Principles of Rodent Aseptic Surgery & Perioperative Care

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Tips, Tips, Many Tips... Practical Tips

The Needle & Tissue Drag

Swaged Needle Eye Needle

Swaged Needle Eye Needle

Tips, Tips, Many Tips...

Multi stranded (braided) Suture

Single stranded (monofilament) Suture

Closure with Non-Suture Material

Tissue adhesives cyanoacrylate

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

Wonders of Acclimation

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 (10⁸ 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)

Summary

Poor rodent aseptic surgery results in:

• Poor experimental results
• Delay to post-op normality
• Physiological changes
• Alterations in fibrinogen, glucose, leukocytes, histology, cytokines, B cells, etc.
• Questionable data

Instrument Cleaning & Lubrication

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

Surgical area setup

Surgical area setup

This?

or

This?

Surgeon Prep

Impermeable arm sleeves
Misted with disinfectant
Sterile Surgery Gloves

Donning Sterile Surgery Gloves Video

Crowded surgical area setup

This is the surgery table

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

Contact points of contamination

Suture and thumb forceps crossing over into non-sterile field

All instruments are on sterile field

Caution with forearm hyperextension – could compromise respiration

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 (I! resistant strains)
- Generally, not recommended – justify
- Best preemptively
- Once pre op usually enough
- Adds one more variable???

Assessing antibiotic need

- Immune deficiency
- Organs with high contamination potential (gut, bladder, lungs...)
- Extensive tissue dissection & blood loss
- Inadvertent contamination
- Biomaterial implantation
- Lengthy procedure
- Stressed, aged or ill
- Inexperienced surgeon
  - 11 experience = ill antibiotic

Protect the Eyes
Skin Prep

Clipping is better than chemical depilation

Chemical depilation (e.g., Nair) – irritating – remove in 45-60 sec – wipe off

Dermatitis produced by chemical depilation 1 minute contact time

Skin Disinfection

Clip in both directions
Skin Disinfection

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

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

Rodent Surgical Draping

The 5 properties of the ideal rodent draping material...
- Sterile
- Adheres to skin
- Impermeable
- Transparent
- Traps body heat...

Bonus property: Cheap!

Draping the Animal

- Sterile
- Adheres to skin
- Impermeable
- Transparent
- Traps body heat
- Cheap!

Draping other parts

Reynolds Aluminum Foil
The Solo Surgeon

- Typical lab: One does it all
- 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

Press’n Seal or aluminum foil
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

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
Here are the 10 Devils:

1. No hair cover
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And one more devil:
11. Did not draped
Hot Bead Sterilizer – Tips Only Technique

Recommendations*:

- Use only when light contamination - Not for gross contamination (such as gut surgery)
- Do not overload sterilizer (not too many instruments)
- Dip x 1 minute at 500 ± 50 °F (260 ± 10 °C)
- Clean instruments between surgeries
- Autoclave instruments on first animal & use for up to 5 serial surgeries only

*Holdridge et al. 2001

Tissue protection

“Wet tissues = Happy tissues”

Monitoring, thermoregulation, recovery & fluid maintenance

Anesthetics & Hypothermia

- All anesthetics depress thermoregulation
- Vasodilation makes it worse
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

- 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
- Administer pre-warmed fluids

Fluid bag kept warm on heating blanket

Temperature monitoring

Heat pad under induction chamber
Insulate with Press’n Seal,

Heat pad under ½ of cage allows rodent to escape excess heat

Heat Lamp: difficult to judge distance (burns), some institutions have banned it

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

Post-op Recovery

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

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

10-20 ml/kg

O₂ Supplementation

- Injectable anesthesia results in hypoxic state
- O₂ supplementation = ↑↑↑ Survival*
- Use vaporizer system with anesthesia turned off to supplement O₂ (0.5 L/min) via facemask when using injectable anesthetics

*Blevins et al, 2021

Hydration

Normothermia + Oxygen = ↓↓↓ mortality
Pharmacological Methods of Pain Control

Preemptive analgesia (before surgery) can reduce post-op hypersensitivity and pain*

*Brumley 2004; 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

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>
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- 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; Perkesopp 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

Mouse in Pain

Rat in Pain (Laparotomy)

Back arching & squinted eyes

Pain Assessment in the Rat
Behaviour following Laparotomy for Bladder Tumour Implant

Courtesy of Paul Flecknell
Grimace Scale

Orbital tightening

Nose bulge

Ear Position

Images from Langford et al, 2010

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