Principles of Rodent Aseptic Surgery & Perioperative Care

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Practice the art of surgery…
- Skills are Developed, Practiced and Refined
- After that, Practice, Practice and Practice

Time = Trauma

“In theory, there is no difference between theory and practice… But in practice there is”
--- Yogi Berra ---

2 + 2 ≠ 4

Tips, Tips, Many Tips…

Magnification
**Lighting**

- Ovarian fat pad
- Ovary
- Uterine horn

**Microsurgical instrument selection**

- Longer instruments allow handles to rest on hands between thumb & 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

**Tissue Drag**

- Swaged Needle
- Eye Needle
- 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

Tissue adhesives (cyanoacrylate)

Note: Skin tissue eversion is OK, inversion IS NOT

Clips, applier & remover

Acclimation

Wonders of Acclimation

The healthy rodent to start with

Food

Cage

Light

Smells

Temperatur e

Humidity

Sounds
Aseptic Technique in Rodents

“The Solution to Pollution is Dilution”

So Why Aseptic Rodent Surgery?

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 (10^6 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

ACLAM: American College of Lab Animal Medicine
Instrument Cleaning & Lubrication

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

Surgeon Prep

Impermeable arm sleeves not required but highly recommended – disinfectant sprayed

Sterile Surgery Gloves

Donning Sterile Surgery Gloves Video

goo.gl/SEqeSH

Surgical area setup

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

стерильные инструменты на нетеррильной поверхности

Stere instruments placed on non-sterile surface

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
- Source of variables???

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 product to eyes

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

Skin Disinfection

- Clip in direction of hair 1st then against it
- #40 Clipper Blade
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

Sterile Draping

The ideal draping material
- Adheres to skin
- Impermeable to moisture
- See through
- Traps body heat

Draping the Animal

Draping with cotton material leads to wicking of fluids and bacteria into the surgical wound

Impermeable material such as Press’n Seal as seen on this rat is a great drape

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 autoclaved aluminum foil prevents cross-contamination of the surgical wound

Covering parts of the stereotaxic apparatus with Press’n Seal or autoclaved aluminum foil

Covering light handles with Press’n Seal or autoclaved aluminum foil

Covering knobs of the surgical microscope with Press’n Seal or autoclaved aluminum foil

Need to use a timer and you are the sole 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. Not 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

All required PPE is in place:
1. Gown
2. Sterile gloves
3. Face mask
4. Hair cover
5. Arm sleeves (not required but highly recommended)

Let's critique this
Survival Surgery

Let's Critique Another One
Cirugía sobreviviente
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 during 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

Heat pad under induction chamber

Insulate with bubble wrap, Press’n Seal

Post-op Recovery

Heat pad under ½ 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

Homeothermic (self-regulating) Pad

Physiosuite
kentscientific.com
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) Pad

Hydration

Like hypothermia, good hydration may be difference bet 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

Pharmacological Pain Control

- Best when administered before insult (a.k.a. preemptive analgesia)
- Multimodal superior to single analgesic

hydration + Normothermia

= ↓ Mortality & Morbidity

*Jirkut, 2017
Local Anesthesia

- Can reduce required overall dose of analgesics
- Prevents hyperalgesia (amplification of pain signal)

1% lidocaine/0.25% bupivacaine (50/50 mix by volume)
- Infuse incision site and underlying tissues
- May need to dilute, esp. for mice – dilution reduces duration of effect*
- Epinephrine prolongs action

*Grant et al, 2000, J.Pharm and Tox Methods, 43, 69-72

Local Anesthesia – Part of Multimodal Mix

<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 (xylocaine)</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>
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- Mix: ½ and ½ volume of each solution
- Administer under incision and surrounding area

Non-Pharmacological Methods of Pain Control

Confortable Environment

Provide comfort & stress free environment
Nestlets, Enviro-dri, huts)

Tasty Calories

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

Bacon Softies
bio-Serv.com

DietGel
clearh2o.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)

Other Non-Pharmacological Methods of Pain Control

- Habituation to procedures
- Fluid therapy
- Thermal support

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 staring 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
Mouse in Pain

Rat in Pain

Back arching & squinted eyes

Courtesy of Paul Flecknell

Grimace Scale

Orbital tightening

Nose bulge

Ear Position

Images from Langford et al. 2010

Grimace Scale

Check bulge

Whisker position

Images from Langford et al. 2010

Resources

Presentations, References & Useful Notes

http://goo.gl/rzR3sl
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