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Letters to the Editor

Castration eliminates conspecific aggression in group-housed CD1 male surveillance mice (*Mus musculus*)

Dear Editor,

I just read the article entitled "Castration eliminates conspecific aggression in group-housed CD1 male surveillance mice (*Mus musculus*)"[1] and would like to comment on the materials and methods and statements.

First, although I applaud the authors' concern for the welfare of fighting mice, surely intermale aggression could be avoided by the simple expediency of using only females. However, even if males must be used and castrated, I have an issue with the statement in the Discussion "the castration method used did not expose a body cavity…"[1] In the description of the surgical castration, the authors state that "An open castration was performed, followed by separate closure of the body wall and skin…",[1] and the photo caption says the vessels were ligated separately. So, the authors have contradicted themselves; an open castration means opening the vaginal tunic, which is the body wall, thus exposing a body cavity. If they had really done a closed castration, exposing the testicles within the vaginal tunic and ligating around the tunic, that would have been preferable, particularly because there was no type of draping material (pulling the testes through a small opening in a disposable drape would have avoided the possibility of contamination via unprepped hair, etc). In the photos, blood was obviously on structures around the prepped area.

Our IACUC banned the use of 2, 2, 2-tribromoethanol several years ago; at that time it was not available in a pharmaceutical grade and required reconstitution. Perhaps this is no longer the case. However, if the 2, 2, 2-tribromoethanol merely induces narcosis and not analgesia, it would seem incumbent on the authors to provide analgesia, as with concurrent buprenorphine, to avoid pain.

Finally, the choice of 5-0 silk as a suture material is a concern. Our institution accepts nonabsorbable suture only for very short-term subdermal use in recovery surgeries. I assume that these mice will stay alive for several months as sentinels. Was histology done on the area of these sutures to look for inflammation? Braided 4-0 absorbable suture would be an affordable and appropriate alternative material.

Sincerely,
M Lynne Kesel, DVM
Clinical Veterinarian, Laboratory Animal Resources
Associate Professor, Animal Science Department
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Reference

1. **Lofgren JLS, Erdman SE, Hewes C, Wong C, King R, Chavarria TE, Discua AR, Fox JG, Maurer KJ.** 2012. Castration eliminates conspecific aggression in group-housed CD1 male surveillance mice (*Mus musculus*). J Am Assoc Lab Anim Sci **51:**594–599.

Response to Dr Kesel's Letter to the Editor:

Thank you for providing the opportunity to address Dr Kesel's comments and concerns regarding our article "Castration eliminates conspecific aggression in group-housed CD1 male surveillance mice (*Mus musculus*)."[4] We appreciate that dialogue among colleagues is essential for continuing to advance our field, particularly in the area of the 3Rs. We would like to highlight that the primary objective of our article was the demonstration of a successful method of reducing animal use and distress in a rodent health-monitoring program.

The use of males in the surveillance program was the central focus of this animal use reduction strategy. As we stated in the article, the Division of Comparative Medicine maintains an in house-bred colony of CD1 mice to support our transgenic core. Prior to conducting our study, additional mice were ordered from vendors to supply our surveillance program. As a reduction initiative, the breeding colony pups produced in excess of the transgenic core needs were cycled into the surveillance program rather than being euthanized. This strategy reduced animal numbers by maintaining a smaller colony and eliminating needless euthanasia of excess animals. While the female mice served well in this role, the males often fought and many required separation or euthanasia. After evaluating factors influencing aggressive behaviors in male mice, we saw an opportunity to retain these males in the surveillance program through castration, thereby reducing the number of mice required to support programmatic needs, obviating the unnecessary euthanasia of male mice, and eliminating the pain and distress experienced as a result of fighting.

We would also like to address the concerns raised regarding our surgical technique and anesthetic and analgesic choices. As reflected by OLAW's FAQ no. 13, the categorization of a major surgery is based not entirely on the tissue layers dissected but by both penetration and exposure of a body cavity, production of substantial impairment of physical or physiologic function, and involvement of extensive tissue dissection or transection; examples provided include laparotomy, thoracotomy, joint replacement, and limb amputation.[7] In contrast, the castration surgery performed required minimal tissue penetration, dissection, and handling, caused no impairment, and is routinely performed on an outpatient basis in veterinary clinical practice.[1,5,9] Further, no true body cavity exposure occurred (even in an open castration the body cavity is not truly exposed, similar to arthroscopy or other procedures with similarly small incisions). Thus, the castration surgery aligns with the 2011 *Guide*'s categorization of a minor surgery.[3]

With specific regard to the use of 2, 2, 2-tribromoethanol, the 7th edition of the *Guide*, our guiding document at the time of the surgeries, did not contain the same requirements for pharmaceutical grade drugs as the 8th edition. We had selected tribromoethanol for this study because the surveillance mice used were generated in our transgenic core, where this agent has had a long and established history of use without any negative incidents.[2,6] In our hands, tribromoethanol provided loss of consciousness, muscle relaxation, and lack of response to painful stimuli consistent with surgical anesthesia and produced an extremely low prevalence (< 1%) of anesthesia-associated morbidity.[8] Furthermore, as stated in our article, all castrated mice received the analgesic buprenorphine before recovery from anesthesia and showed no behavioral changes indicative of postoperative pain. With regard to our choice of suture material, silk suture was commonly and successfully usedin this high throughput surgical setting. Its use in the castration protocol did not result in any postoperative morbidity or gross pathology. The surgeries were performed by skilled microsurgeons in about 5 min each. All mice recovered from surgery without incident

and remained clinically normal. Although Dr Kesel's institution may have banned the use of this anesthetic and may recommend other choices, the policies of one institution may not be ideal for another. Ultimately, determination of appropriateness should be based upon scientific merit and demonstration of surgical and anesthetic competence. Indeed, the use of performance-based standards is one of the core components of laboratory animal medicine. That being said, despite the absence of any observed untoward effects, we have since refined and expanded our anesthesia regimen and suture material options.

We believe that castration of otherwise unused colony-bred-males may serve as a beneficial reduction strategy for programs of sufficient size to breed their own surveillance mice inhouse. In addition to allowing male mice from the breeding colony to be used as surveillance mice, the castration program also obviates the time and effort spent addressing health concerns arising from fighting and the cost of purchasing female mice as opposed to using males that would otherwise be culled.

Sincerely,

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References

1. **Banfield Pet Hospital.** [Internet]. 2012. Puppy and Kitten Health: Neutering. [Cited 10 December 2012]. Available at: http://www.banfield. com/Pet-Owners/Pet-Health/Browse-by-Topic/Life-stages/Puppy-Kitten/neutering-overview
2. **British Veterinary Association Animal Welfare Foundation/Fund for the Replacement of Animals in Medical Experiments/Royal Society for the Prevention of Cruelty to Animals/Universities Federation for Animal Welfare**. 2003. Refinement and reduction in production of genetically modified mice. Sixth report of the BVAAW/FRAME/RSPCA/UFAW Joint Working Group on Refinement, Anesthesia, analgesia and perioperative care. Lab Anim **37(Suppl 1):**17–20.
3. **Institute for Laboratory Animal Research**. 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
4. **Lofgren JLS, Erdman SE, Hewes C, Wong C, King R, Chavarria TE, Discua AR, Fox JG, Maurer KJ.** 2012. Castration eliminates conspecific aggression in group-housed CD1 male surveillance mice (*Mus musculus*). J Am Assoc Lab Anim Sci **51:**594–599.
5. **Looney AL, Bohling MW, Bushby PA, Howe LM, Griffin B, Levy JK, Eddlestone SM, Weedon JR, Appel LD, Rigdon-Brestle YK, Ferguson NJ, Sweeney DJ, Tyson KA, Voors AH, White SC, Wilford CL, Farrell KA, Jefferson EP, Moyer MR, Newbury SP, Saxton MA, Scarlett JM; Association of Shelter Veterinarians' Spay and Neuter Task Force**. 2008. The Association of Shelter Veterinarians veterinary medical care guidelines for spay-neuter programs. J Am Vet Med Assoc **233:**74–86.
6. **Nagy AGM, Vintersten K, Behringer R**. 2003. Manipulating the mouse embryo. A laboratory manual, 3rd ed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press.
7. **Office for Laboratory Animal Welfare.** [Internet]. 2012. Frequently Asked Questions: PHS Policy on Humane Care and Use of Laboratory Animals, Question 13. [Cited 12 November 2012]. Available at: http://grants. nih. gov/grants/olaw/faqs. htm#useandmgmt_13
8. **Papaioannou VE, Fox JG.** 1993. Efficacy of tribromoethanol anesthesia in mice. Lab Anim Sci **43:**189–192.
9. **Väisänen MA, Tuomikoski SK, Vainio OM.** 2007. Behavioral alterations and severity of pain in cats recovering at home following elective ovariohysterectomy or castration. J Am Vet Med Assoc **231:**236–242.

Letters to the Editor

Letters discuss material published in *JAALAS* in the previous 3 issues. They can be submitted through email (journals@aalas. org) or by regular mail (9190 Crestwyn Hills Dr, Memphis, TN 38125). Letters are not necessarily acknowledged upon receipt nor are the authors necessarily consulted before publication. Whether published in full or part, letters are subject to editing for clarity and space. The authors of the cited article will generally be given an opportunity to respond in the same issue in which the letter is published.

Erratum

In abstract P175 published in the Abstracts of Scientific Presentations section of the September 2012 issue of *JAALAS*, the name of the bacterium listed was inadvertently changed during copyediting. The correct title is "Evaluating the Efficacy of a Low-Dose Garlic Compound (Allicin) against Infection with *Aeromonas salmonicida*."