Pathology and a Lot of Other Stuff
Because Let’s All Admit There Isn’t Much Pathology in Minipigs

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Miniature Pigs

- Sus scrofa domestica
- Smaller than 75 kg adult weight, easy to handle
- Require less food, less test material than farm pigs
- Controlled genotype and phenotype

Miniature Pigs

- Common miniature pig breeds used in research: Goettingen, Hanford, Sinclair S-1, Yucatan
- Available as SPF barrier-bred, some are also available as germ-free and GEMS
- Low background lesion incidence in most breeds
- Use of minipigs growing in US; 7.2-10% of non-rodent use (Heining, 2016)

Swine vs Dogs

- Not prone to vomiting
- Better NSAID tolerance
- Tolerate sympathomimetic drugs better, no cardiotoxicity
- Physiologically more similar to man

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Swine vs Primates

- Ethics
- Lower costs
- Early sexual maturity (3-6 months)
- High reproduction rate
- No supply or conservation concern
- Easier to handle, transport, and house
- Fewer zoonotic diseases

Gottingen

- Developed in the 1960’s
- Well-defined and strictly managed genetic background
- White-skinned, short snout, pot belly
- 6-mo weight ~12 kg, adult weight is ~35 kg.
- Barrier raised in US and Europe

Hanford

- Supplied by Sinclair Bio-resources
- Looks like a conventional pig
- White-skin and hair, less SQ fat
- 6-mo weight ~40 kg
- Surgical and cardiovascular research
- Develops adult human-sized organs and structures between 6-8 months of age

Sinclair

- Supplied by Sinclair Bio-resources
- First mini stock developed specifically for research purposes - 1949
- 6-mo weight ~25 kg Adult - ~55-75 kg
- Different colors; easy identification
- White strain now available
- Used for cardiovascular, osteoporosis, diabetes, and fetal alcohol syndrome research
- Melanoma model line

Yucatan

- Naturally occurring breed found in Mexico, Central & South America
- Characterized as research animal in 1970’s
- Grey to black skin, sparse hair, docile
- Supplier: Sinclair BioResources
- Adult weight: 70-80kg
  - Micro-Yucatan: 55-70kg
HUSBANDRY

Behavior
• Prey species
• Herd animals with a social hierarchy
• Highly food motivated
• Rooting behavior
• Nesting behavior
• Vocal
• Clean

Enclosures
• Floor space requirements vary
• Consider natural social behavior & dominance hierarchy
• Group or pair-house if possible (except adult intact males)
• Visual, auditory or olfactory contact if single-housed
  – Single pet pig households have higher incidence of human-directed aggression

Enrichment
► Hay/straw
► Chains
► Balls
► Brown paper
► Social interaction (other pigs or humans)
► Exploration of new environment

Husbandry Issues
• Hoof overgrowth
• Traumatic injury
Husbandry Issues

- Slippery floor → stressed pig
- Ad libitum feed → obese pig
- Lack of water → salt toxicity

Handling & Restraint

- Pigs can learn to willingly accept:
  - Being picked up
  - Sling
  - V-trough
  - Weighing
  - Masking
  - Dermal dosing
- Operant conditioning (clicker training)

Panepinto Pig Sling

Integumentary System

Mental Gland

Carpal Glands
Skin: comparison to humans

- Epidermal thickness
  - Rat: 10-20 µm
  - Pig: 70-140 µm
  - Human: 50-120 µm
  - Remember, epidermal thickness varies with location!
- Dermal collagen and elastic content biochemically similar

Differences:
- Eccrine sweat glands limited to snout and carpal glands
- Less vascular = bloodless incisions

Normal Skin: Pig vs. Human

Normal Skin: Mouse vs. Human

Skin
- Standard model of wound healing/burns (a standard plastic surgery model)
- Heals primarily by reepithelialization rather than contraction
- High surface area available

Drug Administration
Dermal Studies

Continuous subcutaneous infusion (24 hours for a 10-day period) is possible
Folliculitis/Furunculosis common with high viscosity materials or vehicles

Dermal Phototoxicity Studies

Dermal phototoxicity studies following continuous subcutaneous infusion of ciprofloxacin hydrochloride in the novel micro mini pigs.

Aural, Ocular Discharge

Dippity pig syndrome”

- a.k.a. Bleeding back syndrome
- Likely multifactorial etiology (genetics, stress?). 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. Circular, serum-oozing lesions of various sizes are seen on lumbar skin surfaces.
- Recover in several days with restricted activity with or without symptomatic treatment. May recur in some animals.
- Reportedly, these pigs can then develop secondary staph infections of the skin

Dippity Pig Skin Lesions

Bullous pemphigoid

- Reported in Yucatans
- Vesicobullous disease
- Separation of the dermis and epidermis at the lamina lucida of the basement membrane
- May be preceded by local erythema, pruritis
- Few inflammatory cells
Thrombocytopenic Purpura Syndrome

- Severe thrombocytopenia, multiorgan hemorrhages
Porcine juvenile pustular psoriasiform dermatitis (Pityriasis rosea)

- Etiology unknown
- Young white breeds
- Microscopic lesions
  - Early: perivascular eosinophilic and lymphoplasmacytic inflammation, spongiform pustules, & psoriasiform hyperplasia
  - Later: Hyperplastic plaques with little inflammation, resolve completely after 6-8 weeks
  - Can be experimentally induced with IL-23 injections

Melanoma

- Sinclair minipig
- 50% of piglets have tumors at birth, 85% by 6 weeks of age
- Aggressive metastasis
- However: spontaneous depigmentation and regression between 6-12 months of age

Hyperkeratosis

- Brown crusting of the back of the head, neck, & dorsum
- Etiology unknown
- Exacerbated by sun exposure in pet pigs

Sunburn

Cardiovascular

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

- Alternative to dog or primate for anesthetized or conscious cardiovascular profiling and telemetry
- Growth of CV system from birth to 4 months is analogous to that of humans into the mid-teens
- Suitable model to identify effect of compounds on QT interval

**Ventricular septal defect**

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

**Atherosclerotic Plaque**

**Metabolic syndrome**

- Obesity
- Hypertension
- Dyslipidemia
- Insulin resistance
- Increase in C-reactive protein

**Metabolic syndrome in minipigs**

- High caloric diet (high fat/high fructose)
  - Yucatan, Ossabaw
  - Ossabaw - low insulin binding affinity for liver microenzymes
- GEM
  - Increased activity of proprotein convertase subtilisin/kexin (PCSK) ➔ reduced hepatic LDL receptors
Metabolic syndrome in minipigs
- Heart – oxygenation greatly reduced
- Kidney – increased interstitial fibrosis and inflammation
- Adipose tissue – increased abdominal fat tissue volume and adipocyte size
  - Increased fat in salivary glands - hyposalivation
- Increased C-reactive protein

Atherosclerosis
- High lipid/high carb diet or GEM
- Similar pathophysiology of hyperlipidemic vasculopathy in humans

Atrial Thrombosis

Spontaneous arteritis
- Gottingens
- Lesions similar to polyarteritis nodosa in rats
- Degenerative vascular lesions in small-to-medium-sized muscular arteries
- Usually coronary arteries and vessels of the renal pelvis
- Intimal proliferation, medial thickening +/- necrosis, endothelial proliferation, luminal stenosis, thrombosis, disruption and fragmentation of the internal elastic lamina
Inhalation studies

Nasal studies

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.


Respiratory

Porcine Respiratory Disease Complex

- 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
Gastrointestinal System

Gastrointestinal & Hepatobiliary
- 2-3 days to empty
- Torus pyloricus: muscular outpouching at level of pylorus
- Cecal tonsil: 2 cm-wide Peyer's patch opening
- Unbranched pancreatic duct like humans (can study pancreatic secretion)
- P450s CYP 1A, 2A, 3A metabolize same test substrates as humans

Alveolar macrophages
- Increased in gavage studies

Pharyngeal Diverticulum

Gastrointestinal
- Gavage dosing
- Don't tolerate capsules as well as dogs
- Not as sensitive as dogs to ulcer-inducing effects of NSAIDs
- renal papillary necrosis also unlikely
Restraint chair for gavaging

Procedural trauma
- Long soft palate and pharyngeal diverticulum make intubation or gavage difficult
- Edema / acute inflammation in the pharynx, larynx, and cervical region
- May see red frothy fluid following procedure; can bite tongue

Foaming at the Mouth

Background findings
**Gallbladder**
- Cholecystitis (acute or chronic)
- Acute: necrotizing with hemorrhage
- Chronic: Fibrosis with mononuclear cells
- May not be visible grossly (hypoplasia is differential)

**Liver**
- Focal inflammatory cell infiltrates
- Hematopoiesis
- Single cell necrosis
- Nodular hyperplasia in Yucatans (Garlick, 2001)

Cholelithiasis
- Often develops in obese pigs on a poor diet (i.e. table scraps, dog food)
- No clinical signs or biochemical abnormalities
- Incidental finding at necropsy
Gastric ulcer syndrome

- Complex syndrome
- +/- early hyperkeratosis of the esophagus and/or pars esophagea
- Related to anorexia or finely ground, pelleted feed – decreased gastric emptying
- Damage to squamous epithelium → ulceration
- Exacerbated by stress

Stomach erosions

Pig mucus is good for you!

- Mucus from pig stomachs is effective as antiviral agent: May be useful in cosmetics and baby formula


- Broad-spectrum antiviral mucins
- Possible addition to toothpastes, mouthwashes, wound ointments and genital lubricants

Hematopoietic System
Mononuclear cell infiltrates

- Seen in many different organs:
  - Liver
  - Adrenal cortex
  - GI tract
  - Lung
  - Heart
- May indicate normal immune function

Other background findings

Interstitial Nephritis

- Göttingen and Sinclair breeds, especially
- Features include:
  - Interstitial mononuclear cell infiltrates
  - Tubular degeneration and loss
  - Tubular ectasia and proteinosis
  - Basement membrane thickening
  - Glomerulosclerosis
  - Fibrosis

Renal

- Kidney anatomy more similar (even than NHPs) to human
- Spontaneous glomerulonephritis in 2.5% Göttingens

  - Membranous or membranoproliferative
Urinary Issues

- Crystal or urolith formation
- Urinary tract obstruction
- Recurrent UTIs

Urinary Obstruction

- Most often associated with damage to mucosa; often urolithiasis
- Congenital


Lymphoma (kidney)

Renal tumors in diabetic model

Development of a Novel Model for Streptozotocin-induced Renal Cell Tumors and Chronic Diabetes in Goettingen Minipigs (Fukuoka A et al. 2005 J Toxicol Pathol)

- Adenoma and carcinoma 5 years after STZ injection
- A stable diabetes animal model plus a chemical-induced tumor model developed with a single administration of STZ
Reproductive

- Sexually mature in 4-6 months (economic benefit vs. 3-4 years for macaques)
- Not good model for penile or ureteral procedures
- Must clean the preputial diverticulum before surgery
- Diffuse epitheliocorial placentation
- Slowly increasing use in embryo-fetal studies

Reproductive: Male

- Penis ~60 cm, relatively thin
- Coitus lasts up to 30 min; boar is said to “soak”
- Contraction/relaxation of retractor penis muscle is responsible for movements of intercourse; mostly smooth m.; attaches just distal to sigmoid flexure
- Implications of drugs affecting smooth m.; e.g., possible paralysis of smooth m. by sympatholytic drugs

Reproductive: Female

- HOWEVER: Tortereau A et al. showed only 50% of females are mature at about 6.5 months old (11.8 kg bw), and 100% were not mature until about 7.5 months old (13.1 kg). Higher uterine wt with sexual maturity, but ovarian wt not

Reproductive

- Colostrum needed for maternal antibody transfer (not placenta)

Normal Uterus
- Spontaneous uterine leiomyoma occurs at a very high frequency in minipigs (5+ yrs)
- Tumors appear to be hormonally responsive
- Pregnancy is protective
Uterine Carcinoma & Cystic Endometrial Hyperplasia

Epididymal cysts

Atrophy/Hypoplasia

Testes: focal segmental atrophy or tubular hypoplasia

Testicular Tumor

Ulcers in prepuce of piglet; Ureaplasma suspected
Melanosis

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

“Necrotizing myositis”

- Gottingens
- Multifocal skeletal muscle degeneration and necrosis
- Mononuclear cell infiltrates
- +/- myofiber regeneration

Porcine stress syndrome/ Malignant hyperthermia

- Newly diagnosed in a Gottingen (2014)
- Ryanodine receptor defect in farm pigs, may be dystrophin gene in minipigs
- Unregulated release of calcium from sarcoplasmic reticulum → excessive myofiber contraction → increase in body temp
- Triggered by anesthetic agents (halothane) or stress
Porcine stress syndrome / Malignant hyperthermia

Cornell Veterinary Medicine

• Osteochondrosis dissecans

Rare MS incidental findings
• Lingual chondrocytes = ectopic? (McInnes 2015)

Nervous System

Nervous/special senses
• CNS development; brain growth spurt before birth
• Majority of CNS cell division before birth
• Myelination largely postnatal
• Blood-brain barrier relatively immature at birth
• Highly developed eye
• Ideal for teratology studies
• Massive skull bones limit use in cranial surgery

Good model for spinal surgery
• Model for intraspinal delivery of gene and cell therapies due to size, anatomy and vulnerability of the spine/spinal cord
• Surgical process of exposing and manipulating the spinal cord, and closing the wound, is virtually indistinguishable from human
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.

Eye

• Retina, pupil, lens have closest resemblance to human eyes after NHPs.
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Endocrine System

Thyroid Gland

Parathyroid

Pituitary Gland Lesion

Diabetes models
• Provides large animal model of Type 1 and Type 2 diabetes models to study diabetes treatments and wound healing in diabetic patients.
• No spontaneous diabetes in swine
• Induced
  – Streptozotocin (STZ)
  – Severity depends on dosage (commonly 150 mg/kg)
  – Residual insulin-positive cells in pancreas
• Alloxan
  – Similar to glucose in structure
  – When in beta cell, binds glucokinase, inhibiting glucose-induced insulin secretion

Image: Lydia Andrews-Jones
Cataracts in Diabetic Minipigs

Diabetes models

Total pancreatectomy
- Better at removing endogenous insulin, but requires intensive postoperative care
- More health problems than STZ, greater time and resources
- Post-op survival only ~10 days unless receive islet transplants, then live several months

Swine as xenograft models?!
- Human xenografts are not rejected in a naturally occurring immunodeficient porcine line: A human tumor model in pigs

Organ transplants
- α-gal problem:
  1,3-galactose (α-gal) coats the surface of pig blood vessels but is absent from human tissues
  2. Antibodies against this protein → complement → holes punched in membranes of the foreign cells on contact
  3. Progress being made with genetically engineered pigs lacking gene that makes α-gal

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Minipig Research Forum on LinkedIn