

Animal Use Protocol Form – AUP

Revised in 2024

AUP D380a – Dr. Cameron Schmidt

East Carolina University
Institution Animal Care and Use Committee
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READ ALL SECTIONS FOR INSTRUCTIONS. Answer all applicable questions. Answer N/A if the question does not apply. Complete electronically. No hand-written versions accepted. Submit a signed electronic version to the IACUC Director at iacuc@ecu.edu.

Instructions

- All **REQUIRED** sections are marked with an “ * ”
 - Sections **I.** to **IV.** and Sections **VI** and **VII.** **MUST** be completed.
 - In Section **V.**, you **MUST** complete sub-section **V.A.** For sub-sections **V.B.** to **V.S.**, only answer applicable questions, check the N/A box if the question does not apply.
- All sections in the “**Table of Contents**” are hyperlinks that are bookmarked in the document. Please place your mouse on the desired section, press the “**Ctrl**” key on your keyboard and **left click** on your mouse simultaneously. This will take you to the corresponding section in the document.
- Some sections are presented as collapsed. To expand those sections, please click on the ► and answer all applicable questions.
- To come back to the first page of the document, please press the “**Ctrl**” key on your keyboard and the “**Home**” key simultaneously.

! For Mac Users – Instructions!

- In **OUTLINE** Mode:
 - To expand text under heading, use “**Control+Shift+ Plus Sign (+)**”
 - To collapse text under heading, use “**Control+Shift+Minus Sign (-)**”

For IACUC Use Only

AUP #	D380a	PI	Cameron Schmidt, Ph.D.
New/Renewal	Renewal		
Full Review Date	DR/Date		
Approval Date	10/7/2025		
Study Type	Reproduction		
Species	Mouse		
Number of Animals Approved	500		
Pain/Distress Category	C		
Surgery - No	SS	MSS	
Prolonged Restraint			
Food/Fluid Regulation			
Other			
Hazard Approval/Dates	IBC	EHS 9/24/2025	Rad
Non-Centralized Housing			
Exception to the Guide			
Amendments Approved			

SS: Survival Surgery, MSS: Multiple Survival Surgery

Animal Use Protocol Form – AUP

Revised in 2024

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SECTION I. General Protocol Information*

A. Protocol Identification

1. Project Title:
- The Role of Metabolic Microenvironment in Gamete Physiology
2. Project Type:
- ☒ Research

☐ Breeding

☐ Teaching

☐ Training

☐ Other Click or tap here to enter text.
3. Project Funding/Sponsor*:

	Sponsor	Grant/Proposal Number
Federal (NIH/NSF/other)	NIH	HD110170
Federal (DOD)		
Private		
State		
Institutional		
Departmental		
Start-up		
Other		

*If this application is covered on an awarded grant, please attach the grant proposal to this application.

4. Collaboration:
- Will any live animal work for this project be performed at a facility or institution other than ECU?
- ☐ Yes

☒ No

If yes, complete the following
- a. Which institution/facility will have ownership of the animals?

Click or tap here to enter text.

b. What is the nature of the collaborative work being performed, including where and with whom work will be performed?

Click or tap here to enter text.

c. If the collaborative work is being performed as part of an approved IACUC protocol at the other institution, please include a copy of the approval letter with this protocol submission and complete the tracking protocol form.

B. **INDICATE** the type of submission below by checking the applicable box and *follow the bolded instructions*

☐ INITIAL APPLICATION – Complete this form beginning with Section II.

☐ **AMENDMENT** - All changes incorporated into this document must be done using the “Track Changes” feature in Word (under “Review” tab, select “Track Changes”) so the changes are plainly discernible.

- 1. Amendment # Choose an item.
- 2. Type of Modification (check all that apply and describe in question section III.A.5)
 - ☐ Personnel Changes
 - ☐ Location Changes
 - ☐ Species Changes
 - ☐ Animal Number Changes
 - ☐ Procedure Changes
 - ☐ Other

Please proceed to Section III.A., provide the rationale to the amendment, and review the entire document, making changes as needed using “Track Changes”.

☒ **3-YEAR RENEWAL** - All changes incorporated into this document must be done using the “Track Changes” feature in Word (under “Review” tab, select “Track Changes”) so the changes are plainly discernible.

- 1. Over the last 12 months, have you encountered any unexpected outcomes or complications? If so, describe below.
no
- 2. Please provide the number of animals used within the last 12 months.
24
- 3. Please provide a brief update on the progress of this project over the last 3 years.
The project has been impactful, resulting in three publications and three more publications with planned submission dates in Fall 2025. The work has enabled us to better understand how sperm heterogeneity impact male fertility potential providing biological material to advance computer vision methods for sperm motility analysis, validate novel computational/theoretical models of sperm selection during fertilization, and develop new flow cytometric measurements of sperm cell population dynamics that will enable high precision predictive frameworks for next-generation male fertility assessment.
- 4. Please provide a brief rationale for the renewal of the AUP.
We are incorporating human spermatozoa into our research as well, but ultimately need to be able to test the effects of our interventions on fertilizing potential and the predictive value of our models. Mouse epididymal sperm can be used directly for IVF with monitoring of embryo developmental outcomes, something that we are not able to do with human sperm and egg. This is an essential part of our research program, to make parallel measurements of human and mouse sperm, then show proof of principle for physiological relevance by testing our models with mouse IVF. We will continue the work that we have been building on for the first three years of this study by continuing to advance the technological measurement of male fertility potential, the theoretical description of sperm selection, and the foundational science of male reproductive biology.

To incorporate the detailed description of the changes, proceed to Section III., review the entire document and make changes as necessary.

SECTION II. Personnel*

List all personnel (PIs, co-investigators, researchers, students, technicians) that will be working with live animals and describe their qualifications and experience with these specific procedures. If people are to be trained, indicate by whom:

Name/Degree/Certification	Position/Role(s)/Responsibilities	Required Training* (Yes/No)	Relevant Animal Experience/Training (include species, procedures, number of years, etc...)
---------------------------	-----------------------------------	-----------------------------	--

Cameron A. Schmidt PhD	PI/Plan experiments, oversee project	Yes	Cameron has worked extensively with mice during his 8-year career. During his doctoral and postdoctoral work at ECU he consulted with DCM staff to develop IACUC amendments, implemented pre-clinical surgical models of disease, oversaw transgenic breeding programs, weaned pups, conducted euthanasia and terminal procedures, and provided animal care during short term (hours-days) and long term (weeks-months) experiments. His research using mice has been published in peer-reviewed journals and has been supported by extramural grant support. All members of the Schmidt lab undergo appropriate DCM sponsored training in animal care and use, and will receive recurrent lab trainings to optimize care, and minimize distress.
Benjamin Brisard, B.S.	Graduate student; plan and perform experiments, euthanasia, dissection, handling	Yes	Ben is a graduate student and is has been working with laboratory mice for 3 years now. Ben has completed required online and in-person training with DCM.

*Required Training: IACUC online training, enrollment in the Occupational Health program (basic health history form), animal handling training (if applicable), experimental surgery training (if applicable). Find all information on Required Training on the [IACUC Website](#).

SECTION III. Regulatory Compliance*

A. Non-Technical Summary

Provide a non-technical description of the project relating the significance of the animal use to the scientific objective. An effective lay (non-scientific) summary includes a **general overview** of the intended use of animals that provides the **general public** with an understanding of the scientific question being asked, the **species** used and the anticipated benefit.

The lay summary needs to answer the following basic questions below.

1. What is the research or teaching objective of the protocol?

Gametes (sex cells) develop behind a blood barrier, in intimate contact with somatic (non-sex) cells under the influence of a combination of paracrine and endocrine signals *in vivo*. Research in the field of reproductive biology has relied heavily on the use of mice to advance our understanding of the processes necessary for normal germ cell maturation and function during reproduction. Though human sperm cells are relatively easy to obtain, experiments on the downstream effects of gamete variation (i.e. human embryonic development) are controversial and ethically challenging. Additionally, human eggs are very difficult to obtain for experimentation.

Our research group is working to understand how the mammalian reproductive microenvironment influences the physiological function of sperm cells through the metabolism-microenvironment interface. This research holds practical applications in assisted reproductive technologies, contraception, agriculture, and environmental toxicology. This proposal addresses our need to purchase commercially available male and female laboratory mice for use in terminal procedures. More specifically, isolation of gametes (i.e. sperm and egg cells) and other reproductive tissues for study in culture. The procedures must be performed using adult mice because there are no comparable cell lines that can be used for gamete research. Mice are also the most practical laboratory animal for these purposes due to their small size, relatively close evolutionary relationship with other placental mammals of interest (e.g. humans), and well characterized reproductive life cycles.

There are several benefits to the proposed research: graduate and undergraduate students will be trained in advanced cell biology, experimental design and analysis, and laboratory study of mammalian reproduction. The proposed experiments will be accompanied by computational model development which will extend the generalizability of the data and reduce the number of experiments through carefully designed/modeled hypotheses.

LDHC - B6.129S6-Ldhc^{tm1Erg} mice have constitutive (whole-body) deletion of the gene encoding the testis-specific isoform of lactate dehydrogenase (Ldhc). These mice were created at the NIEHS, and their phenotype was reported in 2008 (PMID: 18367675). In short, the wild-type, heterozygous, and homozygous knockout (KO) mice are viable and healthy, but KO males are infertile. These KO mice have normal spermatogenesis and generate sperm, but these sperm have motility defects and thus cannot fertilize females.

LDHC-KO mice serve as an important tool to study the contribution of cellular metabolism in support of fertilization. We have previously published studies indicating that LDHC KO sperm lose the ability to anticipate the proximity of an egg. These mice will be used in an identical manner as the CD-1 mice in the study, as an essential source of sperm that can be isolated and studied in vitro.

2. What species of animal will be used?

Mus musculus, CD-1 outbred mice (strain 022) from Charles River.

LDHC - B6.129S6-Ldhc^{tm1Erg} maintained by the Geyer laboratory in Anatomy and Cell Biology.

3. Please provide a clear, concise, and sequential overview of animal use (do NOT provide a detailed description of the procedures here and do not copy and paste grant abstract).

Outbred CD-1 and LDH-C male and female mice will be euthanized in accordance with approved guidelines. For male mice, the cauda epididymis will be isolated for sperm extraction and purification. Other tissues may also be collected for histological assessment. Sperm will be used to study fundamental cellular organization and responses to changing microenvironmental conditions. For female mice, estrous cycle will be monitored via vaginal lavage and histological assessment of smears. Reproductive tissues and egg cells will be collected in a manner similar to that described for male mice.

4. If successful, how will the study benefit advancement of knowledge, human or animal health, or good of society (or all)?

Infertility affects approximately 15% of couples worldwide. Many of these affected couples seek assisted reproductive therapies (ARTs) like in vitro fertilization (IVF). The cost of IVF is tremendous often exceeding the median annual salary in the U.S. Our research is aimed at improving our understanding of male fertility determinants so that we can improve sperm selection for ARTs. The research is providing cutting edge advancements in sperm motility analysis technology, sperm selection technology, and an improved foundational understanding of male reproductive biology. Together, these advances will contribute toward lowering the cost of IVF by improving outcomes and minimizing the required number of cycles to successful live birth thereby reducing the burden on patients.

5. Amendment

Provide the rationale for the amendment here

B. Ethics and Animal Use

1. Duplication

Does this study duplicate or contain elements that duplicate existing research?

☐ YES ☒ NO

If YES, why is it necessary (note: teaching by definition is duplicative)

Click or tap here to enter text.

2. 3R's – Refinement, Reduction, and Replacement

Address the 3 Rs of refinement, reduction, and replacement. **Refinement** refers to modification of husbandry or experimental procedures to enhance animal well-being and minimize or eliminate pain and distress. **Replacement** refers to absolute (i.e. replacing animals with an inanimate system) or relative (i.e. using less sentient species) replacement. **Reduction** involves strategies such as experimental design analysis, application of newer technologies, use of appropriate statistical methods, etc., to use the fewest animals or maximize information without increasing animal pain or distress.

Refinement: Vaginal lavage procedures will be performed near the home cage to prevent undue stress during the procedure. Soft handling will be prioritized over restrained handling (e.g. ‘scruffing’). **Replacement:** alternative animal models will be explored as a source of gametes for study, but the specific goals of the proposed projects necessitate the use of placental mammals for this research and mice are the most practical/relevant organism for this type of research. **Reduction:** multifactorial experimental designs will be implemented when possible to maximize empirical data gained from each experiment. Samples will be prepared and stored for future analysis (e.g. cell smears and other tissues prepared and stored for future analysis).

3. Alternatives to the Use of Live Animals

Are there less invasive procedures, other less sentient species, isolated organ preparation, cell or tissue culture, or computer simulation that can be used in place of the live vertebrate species proposed here?
☐ YES ☒ NO
If YES, please explain why you cannot use these alternatives.

Click or tap here to enter text.

4. Consideration of Alternatives to Painful/Distressful Procedures

Include a literature search to ensure that alternatives to all procedures that may cause more than momentary or slight pain or distress to the animals have been considered.
a. Please list **all** the potential painful or distressful procedures in the protocol

.

b. For the procedures listed above, provide the following information (do not submit search results but keep them for your records)

Date search was performed:	
Database(s) searched (i.e. PubMed, Google Scholar, etc):	
Time period covered by the search (i.e. 2000 - present)	
Search strategy (including scientifically relevant terminology)	
Other sources consulted	

c. In a few sentences, please provide a brief narrative indicating the results of the search(es) to determine the availability of alternatives and explain why these alternatives were not chosen.

Click or tap here to enter text.

C. Hazardous Agents

1. Protocol Related Hazards (chemical, biological or radiological)

Please indicate if any of the following are used in animals and the status of review/approval by the referenced committees:

Hazards	Oversight Committee	Status (Approved, Pending, Submitted)/Date	AUP Appendix I Completed?
Radioisotopes	Radiation		Choose an item.
Ionizing radiation	Radiation		Choose an item.
Infectious agents (bacteria, viruses, rickettsia, prions, etc.)	IBC		Choose an item.
Toxins of biological origins (venoms, plant toxins, etc.)	IBC		Choose an item.
Transgenic, Knock In, Knock Out Animals---breeding, cross breeding or any use of live animals or tissues	IBC		Choose an item.
Human tissues, cells, body fluids, cell lines	IBC		Choose an item.
Viral/Plasmid Vectors/Recombinant DNA or recombinant techniques	IBC		Choose an item.
Oncogenic/toxic/mutagenic chemical agents	EH&S		Choose an item.
Nanoparticles	EH&S		Choose an item.
Cell lines, tissues or other biological products injected or implanted in animals	DCM		Choose an item.
Other	EH&S	Approved	N/A

2. Incidental Hazards

Will personnel be exposed to any incidental zoonotic diseases or hazards during the study (field studies, primate work, etc)? If so, please identify each and explain steps taken to mitigate risk.

Click or tap here to enter text.

SECTION IV. Animals and Housing*

A. Species and Strains Mice: CD1 and LDHC - B6.129S6-Ldhc<tm1Erg>

B. Age, Sex, and/or Weight

Please check ALL that apply.

- ☐ Young (rodent fetuses, pre-weaned pups; fish embryos/larvae; tadpoles; hatchling/fledgling)
- ☒ Adult
- ☒ Female
- ☒ Male

If you are using a species other than rodents, fish, amphibians, reptiles, or birds, please indicate weight range and/or age range

Click or tap here to enter text.

C. Animal Numbers

1. Please complete the following table:

Number of Animals in Treatment and Control Groups	Additional animals (Breeders, substitute animals, training)	Total Number of Animals Used for this Project
500		=500

2. Justify the above species and animal numbers (use statistical justification when possible):

Please also include all animals born even if their genotype is non-desirable or numbers not needed for experimental purposes, also include fetuses if used for experiments.

Research in the field of reproductive biology has relied heavily on the use of mice to advance our understanding of the processes necessary for normal germ cell maturation and function during reproduction. Mice are the least sentient species that will allow us to understand how the mammalian reproductive microenvironment influences the physiological function of sperm cells through the metabolism-microenvironment interface . Our preliminary data and power analysis indicate that approximately 6-10 mice must be used for each type of experiment in order to obtain confident estimates for effect sizes. There are a variety of experiments/measurements involved in developing accurate metabolic models of gamete function. To reduce numbers needed for the study, computational models will be used throughout to create more informed hypotheses prior to any mouse experiments.

Assuming an average sample size per experiment of 8 animals, the total number of animals requested equals about 60 experiments. Over a three-year period, this is approximately two experiments per week. The research involves many different experiments involving development of microfluidics devices for sperm selection, studying cell subpopulation adaptation using flow cytometry, IVF with frozen mouse oocytes, and various metabolic assays to better understand microenvironmental effects on fertility. The provided sample sizes are a conservative estimate, and we will use animals only as needed while minimizing the number as much as possible.

3. For breeding situations, will you be following the DCM breeding guidelines as shown on the DCM website?

☐ Yes ☐ No ☒ N/A

If NO, describe and justify.

Click or tap here to enter text.

4. If genotyping, will you be following IACUC genotyping guidelines as shown on [IACUC Website](#)?

☐ Yes ☐ No ☒ N/A

If NO, describe and justify.

Click or tap here to enter text.

5. Will the phenotype of mutant, transgenic or knockout animals predispose them to any health, behavioral, physical abnormalities, or cause debilitating effects in experimental manipulations?

☐ Yes ☒ No

If YES, describe.

Click or tap here to enter text.

6. Are there any deviations from standard husbandry practices as shown on the DCM website?

☐ Yes ☒ No

If YES, describe conditions and justify the exceptions to standard housing (temperature, light cycles, sterile cages, special feed, prolonged weaning times, wire-bottom cages, etc.).

Click or tap here to enter text.

7. The default housing method for social species is pair or group housing (including mice, rats, guinea pigs, rabbits, dogs, pigs, monkeys). Is it necessary for animals to be singly housed at any time during the study?

☒ Yes ☐ No

If YES, describe housing and justify the need to singly house social species.

Female mice can be co-housed. The adult male mice are single housed. Often the mice are retired breeders having been single housed males previously. Housing them together with non litter mates will result in distress to the animals due to fighting and unnecessary injuries.

8. Are there experimental or scientific reasons why routine environmental enrichment should not be provided?

☐ Yes ☒ No

If YES, describe and justify the need to withhold enrichment.

Click or tap here to enter text.

9. If wild animals will be captured or used, provide permissions (collection permit # or other required information)

Click or tap here to enter text.

10. List all laboratories or locations outside the animal facility where animals will be used. Note that animals may not stay in areas outside the animal facilities for more than 12 hours without prior IACUC approval. For field studies, list location of work/study site.

Life sciences and biotechnology building 2nd floor tissue culture facility (room 2204- vented fume hood). 1st floor vivarium, rooms 1206 and 1208- vented fume hoods. Where mice will be euthanized for tissue/cell collection.

SECTION V. Animal Procedures

A. Experimental Design*

Outline the experimental design including all treatment and control groups and the number of animals in each. Tables or flow charts are particularly useful to communicate your design. Briefly state surgical plans in this section. Surgical procedures can be described in detail in V.S. If applicable, you can reference Standard Operating Procedures (SOPs) listed on the [IACUC Website](#). If deviation from approved SOP – please describe.

The role of microenvironment in regulation of mouse sperm motility- Sperm will be isolated from sexually mature CD-1 or LDH-C KO mice following euthanasia. Control groups are within the specific assays, rather than animal control groups (none of the animals are placed in treatment groups for this design).

Comprehensive metabolite screening- (N=120 male mice): Combinatorial screens will be performed using multiplexed fluorometry assays. Requires pooled sampling strategy from three mice per replicate. The purpose of these screening protocols will be to comprehensively identify microenvironmental parameters that impact the motility and fertility competency of live mouse sperm. This screening approach reduces the number of metabolic experiments that would be needed for more detailed characterization of sperm metabolic phenotype using alternative approaches such as force-flow analysis. The pooled sample size estimate includes both CD-1 and LDH-C mice. LDH-C mouse availability will depend on breeding success.

Validation of metabolic/motility models- (N=150 male mice): Kinetic measurements of mitochondrial membrane potential, calcium dynamics, mitochondrial force-flow analysis, and light microscopy-based kinematic motility analysis. Requires pooled sampling, 3 mice per replicate. These experiments will validate the screening assays described earlier and will

provide important parameters for development of metabolic flux models. Pooled samples will facilitate approximately 5-10 sets of measurements using a replicate size of 6-10 per measurement. Developing accurate predictive metabolic models reduces the number of experiments that will be necessary in the future. The pooled sample size estimate includes both CD-1 and LDH-C mice. LDH-C mouse availability will depend on breeding success.

Development of live sperm cell force-flow analysis using attenuation absorbance

spectrometry and respirometry- (N=150 male mice): These important experiments will develop a novel system for correlating metabolic and mechanical energy transduction in live cells. As above, pooled samples will facilitate approximately 5-10 sets of measurements using a replicate size of 6-10 per measurement. The pooled sample size estimate includes both CD-1 and LDH-C mice. LDH-C mouse availability will depend on breeding success.

Quantitation of the mouse female reproductive tract chemical microenvironment- (80 female mice):

Very little is known regarding the influence of the female reproductive tract microenvironmental variation on fertility. In these experiments, estrous cycle dependent changes in key metabolite concentrations in the vaginal, uterine, and oviductal fluids will be determined using microfluorimetry. Tissues will be collected for histochemical characterization of localized metabolism within the tissues. These samples are not pooled. Provides for 8-16 measurements with a replicate size of 6-10 per measurement.

In sections V.B-V.S below, please respond to all items relating to your proposed animal procedures. If a section does not apply to your experimental plans, please check N/A.

Please refer to [DCM Website](#) and [IACUC Website](#) for relevant guidelines and SOPs.

B. Anesthesia/Analgesia/Tranquilization/Pain/Distress Management for Procedures other than Surgery

☒ N/A

C. Use of Paralytics

☒ N/A

D. Blood or Body Fluid Collection

☐ N/A

If blood and/or body fluid will be collected, please fill out the appropriate sections in the table below.

	Location on animal	Needle/catheter size	Volume Collected	Frequency of Procedure	Time interval between collections
Blood Collection					
Body Fluid Collection					
Other (Vaginal Lavage)	Vulva/vagina	Micropipette tip (200uL) <1 mm at the tip	50 uL	Every 24 hours; No more than 4 times total	24 hrs

E. Injections, Gavage, and Other Substance Administration

☒ N/A

F. Prolonged Restraint with Mechanical Devices

☒ N/A

G. Tumor Studies, Disease Models, Toxicity Testing, Vaccine Studies, Trauma Studies, Pain Studies, Organ or System Failure Studies, Shock Models, etc.

☒ N/A

H. Treadmills/Swimming/Forced Exercise

☒ N/A

- I. Projects Involving Food and Water Regulation or Dietary Manipulation

J. Endoscopy, Fluoroscopy, X-Ray, Ultrasound, MRI, CT, PET, Other Imaging

K. Polyclonal Antibody Production

L. Monoclonal Antibody Production

M. Temperature/Light/Environmental Manipulations

N. Behavioral Studies

O. Capture with Mechanical Devices/Traps/Nets

P. Manipulations of Wild-Caught Animals in the Field or Laboratory

Q. Wildlife Telemetry/Other Marking Methods

R. Other Animal Manipulations
- ☒ N/A

☒ N/A

☒ N/A

☒ N/A

☒ N/A

☒ N/A

☒ N/A

☒ N/A

☒ N/A

☐ N/A

1. Describe methodology

Click or tap here to enter text.

Vaginal lavage of female mice will be used to determine the stage of the estrous cycle, female mice will undergo vaginal lavage and smears will be prepared for histological analysis. PI-Schmidt has performed lavage procedures previously. They are very low impact and do not require anesthesia. Mice are held by the tail, placed on a cage top so they can grip with their front paws, and using a pipette and small pipette tip, ~50uL of autoclaved ddH2O is placed into and then gently aspirated from the vulva to obtain cells for smears. A 100uL sterile plastic micro-pipette tip is used for the procedure. The procedure will be repeated only once within a 24 hour period (guided by the approximate time in each of the estrous phases detailed in table 1). Once the initial phase is determined, the procedure may be performed once every 24 hours until the desired cycle is reached (maximum of 4 times).

Estrous Phase:	Time (hrs):
Proestrous	<24
Estrous	12-48
Metestrous	8-24
Diestrous	48-72

Table 1: Timing of mouse estrous phases

2. Describe methods to ensure animal comfort and well-being
- The tip is not inserted into the vagina, as this may induce pseudopregnancy or cause discomfort.
3. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study)
- There should be no adverse effects but should a mouse have bleeding or other complications from lavage, we will contact DCM vet staff for consultation or the mouse will be euthanized.

S. Surgical Procedures

SECTION VI. Euthanasia*

☒ N/A

Please refer to the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition and DCM Guidelines on the [IACUC Website](#) to determine appropriate euthanasia methods.

A. Euthanasia Procedure

All investigators, even those conducting non-terminal studies, must complete this section in case euthanasia is required for humane reasons.

1. Physical method

If a physical method is used, the animal should be first sedated/anesthetized with CO₂ or other anesthetic agent. If prior sedation is not possible, a scientific justification must be provided.

2. Inhalant method

☐ Carbon Dioxide ☐ Other

If OTHER, describe the agent and delivery method

Mice are euthanized with isoflurane in a special open drop chamber (that has been inspected by DCM). Euthanasia is confirmed with lack of response to toe pinch and pedal withdrawal reflex. Mice are then euthanized by cervical dislocation (secondary method) followed by thoracotomy (tertiary method). Isoflurane in a sealed container. 200uL isoflurane on a cotton swab in a 'tea strainer' so that the mice cannot touch the cotton swab since isoflurane is an irritant.

3. Non-inhalant pharmaceutical method (injectables, MS-222, etc.)

Please provide the following

a. Agent

Click or tap here to enter text.

b. Dose or concentration

Click or tap here to enter text.

c. Route

Click or tap here to enter text.

B. Method of Ensuring Death

The method can be physical method, such as pneumothorax or decapitation for small species and assessment method such as auscultation for large animals)

Mice are euthanized by cervical dislocation (secondary method) followed by thoracotomy (tertiary method).

C. Describe disposition of carcass following euthanasia

Carcasses will be stored in a -20C freezer and disposed of via incineration as medical waste.

SECTION VII. Assurance and Signatures*

I acknowledge that humane care and use of animals in research, teaching and testing is of paramount importance, and agree to conduct animal studies with professionalism, using ethical principles of sound animal stewardship. I further acknowledge that I will perform only those procedures that are described in this AUP and that my use of animals must conform to the standards described in the Animal Welfare Act, the Public Health Service Policy, The Guide for the Care and Use of Laboratory Animals, the Association for the Assessment and Accreditation of Laboratory Animal Care, and East Carolina University.

Please submit the completed animal use protocol form via e-mail attachment to iacuc@ecu.edu. You must also carbon copy your Department Chair.

PI Signature:  Date: 9/28/2025

Veterinarian:  10/8/2025 | 10:12 AM EDT Date: _____

IACUC Chair:  Date: 10/7/2025

Animal Use Protocol Form – Appendix 1

Revised in 2024

East Carolina University
Institution Animal Care and Use Committee
212 Ed Warren Life Sciences Building | Greenville NC 27834 – 4354
252-744-2436 office | 252-744-2355 fax
iacuc@ecu.edu

READ ALL INSTRUCTIONS. Answer all questions and fill out applicable page(s). Complete electronically and submit a copy to the IACUC Director at iacuc@ecu.edu. You will date and sign after review and approval by subject matter experts.

Principal Investigator:**AUP#:****Department:****E-mail:****Phone #:**

(best # to contact in case of emergency)

Secondary Contact:**Department:****E-mail:****Phone #:**

(best # to contact in case of emergency)

THE FOLLOWING HAZARDOUS AGENT(S) WILL BE USED – check all that apply

- ☐ CHEMICAL AGENTS – fill out the top portion of the [blue page](#)
 - ☐ BIOLOGICAL AGENTS – fill out the top portion of the [orange page](#)
 - ☐ RADIATION – fill out the top portion of the [yellow page](#)
1. **Verify** that all planned hazardous agents use matches what is in the approved AUP (and Lab Safety Plan).
 2. Prior to **EACH** hazard use, **notify** the Facility Manager Aaron Hinkle by phone (744- 2997) or email (hinklea@ecu.edu) at least one week before planned hazard use (two weeks if this is the first time you are using the agent).
 3. On the day of hazard use, **flip** this door sign over and display all applicable Appendix 1 hazard signs that describe the hazard(s) and associated handling procedures so they are visible to all personnel.
 4. **Place** the appropriate hazard stickers on each animal cage after the hazard has been administered.
 5. **Record** the hazard administration and related information on the yellow procedure card for each cage and continue per Yellow Procedure Card PowerPoint (on IACUC website).
 6. If there are any questions, **contact** the Facility Manager (744-2997).

PI Signature: _____ Date: _____

When HAZARD in USE
Display ALL that apply

CHEMICAL HAZARD	
Principal Investigator: AUP#: Department: Email: Phone#: (best # to contact in case of emergency)	Secondary Contact: Department: Email: Phone#: (best # to contact in case of emergency)
Chemical Agents used:	
Below this line – FOR IACUC USE ONLY	
PERSONAL PROTECTIVE EQUIPMENT REQUIRED:	
Route of Excretion:	
Precautions for Handling Live or Dead Animals:	
Animal Disposal:	
Bedding/Waste Disposal:	
Cage Decontamination:	
Additional Precautions to Protect Personnel, Adjacent Research Projects including Animals and the Environment:	
Safety/Subject Matter Expert Signature & Date 	

**Principal Investigator:****AUP#:****Department:****Email:****Phone#:**

(best # to contact in case of emergency)

Secondary Contact:**Department:****Email:****Phone#:**

(best # to contact in case of emergency)

Biological Agents used:**Below this line – FOR IACUC USE ONLY****PERSONAL PROTECTIVE EQUIPMENT REQUIRED:**

Route of Excretion:

Precautions for Handling Live or Dead Animals:

Animal Disposal:

Bedding/Waste Disposal:

Cage Decontamination:

Additional Precautions to Protect Personnel, Adjacent Research Projects including Animals and the Environment:

Safety/Subject Matter Expert Signature & Date

**Principal Investigator:****AUP#:****Department:****Email:****Phone#:**

(best # to contact in case of emergency)

Secondary Contact:**Department:****Email:****Phone#:**

(best # to contact in case of emergency)

Radiation:**Below this line – FOR IACUC USE ONLY****PERSONAL PROTECTIVE EQUIPMENT REQUIRED:**

Route of Excretion:

Precautions for Handling Live or Dead Animals:

Animal Disposal:

Bedding/Waste Disposal:

Cage Decontamination:

Additional Precautions to Protect Personnel, Adjacent Research Projects including Animals and the Environment:

Safety/Subject Matter Expert Signature & Date
