Principles of Rodent Aseptic Surgery & Perioperative Care

Marcel Perret-Gentil
University Veterinarian & Director
Laboratory Animal Resources Center
The University of Texas at San Antonio
marcel.perret@utsa.edu  (210) 458-6692
http://research.utsa.edu/research-funding/laboratory-animal-resources-center/training/
Resources

LARC Training Page

Key resource
Your Veterinarian
Practicing the art of surgery...

- Skills are Developed, Practiced and Refined
- After that, Practice, Practice and Practice

So that what happened to Ms. Rita doesn’t happen to you...
Ms. Rita is 85

An exemplary nurse

Just a Little bit out of practice
Tips, Tips, Many Tips...
Magnification
Lighting

- Ovarian fat pad
- Ovary
- Uterine horn

Poor lighting vs. Good lighting
Microsurgical instrument selection

- Longer instruments allow handles to rest on hands between thumb and index (web of the hand) for greater control.
- Rounded handles (vs. flat) allow rotation around the axis for greater control.
Needle & Suture
Needle Anatomy

Needle loading zone

Weak zone
Swaged Needle

Eye Needle

Tissue Drag

Swaged Needle

Eye Needle

😊

😢
Absorbable vs. non-absorbable

Various suture material

Double packaging

Expiration date
Multi stranded (braided) Suture

Single stranded (monofilament) Suture
Closure with Non-Suture Material

Wound clips

Note: Skin tissue *eversion* is OK, *inversion* IS NOT

Tissue adhesives
cyanoacrylate

Clips, applier & remover
Acclimation
Wonders of Acclimation
The healthy rodent to start with
Aseptic Technique in Rodents

“The Solution to Pollution is Dilution”
Rodents don’t get infections???
Why Aseptic Rodent Surgery?
Survival alone not a valid criterion for success

Success should be based on absence of altered physiological function, immune responses and behavioral changes

(Cunliffe-Beamer, 1993)
Aseptic technique increases success of ovarian transplants in mice & speeds return to post-op normal (Cunliffe-Beamer 1972-73; Cunliffe-Beamer 1990)

Contamination activates macrophages (Bancroft, Schreiber et al. 1989), and leads to changes in cytokines & B cells levels (Abbas, Lichtman et al. 1991)

SUBCLINICAL infections induce physiological changes (Committee on Infectious Diseases of Laboratory Rats and Mice 1992)

Although NO CLINICAL SIGNS observed, experimentally inoculated rats (10^8 S. aureus or P. aeruginosa) had significant alterations in plasma fibrinogen, serum glucose, total white blood cell counts, and wound histology scores (Bradfield, John et al. 1992)
ACLAM Position Statement on Rodent Surgery

Aseptic technique is required for survival surgery since animals can develop either gross or unapparent surgical site infection that may affect research outcomes and animal welfare.

JAALAS 55(6):822-823
Summary

Poor rodent aseptic surgery results in:

- Poor experimental results
- Delay to post-op normality
- Physiological changes
- Alterations in fibrinogen, glucose, leukocytes, histology, citoguines, B cells, etc.
- Questionable data
Instrument Cleaning & Lubrication

Cleaning steps:
1. Soak in enzymatic sol  
   (ultrasound if available)
2. Brush/clean
3. Rinse
4. Lubricate & dry on
Contact points y contamination
Surgeon Prep

Impermeable arm sleeves
Misted with disinfectant
Sterile Surgery Gloves
Donning Sterile Surgery Gloves Video

Click Here
Surgical area setup

This? or This?

or

This?
This is the surgery table

Clipping hair where surgery is going to take place
NO NO!!!

Surgery in animal prep area = Contamination
This is where you clip hair, away from surgery table to avoid debris from falling into the surgical wound.

Surgery location ≠ Animal prep area
Sterile instruments placed on non-sterile surface.
All instruments are on sterile field

😊
Suture and thumb forceps crossing over into non-sterile field
Suture and all instruments are within the sterile field
Post-op recovery cage is dirty
Post-op recovery cage is clean, to minimize post-op infections
Patient preparation
Antibiotics?
Antibiotic use

- Not a substitute for proper asepsis (...false sense of security...)
- Use judiciously – resistant strains
- Consider antibiotic rotation (↓↓resistant strains)
- Generally not recommended - justify
- Best preemptively
- Once pre op usually enough
- Adds one more variable???
Assessing antibiotic need

- Immune deficiency
- Organs with contamination potential (gut, bladder, lungs...)
- Extensive tissue dissection & blood loss
- Inadvertent contamination
- Biomaterial implantation
- Lengthy procedure
- Stressed, aged or ill
- Inexperienced surgeon
  - ↑↑ experience = ↓↓ antibiotic
Protect the Eyes
Do not apply non-ophthalmic products
Only **ophthalmic** ointment to protect eyes when an animal is anesthetized

Apply soon after anesthesia induction
Skin Prep

Clipping is better than chemical depilation

Chemical depilation (e.g., Nair) – irritating – remove in 45-60 sec – wipe off
Clip in direction of hair 1st then against it

#40/50 Clipper Blade
Skin Disinfection
Skin Disinfection

1. Remove hair
2. Chlorhexidine or povidone iodine SCRUB followed by 70% alcohol
3. Repeat previous SCRUB & alcohol step 2 more times
4. After last alcohol, paint area with chlorhexidine or povidone iodine SOLUTION (NOT scrub)
5. Allow SOLUTION to dry on – lethal effects on bacteria maximized by combination of chemical + desiccating activity
Skin Disinfection Video

Click Here
Draping with cotton material leads to wicking of fluids and bacteria into the surgical wound.
The 5 properties of the ideal rodent draping material...

- Sterile
- Adheres to skin
- Impermeable
- Transparent
- Traps body heat...
Draping the Animal

Sterile
Adheres to skin
Impermeable
Transparent
Traps body heat
Cost Comparison

Glad Press’n Seal
- $3.69 - 70 sq. ft. roll (Target)

Others
- ETO sterilized Press’n Seal
  ✓ $18.94 - 70 sq. ft. roll (SAI)
- Packaged sterile drape
  ✓ $45.90 - Rodent Surgical Drapes 5pk (SAI)
Draping other parts

Reynolds Aluminum Foil
Tail kept on warming pad
Easy Table & Animal Draping Technique without Sterile Gloves Video
The Solo Surgeon

- Frequently investigators assign surgery duties to a member of their lab without additional help from another person. The lab member ends up performing every aspect of the surgery alone.

- So what happens if non-sterile items such as the anesthesia dials, stereotaxic apparatus knobs, light handles and microscope dials have to be manipulated during surgery?

- The next slides provide practical solutions to the “Solo Surgeon” problem.
Touching non-sterile parts with sterile gloves
Covering the anesthesia machine dials with Press’n Seal or aluminum foil prevents cross-contamination of the surgical wound.
Covering parts of the stereotaxic apparatus
Light handles
Surgical microscope
Need to use a timer and you are the solo surgeon?
When need to write notes and you are the solo surgeon
There are many devils in this picture. See if you can identify them before going to the next slide.
Here are the devils

1) No hair cover

2) Touching contaminated object (glasses) with gloves

3) Using non-sterile (nitrile) gloves

4) Syringe outer package on sterile field

5) Tube of ophthalmic ointment on sterile field

6) Suture outer package on sterile field

7) Anesthesia hoses & animal not covered with sterile drape

8) Sterile glove outer packaging on sterile field

9) Red top blood tube on sterile field

10) Disinfectant bottle on sterile field

11) Dial not draped
UTSA required rodent surgery PPE:
1. Gown
2. Sterile gloves
3. Face mask
4. Hair cover
5. Arm sleeves
More Tips...
Tissue protection

“Wet tissues = Happy tissues”
Monitoring, thermoregulation, recovery & fluid maintenance
Normal Temperature

<table>
<thead>
<tr>
<th></th>
<th>°F</th>
<th>°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOUSE</td>
<td>99</td>
<td>37</td>
</tr>
<tr>
<td>RAT</td>
<td>99.5</td>
<td>37.5</td>
</tr>
</tbody>
</table>
Anesthetics & Hypothermia

- All anesthetics depress thermoregulation
- Vasodilation makes it worse
Fear
Hypothermia
Fear Hypothermia

- Rodents lose heat rapidly (high surface area/body wt ratio)
- Starts at induction
- Exacerbated by cold, dry gases, shaving, skin prep solutions & admin of cold fluids
Fear Hypothermia

- Prolongs recovery
- ↑ potency of volatile anesthetics
- Leads to hypoventilation
- ↓ platelet function
Fear Hypothermia

- Bradycardia, fatal arrhythmias, myocardial infarction
- ↑ blood viscosity = ↓ gas exchange
Fear Hypothermia

- ↑ stress response to surgery
- ↑ risk of infection
- Impaired wound healing
- CNS depression
Fear Hypothermia

- Interferes with metabolism of some drugs
- Special concern with hairless strains and neonates

May be difference bet life & death, reliable & unreliable data, success & failure
Preventing Hypothermia
• Monitor temp – otherwise how will you know?
• Cutaneous warming (lamps, warming blanket) – Preheat prep (scrub) solutions
• Airway heating
• Administer pre-warmed fluids

Temperature monitoring

Fluid bag kept warm on heating blanket
Heat pad under induction chamber

Insulate with bubble wrap, Press’n Seal
Heat pad under $\frac{1}{2}$ of cage allows rodent to escape excess heat.
Warming systems in order of preference

- 😊😊😊😊😊 Far Infrared Homeothermic Pad (self regulating)
- 😊😊😊 Circulating Water Heating Pad
- 😊😊 Isothermal Pads
- 😊😊 😡 Electric Pad: focused warming areas, elements may burn
- 😊 😡 😡 Heat Lamp: difficult to judge distance (burns), some institutions have banned it
Electric Pad Warning!

Electric Pad Burn - Rat
Physiosuite (Kent Scientific)

- Homeothermic pad (warms rodent at exact temp through far infrared arming that goes beyond cutaneous warming by heating deep into rodent’s body with temp feedback)
- Pulse oximeter and heart rate
- Automatic ventilator (enter animal weight & press run)
- End tidal CO₂ monitor
Homeothermic (self-regulating) system
Homeothermic (self-regulating) Pad
Hydration

Like hypothermia, good hydration may be the difference between life/death, success/failure.
Hydration

Make it a habit of administering WARMED fluids before surgery – Lactated Ringer’s solution or Normal saline

- Mice 0.5-1 ml SC/IP
- Rats 5-10 ml SC/IP
Hydration + Normothermia

↓↓ Morbidity & Mortality
Pharmacological Methods of Pain Control
Preemptive analgesia (before surgery) can reduce magnitude of post-op hypersensitivity and pain*

*Bromley 2006; Gonzalez et al. 2000; Lascelles et al. 1995, 1997; Reichert et al. 2001
Multimodal approach provides superior analgesia compared to use of a single analgesic*

Combinations of:

- NSAIDs
- Opioids
- Local anesthesia

*Recognition & Alleviation of Pain in Laboratory Animals, NRC 2009
Avoidance of peaks and valleys in analgesic dosing*

- Best accomplished by administration of continuous or overlapping regimes
- Sustained Release (SR) formulations (e.g., Buprenorphine SR, Meloxicam SR)

*Recognition & Alleviation of Pain in Laboratory Animals, NRC 2009
Local Anesthesia

- Can reduce required overall dose of analgesics
- Prevents hyperalgesia (amplification of pain signal)
Local Anesthesia – Part of Multimodal Strategy

<table>
<thead>
<tr>
<th>Local Anesthetic</th>
<th>Onset</th>
<th>Duration</th>
<th>Do not exceed (toxic dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lidocaine</td>
<td>1-3 min</td>
<td>~40 min</td>
<td>10 mg/kg</td>
</tr>
<tr>
<td>Bupivacaine</td>
<td>~20 min</td>
<td>~4-6 hours</td>
<td>5 mg/kg</td>
</tr>
</tbody>
</table>

- Mix: ½ and ½ volume of each solution
- Administer under incision and surrounding area
Non-Pharmacological Methods of Pain Control

Treating the mind... & other parts

Simkin & O’hara, 2002; Chia et al, 2017
Fear & Anxiety Enhance Pain Response

Providing a stress-free environment must be included in the pain management program

Linton 2000; Morley et al. 1999; Munro et al. 2007; Panksepp 1980; Perkins and Kehlet 2000; Ploghaus et al. 2001
Minimizing Environmental Stress

Minimizes stress-induced hyperalgesia*

*Mogil, 2017
Delicious Calories

• Tasty, energy dense food supplements
• If necessary, place at floor level

DietGel
clearh2o.com

Bacon Softies
bio-Serv.com
Social Housing

- **Single housing – The post op norm, however...**
  - Rats spinal cord injury – 20% less chance of survival when housed individually
  - Telemetric implant surgery, female mice - When housed socially needed less time to fully recover

(Van Loo et al., 2007)
The sick & painful rodent

Both mouse and rat have starey (piloerection of guard hairs) coats and a poor body condition.
The sick, stressed & painful rodent

Red tears (chromodacryorrhea) – Porphyrin, red-brown pigment secreted from Harderian glands in rats. Occasional low levels of staining may be normal. Overproduction indicates stress, sickness, poor nutrition or pain. Porphyrin drains through nasolacrimal duct. Rat may smear around nose and fur with paws during grooming.
The painful rodent
Abdominal presses and extension of the hind-limbs indicative of abdominal pain
Grimace Scale

Orbital tightening

Nose bulge

Ear Position

Images from Langford et al, 2010
Grimace Scale

Cheek bulge

Whisker position

Images from Langford et al, 2010
Training

The LARC is pleased to offer instruction in the various techniques used in studies involving laboratory animals. The LARC provides the hands-on and instructor-led portions of the UTSA Animal User Training Program Requirements Checklist (PDF). Please feel free to contact the LARC at larc@utsa.edu to see how we can assist you with your animal research.

Training Handouts and Videos

Rodent Surgery, Anesthesia and Analgesia

Rodent Aseptic Surgery Techniques and Videos

Dos and Don'ts of Rodent Aseptic Surgery (PDF)

Draping Technique for Stereotaxic Surgery (PDF)

Draping a Rodent for an Orthopedic Procedure (PDF)

Survival Rodent Surgery Checklist (PDF)

Aseptic Rodent Surgery Videos

Open Gloving Technique for Survival Surgery (YouTube)

Cutting Press'n Seal Surgical Drape over the Animal (YouTube)

Draping the Table & Animal for Rodent Surgery with Press'n Seal with Non-Sterile Gloves (YouTube)

Placing Sterile Instruments & Surgical Supplies on Sterile Field (YouTube)

Skin Surgical Prep in Rodents (YouTube)

Draping Rodent with Press’n Seal after Table is Draped (YouTube)

Removing a Drape Piece from an Already Opened Press’n Seal Box (YouTube)
Resources

Presentations, References & Useful Notes

http://research.utsa.edu/research-funding/laboratory-animal-resources-center/training/
Marcel Perret-Gentil, DVM, MS
University Veterinarian & Director
Laboratory Animal Resources Center
(LARC)
The University of Texas at San Antonio
San Antonio, Texas

marcel.perret@utsa.edu

vpr.utsa.edu/larc