Veterinary Care of Germfree Mice and Rats

Taconic

Smart Solutions To Improve Human Health

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Objectives

- Discuss basic anatomy, physiology, biology and disease of germfree rodents (mice and rats)
- Discuss species differences and its importance in veterinary care
- Provide an overview of germfree versus conventional rodents in Biomedical Research
- Discuss relevant regulatory guidelines for care and use of germfree rodents
- Discuss nutritional requirements and metabolism of germ free rodents
- Provide guidance on overcoming challenges regarding methodologies, management, and transport of germfree rodents (i.e., anesthesia; diet; shipment)

Basic Definitions

- **Germ free – free of all foreign life forms (e.g., bacteria, viruses, etc) apart from itself**
- **Conventional – animal harbors normal indigenous but undefined microflora**
- **Gnotobiotic – any animal or system in which all life forms are known**
- **Specified pathogen free (SPF) – free from pathogens, which can be specified, but otherwise with an undefined microflora**

Germfree Use in Biomedical Research

- **Germfree animals are major means of obtaining data about the relationship between host and its microflora**
  - Cardiovascular research
  - Dental research
  - Diet and Nutrition
  - Gastrointestinal disease
  - Infectious Disease/Mechanism of Disease Research
  - Immunological research
  - Neurological Research
- **Provide a nucleus for specific pathogen free colonies**

Conventional vs. Germfree Rodents

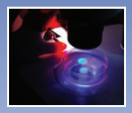
Conventional

- 10^{11} bacteria reside in normal cecum of healthy conventional and SPF mice
- 95% of total number of intestinal bacteria are obligate anaerobes
- Conventional animals have stratified organization of the gut flora

Germfree

- Germfree rodents adapt anatomically and physiologically to environment devoid of microflora
- Have greater nutritional requirements
- Germfree rodents grow and breed similar to rodents raised in conventional settings
- Germfree rodents have a longer life span when fed *ad libitum* diet compared to conventional animals

Disease Prevention - Cesarean vs. Embryo Transfer in Germfree Development



CD

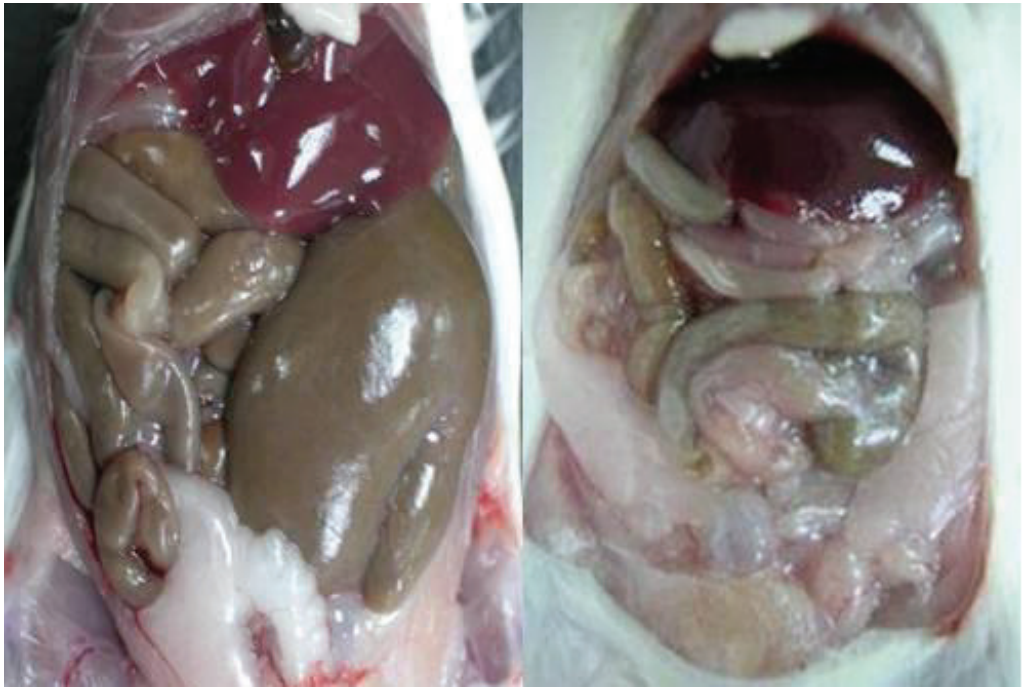
- **Hysterectomy does not eliminate pathogens that may contaminate fetus after uterine implantation or that are vertically transmitted**
  - LCMV
  - LDH
  - *Pasteurella pneumotropica*
  - *Mycoplasma* spp

ET

- **2-cell stage**
- **Eliminate issues associated with vertical transmission**

Anatomy and Physiology of Germfree Rodents

- **Enlarged cecum – primary anatomical difference**
  - 5 x normal size
  - Poor reproductive performance due to restricted abdominal space
  - More urea; little ammonia in intestinal contents

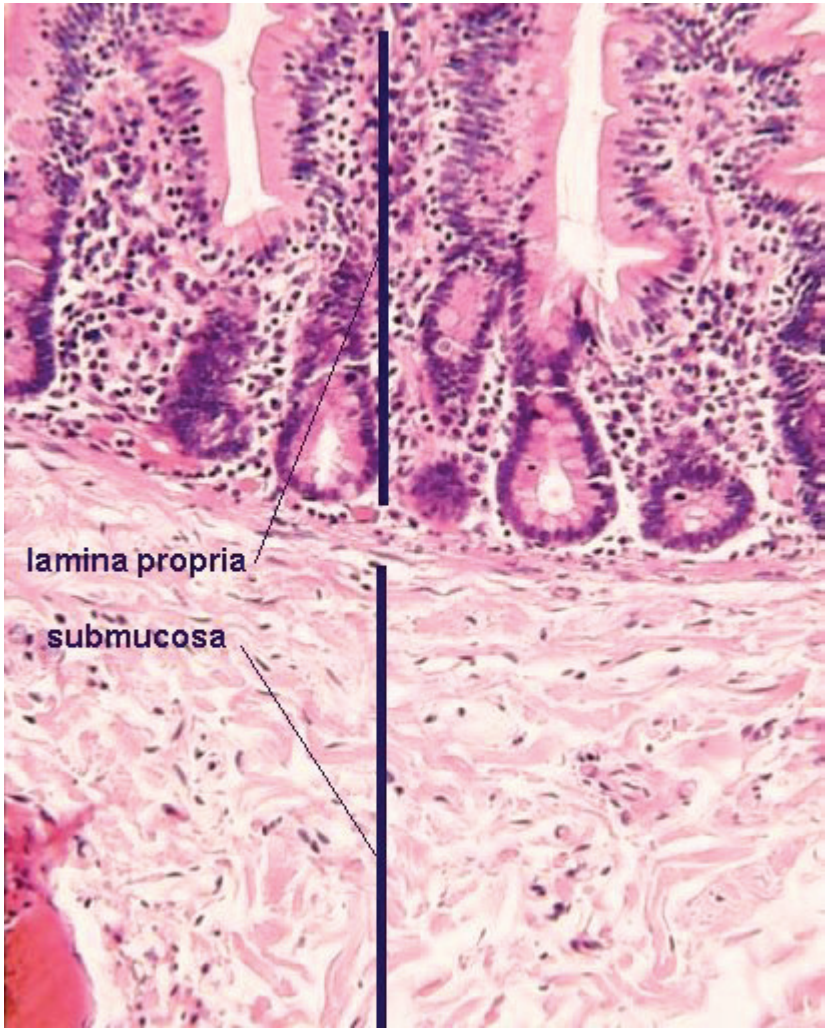


Germ free mouse **Conventional mouse**

Germfree Anatomy and Physiology

- **Intestinal atonia**
  - Altered myenteric neurons; Reduction in smooth muscle tone of lower bowel
  - Cecal volvulus →intestinal strangulation and death
  - Produce semisolid stools ("chronic diarrheal state") – large amount of mucin in feces
- **No urobilin in urine**
- **High urinary calcium and citrate; low phosphate**

Germfree Anatomy and Physiology



- **Thinning of wall of small intestine**
  - Thinning of lamina propia
  - Sparse numbers of plasma cells – decreased IgA
  - Decreased mucosal surface
  - Reduced renewal rates of intestinal epithelium

Anatomy and Physiology

- **Immunology**
  - Lymph node morphology
    - Contain 1/12th blast and antibody-producing cells as conventional mice
  - Immune response of mice is equal to and in many cases greater than conventional mice
  - Reduced circulating leukocytes
  - Lower levels serum immunoglobulin due to decreased antigenic stimulation
  - Good graft vs. host reaction

Germfree Anatomy and Physiology

- **Heart, lungs and liver of the germfree mouse are smaller than conventional mice**
- **Cardiac output is 1/3 lower**
- **Reduced blood volume and flow to organs**
- **Reduced vascular response to catecholamine**
- **Increased RBC count and Hematocrit**
- **Thinner alveolar and capsule wall**

Germfree Anatomy and Physiology

- **Decreased body fat percentage**
- **Decreased basal metabolic rate**
- **Voluntary intake of food is normal or increased**
- **Increased water intake**
- **Altered endocrine function**
  - Decreased iodine uptake in thyroid
  - Decreased motor activity (hypo responsive to epinephrine, norepinephrine, and vasopressin)
- **Increased exocrine pancreatic function**
- **Proglonged diestrus period in germfree females; reduced frequency of estrus and therefore copulation and implantation rates**

Anatomy and Physiology – Germfree Rat

- **Soft tissue calcification**
  - Rats have greater absorption and retention of calcium and magnesium
  - Increased intestinal bile acids → micelle formation with mineral-carrying lipid complexes

- **GF rats feed high cholesterol diet develop 2x levels of blood cholesterol compared to conventional rats; females > males**

Germfree Effects and Onset of Disease

- **Elimination of gastrointestinal microflora increases host susceptibility to infectious disease**
  - Death of GF animals within days of removal from isolator
  - Colonization of rodents with a limited, defined gut microflora establishes near-natural homeostasis
- **Increased susceptibility to:**
  - Shigella flexneri
  - Bacillus anthracix
  - Listeria
  - Influenza A
  - Coxsackie B
- **Varied responses to parasitic infections; viral infection results in comparable or slightly higher interferon production**

Shipment/Transport of GF Rodents

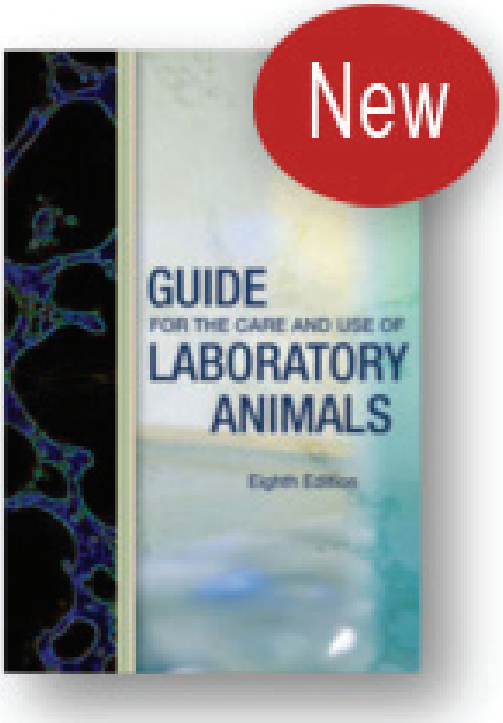
Taconic Germfree Shipper

Husbandry – Ensuring Health and Welfare of Germfree Rodents

- **Diet – fortified with vitamins**
  - Vitamin K deficiency – abnormal clotting time
  - Use autoclavable diets fortified with additional vitamins
  - Irradiated diets – beware risk factors; primary contaminant seen is Aerobic Spore Forming rods

- **Environmental Considerations**
  - Temperature and Humidity Controls
    - Temperature is usually higher and humidity lower than the rearing room
  - Ventilation
    - Ensure airflow through isolator which uniformly ventilates every cage; check for condensate
  - Vibration and pressure differences should be considered to avoid adverse effects on health, welfare, and integrity of the isolator
  - Water Quality

Animal Care and Use – Impact New Guide



New/Expanded Recommendations

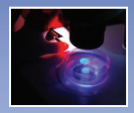
- Requirements for Performance Standards
- Cage sizing
- Cage sanitation
- HVAC controls
- Environmental Monitoring

GF Methodologies – Preventing Disease & Ensuring Health and Welfare

- **Animal Transportation**
  - Transfer containers
  - Transit time
- **Sterilization**
  - Methods: Steam vs. Ionizing Radiation
  - Equipment and supplies
    - Transfer containers
    - Liquids
    - Special diets

GF Methodologies – Preventing Disease & Ensuring Health and Welfare

- **Reliable Genetic Control**
  - Tail biopsies
  - Anesthetic Protocol Required for non weanling animals
- **Anesthesia and Analgesia**
  - Altered response to anesthesia
  - Methods:
    - Parenteral injection
    - Non-flammable gases through filtered gas lines
    - Local anesthesia



The End

QUESTIONS?

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