



Policy for survival surgery

Successful surgical outcomes in research animals of all species require the same techniques and procedures as in any veterinary practice. Aseptic and minimally invasive surgical technique and optimal perioperative and postoperative care are prerequisites to achieve surgical success and best possible animal welfare outcomes. Good surgical practice will not only improve the animal's postoperative recovery, but also study outcome and validity.

The purpose of this policy is to clarify the requirements of the researchers concerning survival surgical procedures performed on rodents and other animals. All researchers performing survival surgery must adhere to the minimum standards addressed in this policy. The policy is approved by the Animal Welfare Body and has been presented for the Faculty Management Team (FLT) for their information.

This policy includes requirements for education and training of personnel, designated surgical area, use of approved anesthesia and analgesia, aseptic technique including preparation of the animal and surgeon, sterilization of surgical instruments and equipment, intraoperative monitoring and postoperative care.

Definitions

Survival surgery: Any surgery conducted on animals that are expected to recover from anesthesia.

Non-survival surgery: Any surgery conducted on animals that are not allowed to regain consciousness. This includes terminal vascular perfusion.

Responsibility

It is the responsibility of the license holder to ensure competences of personnel and thus, to ensure that all personnel are adequately educated and trained in anesthesia administration and monitoring, aseptic technique, good surgical technique, and postoperative care during the recovery period.

Education and training of personnel

Surgical course or dispensation

The basic course in laboratory animal science (FELASA EU function ABD) does not include theoretical or practical training in surgery. Therefore, in order to ascertain that persons engaged in surgical activity are competent and have the best prerequisite for surgical success, researchers must complete one or more relevant courses, and document their surgical skills by self-evaluation (Appendix 1) before engaging in surgical activities.

The department offers courses that covers basic principles of experimental surgery as well as advanced surgical techniques and microsurgery (<https://emed.ku.dk/courses>).

Peer-to-peer training cannot replace an official surgical course, because there is a risk of incorrect practices being passed on. Furthermore, peer-to-peer training often lacks systematics and completeness, and performance evaluation may not occur.

A dispensation from the requirement to complete a surgery course can be granted by AEM's veterinarian to:

- Veterinarians and medical doctors with documented surgical skills (by self-evaluation)
- Researchers with surgical experience and where AEM's veterinarians, after evaluating the researcher's surgical skills, find these sufficient

Evaluation of surgical skills is initially done by the use of self-evaluation (Appendix 1) and can be followed by direct observation by AEM's veterinary personnel. If the surgical skills are found overall to be insufficient, completion of a surgical course and a re-evaluation are necessary before surgical activity can be resumed.

Despite a researcher having previous experience and/or relevant course activity, AEM veterinarians can, if necessary, stop surgical activities and require researchers to obtain relevant competences before resuming surgical activity.

Surgical training

In addition to basic knowledge and understanding of surgical principles, surgical success requires training. To avoid compromised animal welfare, inexperienced surgeons are required to practice on dummies and perform non-survival surgery until confidence is reached and experience is sufficient to perform survival surgery. Initially, only a single animal should be operated at a time, and in the beginning of the work week, and observed for some time after surgery depending on the type of surgery. If the outcome is not as intended, the procedure must be reviewed and possibly changed. The animal may have to be euthanized if it is unsuitable for the research project. AEM veterinarians can be contacted for advice at emed-vet@sund.ku.dk.

It is advised to consult with a model- and species-specific resource person all the way from planning to execution of the study to increase the chance of success and improve study outcome and animal welfare.

Recognition and assessment of pain

To effectively treat pain in animals, it is imperative to be able to recognize signs of pain. Without a knowledge of the animal's normal and abnormal behavior and appearance, assessment of pain in animals is very difficult. Surgeons must be properly educated and trained in recognition and assessment of pain in the species they are working with. AEM offers a range of courses in animal experimentation which include recognition and assessment of pain. For species not covered by AEM courses knowledge must be achieved by other means e.g. by consulting with a species-specific expert and/or attending a species-specific course.

Designated surgery area

- For rodent surgery a dedicated facility is not required. A rodent surgical area can be a room or portion of a room that is easily sanitized. The immediate surgical area must not be used for other purposes during the time of surgery. For large animals, a proper surgical room is necessary.
- Surgery must be conducted in an uncluttered, clean area. The work surface/laboratory bench should be wiped with an appropriate disinfecting agent e.g. Virkon S before surgery. Clean absorbent pads or towels should be placed under the animal. Replace these materials after each surgery session or when soiled.
- The surgery area must be separate from the area where hair/fur is removed.

Anesthesia and analgesia

- A clear plan for providing pharmaceutical grade injectable or inhalation anaesthetics and a description of how and when analgesics will be administered must be described in the project plan and followed accordingly.
- Injectable anesthetics: Animals must be weighed prior to surgery to calculate the appropriate dose of anesthetics. To shorten the recovery time, an antidote can be administered to reverse the anesthesia after surgery has ended.
- Analgesics: Without analgesia, surgery induces nociception which will induce intensified pain and poor healing postoperatively. To prevent this, analgesia must be administered before a noxious stimulus is induced, i.e. before the incision is made, and in appropriate time to have effect. Analgesia must also be administered postoperatively, with the correct time intervals depending on the analgesic and surgical procedure. In addition, the use of analgesia in an anaesthetic regime often requires lower doses of each drug to reach a surgical plane, which will minimize the adverse effects of the individual drugs used.
- Anesthesia and analgesic compounds must never be used after the date of expiration.

Aseptic technique

- Successful aseptic technique involves adequate preparation of the area where surgery is to be performed, of the equipment, utensils and instruments, of the animal itself, and of the surgeon. Careful pre-planning of these elements can significantly improve surgical outcomes.
- The use of a non-sterile assistant is highly recommended.

Surgical instruments and equipment

- All materials, equipment, implants and surgical instruments used in surgery and/or placed inside the animals must be either sterile single-use (disposable) or sterilized prior to each use. Before sterilization, any organic material must be removed. Preferred initial sterilization methods are steam autoclave, ethylene oxide gas, or hydrogen peroxide vapor sterilization. Alcohol is not recommended for sterilizing surgical materials principally because it lacks sporicidal action. Autoclaved packages should be labelled with date of sterilization and stored in a clean dry location to preserve package integrity and avoid need for re-sterilization. Packages should be visually inspected prior to use to ensure they are intact.

Sterile instruments must be handled aseptically to minimize contamination. Instrument tips should not touch non-sterile surfaces. A glass bead sterilizer may be used mid-procedure if an instrument becomes contaminated. Clean the instruments with a wash solution and then re-sterilize per the manufacturer's instructions. Make sure instruments are cool to the touch prior to any contact with the animal. Hot instruments are extremely damaging to tissue.

- It is recommended to use a new set of autoclaved instruments for each animal. However, it is acceptable that instruments are used for a series of similar surgeries, – up to five animals in the same experimental cohort – provided they are cleaned and re-sterilized using a glass bead sterilizer between animals. Gloves should be changed if soiled. Keep sterile instruments on a sterile field when not in use.

Preparation of the animal

- Check that the animal is healthy and fit for surgery.
- The animal must be anesthetized with a suitable anesthetic using the doses and procedure as described in the project plan and the animal experiment license.
- A non-medicated ophthalmic ointment (eye lubricant) should be applied to the animal's eyes to prevent corneal damage.
- After the animal is anesthetized, the fur must be shaved/removed from the surgical field/incision site. No hair is allowed to protrude from underneath the drape into the surgical field. This procedure should be performed in a location separate from the surgery area (i.e. a separate procedure bench or a segregated area on the same procedure bench).
- Aseptic preparation of the surgical site: the skin should be washed with a soap solution – preferably chlorhexidine soap – and rinsed with water to remove soap residuals. After washing the skin should be disinfected. A combination of chlorhexidine and alcohol is recommended, as the combination will prolong the disinfecting properties. The chlorhexidine concentration should be $\geq 0.5\%$ and the alcohol should be 70%. Commercial

combinations are available. Other antiseptics can be used for aseptic preparation, but one should be aware of different properties. E.g. Iodine solutions can be a skin irritant and is inactivated by organic matter such as blood. Allow the skin to dry as the lethal effect on bacteria is maximised by combination of chemicals and desiccating activity. Soap solution and disinfectant should be applied from the centre toward the periphery of the surgical site to avoid introduction of microorganisms into the surgical site. Avoid soaking the animal as this can cause hypothermia.

- The animal must be provided with a heat source to maintain normal body temperature for the duration of the procedure. Always use a towel or drape between the heat source and the animal to avoid skin burn.
- A sterile drape should be placed over the animal to prevent contamination of the surgical field. Furthermore, using a drape expands the sterile area giving the surgeon more workspace e.g. to place sutures, catheters, and instruments.
- Ensure sufficient oxygen supply (via nose cone, face mask or endotracheal tube) under the drape to avoid suffocating the animal.

Preparation of the surgeon

- Surgeons must wear hair cover and face mask and preferably a sterile gown, alternatively a clean lab coat or scrub top. Perform surgical hand scrub before gloving and disinfect for 30 seconds with 70% ethanol. Sterile gloves are required if you manually manipulate animal tissues, sutures, sterile implants, the tips of your sterile instruments, and/or sterile areas with your hands. Once sterile gloves are put on, remember to only touch sterile items. If sterility is compromised i.e. from touching non-sterile items or the non-sterile part of the animal, new sterile gloves must be put on.
- “Tips-only” surgery relies on the surgeon’s ability to only use the sterile tips of the instruments for all surgical manipulations without touching the animal. The technique requires extensive experience, meticulous planning and focus on not compromising sterility during the whole procedure. If this can be done, non-sterile gloves can be acceptable.

Intraoperative monitoring

- The non-sterile assistant or the surgeon should continuously monitor the animal’s basic physiological function for the duration of the procedure – from induction through recovery.
- Common monitoring parameters to assess anesthetic depth are: response to surgical manipulation, tail/toe pinch, respiratory rate/quality, oxygen saturation, mucous membrane color, heart rate.
- Many monitoring devices exist for use in large animals. Such devices are difficult to use in small rodents but may be required with certain procedures.

- Changes in vital signs may require modifications in anesthetic dosing. The most common way to test the depth of anesthesia is by checking pedal withdrawal reflex (pinch the skin between the toes. Do not pinch across the bone) on hind limbs. If there is a response, adjust your anesthesia accordingly, and re-test before making your incision. Remember to work aseptically and not touch non-sterile parts with sterile gloves or instruments.
- If, at any time, the animal begins to respond to noxious stimuli during surgery, the procedure must be stopped until additional anesthetic is administered, and pedal withdrawal reflex test elicits no response.
- Anesthetized animals must not be left unattended.
- Dehydration of exposed tissue should be prevented using sterile saline during surgery. If abdominal organs are placed outside abdominal cavity, sterile gauze wetted with sterile saline can be used to cover the organs.
- Incisions must be closed using appropriate techniques and materials. Each anatomical layer must be closed individually, for example the muscle layer and the skin layer. The usage of multifilament, absorbable suture is recommended for the muscle layer and monofilament, non-absorbable suture for the skin. Note: Organic suture (e.g., silk) is not recommended as it elicits an intense tissue inflammatory response.

Postoperative care

- Surgeons should stay in the room to monitor anesthetized animals until they are fully recovered from anesthesia (when able to ambulate and maintain core body temperature). Animals should be placed in a separate cage or pen for recovering animals with half the cage or floor on a heat source and observed every 10-15 minutes until awake and ambulatory. Animals may then be returned to standard housing.
- Post-operative monitoring: An initial postoperative monitoring should be performed upon animal's recovery from anesthesia. The animal must be monitored again at regular intervals and as a minimum as described in the project plan. Observe for signs of pain, distress or discomfort, such as: abnormal posture (hunched) or movement (restlessness, reluctance to move), weight loss, decreased body temperature, decreased nest building (mice), increased attention to surgical site (licking and scratching), eye squinting, vocalization, unkempt fur, piloerection, and porphyrin (red pigment) staining around eyes and nose (rats). React if the animal shows signs of pain, distress or discomfort. Document any problems and consult AEM veterinarians if there are recurring problems with recovery.
- Postoperative analgesics must be administered as described in the project plan and animal experiment license, and potentially more if an animal is showing signs of pain. Animals experiencing post-procedural complications that cannot be alleviated should be euthanized. Postoperative analgesics must be registered after each treatment using the card below. If using analgesics with prolonged effect such as sustained release opioids, each observation

of the animals must also be registered on the card. The card must be put on the cage and removed when skin sutures or clips are removed.

Treatment	Date	Time	Initials

- Fluid administration: Administration of sterile saline warmed to body temperature can help animals maintain blood pressure, body temperature and speed up recovery from anesthesia and surgery. Fluids may be given subcutaneously, intravenously, or intraperitoneally. Contact AEM veterinarians for questions regarding fluid administration.
- Consider supportive care like soaked food, chow in cage bottom, extra nutrition etc.
- A “Newly Operated Animal” label must be put on the cage and filled out with date and initials. The label is removed when skin sutures or clips are removed.



- Wound clips or skin sutures must be removed 7-14 days post operatively. Failure to heal within this time span is indicative of poor aseptic technique or an active infection.
- In case of complications, contact AEM veterinarians at emed-vet@sund.ku.dk or at telephone +45 51 19 33 58 if urgent.

Non-survival surgery

Non-survival surgeries must be performed in a clean area, free of clutter but neither aseptic technique nor dedicated facilities are mandatory, provided that animals are not anesthetized for so long that they develop infections, and study data are not impacted by non-aseptic procedures. Fur removal and supportive care should continue to be performed.

It cannot be excluded that use of non-sterile technique can confound study data.

No expired anesthetics, analgesics and replacement fluids are allowed.

Appendix 1: Self-evaluation form for assessment of skills

Name:	E-mail address:	Date:
Qualifications/training – check relevant box(es) and fill out additional information: <input type="checkbox"/> Medical Doctor or Doctor of Veterinary Medicine with previous surgical experience <input type="checkbox"/> Attended course in experimental surgery including aseptic technique – <i>Please provide details about the course(s) you have attended:</i> <input type="checkbox"/> Previous surgical experience – <i>Please provide details about</i> <ul style="list-style-type: none"> - <i>which surgical procedures you have experience with</i> - <i>where you have performed surgery</i> - <i>when you have performed surgery</i> <input type="checkbox"/> None of the above		
Surgical procedure(s) you are planning to perform:		

Required skill	Assessment of skill		
	Unable to do	Can do, but need to practice	Confident
Pre-operative procedures			
Preparation of the work surface/surgery area (tidy and clean)			
Preparation of material (cleaning and sterilization of instruments and material)			
Check that the animal is fit for surgery			
Induction of anesthesia			
Application of eye lubricant			
Provision of heat source			
Provision of bodywarm saline bolus			
Preparation of the surgical field (shaving, washing, disinfection and use of surgical drape)			
Ensure sufficient oxygen supply			
Administration of analgesia (local and systemic)			

Preparation of surgeon (surgical hand wash and disinfection, clean clothes or gown, hair cover, face mask and sterile gloves)			
Operative procedures			
Maintenance of anesthesia (test of depth of anesthesia prior to incision and during surgery, dose adjustment, re-dosing and monitor animal's vital signs)			
Monitor for reaction to noxious stimuli and react if observed (stop procedure until additional anesthetic is administered and withdrawal reflex elicits no response)			
Correct use of surgical instruments			
Tissue handling			
Use of aseptic technique			
Tissue hydration during surgery			
Suture technique and material			
Competent to perform the specific procedures			
Post-operative procedures			
Monitoring of the animal until fully recovered			
Supportive care (soaked food, food in cage bottom, extra nutrition etc.)			
Plan for post-operative monitoring (Observe for signs of distress and pain and pay attention to surgical site)			
Recognize sign(s) of pain and react if present			
Plan for administering post-operative analgesics			
Plan for removal of suture/surgical clips			

Perioperative conditions	No	Yes
Appropriate design of the surgical room		
Preparation of clean heated cage for recovery		
Place "Newly Operated Animal" label on cage		
Place "Pain Treatment" card on cage		

Overall evaluation: