2006;43:1037-1040
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Spontaneous Arteritis
- Göttingens
- Lesions similar to polyarteritis nodosa in rats
- Degenerative vascular lesions in small- to medium-sized muscular arteries
- Coronary arteries and vessels of the renal pelvis most commonly involved
- Intimal proliferation, medial thickening +/- necrosis, endothelial proliferation, luminal stenosis, thrombosis, disruption and fragmentation of the internal elastic lamina

Ventricular septal defect
- Specific Yucatan lines
- ~ 20% of fetuses
- 65% are high membranous defects
- Polygenic defect

Atherosclerosis
- Inducible by high lipid diet over short period
- Similar pathophysiology of hyperlipidemic vasculopathy in humans
- Diabetic, hypercholesterolemic pig model induced by streptozotocin (STZ)

VSD
- Small defects may close spontaneously
- Large defects create L→R shunt → pulmonary hypertension and respiratory distress
- +/- failure to thrive
- Model for human disease
**Lung**
- Alveolar histiocytosis
  - More common in 2-6 month old animals than those >6mo
- Inflammatory cell infiltrates
- Mineral
- Pleural adhesions, associated fibrosis
- Pigmented macrophages: only in younger animals

**Alveolar histiocytosis**
- Increased numbers of foamy macrophages in alveoli
- May be grossly visible as white areas in lung
- Can be increased in gavage studies or can be normal background finding in control pigs

**GI tract**
- Salivary glands: inflammatory cell infiltrates, mineral
- Stomach:
  - Non-glandular portion: erosions and ulceration
  - Glandular portion: inflammation and infiltrates
- Few changes seen in intestines

**Stomach erosions**
Liver / Gall Bladder

Liver
- Focal inflammatory cell infiltrates
- Hematopoiesis
- Single cell necrosis

Gallbladder
- Aplasia, hypoplasia
- Cholecystitis (acute or chronic)
- Acute: necrotizing with hemorrhage
- Chronic: Fibrosis with mononuclear cells
Tension lipidosis

Renal
- Kidney anatomy more similar (even than NHPs) to human
- Spontaneous glomerulonephritis in 2.5% Gottingens
  - Membranous or membranoproliferative
  - Etiology unknown, possible Ag/Ab complexes

Kidney
- Mononuclear inflammatory-cell foci
- Minimal to mild tubular-cell basophilia and dilation
- Focal chronic interstitial nephritis
- Renal changes seen more commonly in females than males

Tubular basophilia
Bone Marrow
- Serous atrophy of fat
- Primarily seen in males
- Incidence increases with age
- Seen in animals from EU, not in NA
- Only reported in Göttingens

Skeletal Muscle
- Gottintens
- Multifocal skeletal muscle degeneration and necrosis
- Mononuclear cell infiltrates
- May see regeneration
**Integument**
- Hyperkeratosis
- Hyperplasia
- Inflammation
- Inflammatory cell infiltrates

**Hyperkeratosis**

**Skin Exudate**

**Reproductive**
- Sexually mature in 4-6 months (economic benefit vs. 3-4 years for macaques)
- Not good model for penile or ureteral procedures
- Diffuse epitheliochorial placentation
- Slowly increasing use in embryo-fetal studies
Reproductive: Female

- Age at sexual maturity: Breed dependent.
- Ovaries - mineral
- Göttingen:
  - 50% of females mature at 6.5 months old (11.8 kg bw)
  - 100% mature at 7.5 months old (13.1 kg).
  - Higher uterine wt with sexual maturity, but ovarian wt inconsistent

Reproductive: Male

- Testicle: atrophy, degeneration, hypoplasia (25-37% of Göttingens)
- Epididymis: aspermia / oligospermia, luminal debris, cysts
- Prostate: mineral (Göttingens)

Testes: focal segmental atrophy or tubular hypoplasia

Epididymal Cysts
**Retinal dysplasia**
- Göttingen
- Rosettes or folds in retina
- Congenital

**Eye**
- Retina, pupil, lens have closest resemblance to human eyes after NHPs
- No tapetum
- Harderian glands
- Transcleral drug delivery
- Retinal rosettes less common than in rats

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- Aaron Seargent
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