



Guidelines for the Use of Embryos of Egg-laying Vertebrate Species

Objective:	To provide parameters for research with embryos of egg-laying species
Author:	Office of Research Protections & Integrity
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Version:	1.0

I. General Purpose

Research and teaching activities using embryonated eggs may require IACUC oversight under certain circumstances, and always require humane euthanasia regardless of stage of development. UNC Charlotte is committed to observing federal policies and regulations and the Association of Assessment and Accreditation of Laboratory Animal Care (AAALAC) International standards for the humane care and use of animals. OLAW expects Assured Institutions to have policies and procedures in place that address the care or euthanasia of egg-laying vertebrate species that hatch unexpectedly.

The intent of this policy is to describe euthanasia and disposal procedures for live embryonated eggs of avian and other egg-laying vertebrate species in various stages of development prior to hatching, as well as to ensure euthanasia procedures are in place for inadvertently hatched avian and other egg-laying vertebrate species.

These guidelines refer to all egg-laying vertebrate species regardless of internal or external fertilization.

Domestic chicken embryos, which hatch in approximately 21 days, are considered the model species. If other species are used, the guidelines should be adjusted based on relative time to hatch for that species and stated (per below).

II. Defined Terms

Assured Institution: An Institution with Animal Welfare Assurance to receive Public Health Service funds.

Avian: a bird.

Embryonated: having an embryo.

Embryo: an unborn (or unhatched) offspring at any stage of development prior to hatching.

Hatching: to bring forth offspring from the egg.

Euthanasia: a method of killing that minimizes pain, distress, and anxiety experienced by the animal prior to loss of consciousness and causes rapid loss of consciousness followed by cardiac or respiratory arrest and death.

III. Background

Vertebrate embryos are not considered live animals by U.S. regulatory agencies. Nonetheless, there is a consensus in the scientific community that embryos greater than approximately 67-80% through embryonic development may have developed a neural tube sufficient to experience pain. Additionally, if embryos of egg-laying species hatch, intentionally or unintentionally, they are live vertebrate animals and thus require oversight by the IACUC. Consequently, the IACUC has adopted the following guidelines that apply to all vertebrate egg-laying species.

IV. Covered Parties

This policy applies to all persons responsible for conducting research, teaching, training, breeding, and/or related activities involving live vertebrate animals conducted at or under the auspices of UNC Charlotte.

All individuals to whom this policy applies are responsible for becoming familiar with and following this policy. The PI is responsible for taking appropriate steps (e.g., training staff) to help ensure compliance with this policy.

V. Oversight

The Animal Care and Use Program, Attending Veterinarian, Office of Research Protections & Integrity, and Institutional Animal Care and Use Committee are responsible for promoting, overseeing implementation of, and ensuring compliance with this policy.

If considering work with embryonated eggs, consultation with the Attending Veterinarian is mandatory prior to beginning any project.

VI. Guidelines

A. New studies using embryos that will be euthanized prior to completing 80% of the total expected incubation period:

Principal Investigators (PI) must inform the IACUC of their intent to use live embryonated eggs of egg-laying vertebrate species by submitting a “**Notice of Intent to Use Live Embryos**” form. This form serves as a record of embryo use for the IACUC. (Appendix A)

The “**Notice of Intent to Use Live Embryos**” form will be reviewed by the ORPI staff and the Attending Veterinarian (AV). Clearance to engage in the proposed activities will be provided to the PI by the ORPI after the AV determines that IACUC approval is not required for the proposed activities. The PI MUST obtain this clearance prior to engaging in the proposed activities.

Note: Eggs prior to completing 80% of incubation, including those used in wildlife field studies, must be euthanized as described in the current AVMA Guidelines on Euthanasia. (e.g., for domestic chickens with a 21-day incubation period, research ending in euthanasia on or before day 16 of incubation (E16) would not require an IACUC animal use protocol but would need to follow the AVMA Guidelines for Euthanasia.)

B. New studies using pre-hatched embryos after 80% of incubation, or using hatchlings, and any use of embryonated eggs that will subsequently be allowed to hatch:

Must be reviewed by the IACUC in accordance with its procedures for all vertebrate animals via **submission of an IACUC Animal Use Protocol** and must receive IACUC review and approval to ensure that appropriate plans are in place to address the care and euthanasia of animals that may/will hatch ([SOP Determining when IACUC Approval is Required](#)).

IACUC protocols that utilize either egg-laying adult animals, including breeding to obtain embryos, embryos with >80% of incubation, must describe in an IACUC Animal Use Protocol:

- A succinct explanation and justification of the purpose of the activity and the procedures involved,
- A clear indication of the stage of incubation during which live embryonated eggs will be used or disposed of,
- the method of disposition of embryonated eggs consistent with the [AVMA Guidelines on Euthanasia](#),
- the steps that will be taken (i.e., veterinary staff intervention, euthanasia) in the event that an egg, at any stage of development, inadvertently hatches, and
- How and when egg-laying adults will be euthanized.

(e.g., for domestic chickens with a 21-day incubation period, day \geq 16 for chicken embryos or 4 days post-fertilization (dpf) for zebrafish larvae).

C. Amending Active/Approved IACUC Protocols:

If the PI has an approved protocol to use egg-laying adult animals, including breeding to obtain embryos, the information below may be incorporated into the protocol as an amendment in lieu of submitting a “Notice of Intent to Use Live Embryos” form:

- A succinct explanation and justification of the purpose of the activity and the procedures involved,
- A clear indication of the stage of incubation during which live embryonated eggs will be used or disposed of,
- the method of disposition of embryonated eggs consistent with the [AVMA Guidelines on Euthanasia](#),
- the steps that will be taken (i.e., veterinary staff intervention, euthanasia) in the event that any egg, at any stage of development, inadvertently hatches, and
- How and when egg-laying adults will be euthanized.

D. Amending previously approved embryonic research protocols / projects previously approved with a “Notice of Intent to Use Live Embryos” form (and no approved IACUC protocol):

- Once clearance has been provided for embryonic egg research, the PI is responsible for submitting an updated “Notice of Intent to Use Live Embryos” form if changes are made.
- Changes including embryos after 80% incubation, hatchlings, and/or use of embryonated eggs that will subsequently be allowed to hatch must be documented in an approved IACUC protocol or approved IACUC protocol amendment **not** a “Notice of Intent to Use Live Embryos” form.

VII. Euthanasia Methods

The IACUC recognizes that inadvertent hatching may occur. As such, Principal Investigators are asked to describe their methods for humane euthanasia of hatchlings in IACUC animal use protocols or the "Notice of Intent to Use Live Embryos" form, whichever is most appropriate.

- A. **Embryos that have been exposed by removal of all or part of the shell** can be decapitated. (AVMA guidelines on Euthanasia 2020, S3.4.4.)
- B. **Eggs/embryos <80% incubation** (e.g., chicken eggs younger than embryonic day 16 (E16))
 - must be destroyed by prolonged exposure (> 20 minutes) to CO₂, cooling (< 4°C for 4 hours), or by freezing (-20°C).
- C. **Eggs/embryos >80% incubation** (e.g., chicken embryos older than embryonic day 16 (E16))
 - should be treated like avian neonates and must be euthanized by prolonged exposure (>20 minutes) to CO₂, anesthetic overdose, or decapitation. Avian embryos are resistant to CO₂. Therefore, embryonated eggs must be exposed to 90% CO₂ for a minimum of 20 minutes, preferably through the air cell at the large end of the egg. Dry ice is unacceptable as a source of CO₂ for euthanasia.

"Bird embryos that have attained > 80% incubation demonstrate EEG activity that is sustained, with increases in amplitude suggesting the potential for pain perception in conscious embryos; therefore, they should be euthanized by similar methods used in avian neonates." (AVMA Guidelines on Euthanasia, 2020, S5.3).

D. Eggs/embryos from other species

"Because research is still evolving and there are species-specific differences in development, euthanasia of embryos should be performed based on the best available data and with attention to assuring, as best as possible, that conscious suffering does not occur." (AVMA Guidelines on Euthanasia, 2020, S5.3).

- Zebrafish embryos less than three (3) days post-fertilization (dpf) that are being disposed of should be treated with sodium hypochlorite to prevent further development.
 - Use of other species and methods of euthanasia should be documented in the "Notice of Intent to Use Live Embryos" form or the IACUC Animal Use Protocol, whichever is most appropriate.
- E. Additional humane methods of euthanasia may be considered in consultation with the UNC Charlotte Attending Veterinarian.

VIII. Consequences for Violating this Policy

Violation of this policy may be considered a serious event of noncompliance that is reportable to the IACUC, funding and accrediting agencies, as well as other regulatory agencies. Violation of this policy is a serious matter that may adversely affect both the ability to perform animal work and acquire funding sources.

Failure to comply with this and related policies is subject to disciplinary action, up to and including suspension without pay, or termination of employment or association with the University, in accordance with applicable (e.g., staff, faculty, student) disciplinary procedures.

IX. Related Information

UNC Charlotte is a community in which employees are encouraged to share workplace concerns with University leadership. An individual may call the Office of Research Protections & Integrity at 704-687-1872 or -1876. Additionally, the [Animal Welfare Concern Report](#) allows anonymous and confidential reporting on matters of concern online.

X. References

- <https://olaw.nih.gov/faqs#/guidance/faqs>
- <https://olaw.nih.gov/faqs#/guidance/faqs?anchor=50290>
- <https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf>
- https://oacu.oir.nih.gov/system/files/media/file/2021-02/b17_zebrafish.pdf

Approval Date February 1, 2024

APPENDIX A



NOTICE OF INTENT TO USE LIVE EMBRYOS

I. Project Title:

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II. Principal Investigator or Course Instructor:

Name (please print):	
Email:	
Phone:	
Department:	
Research Building & Room #:	

III. Species

Species:	
Source: (If obtained from another laboratory, please provide approved AUP number)	
Standard embryonic (incubation) period for this species:	
Maximum age(s) of embryos for use:	
Age at which unused embryos will be discarded:	

IV. Method of Euthanasia (following the AVMA Guidelines on Euthanasia)

Embryos <80% incubation	
Embryos >80% incubation	
Procedure for euthanasia of inadvertently hatched chicks	

V. Description of Project:

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VI. PI Assurances (initials):

Check	Assurance
	I have read the IACUC "GUIDELINES FOR THE USE OF EMBRYOS OF EGG-LAYING VERTEBRATE SPECIES IN RESEARCH & TEACHING," and I agree to abide by it.
	Laboratory staff and researchers under my supervision have read the IACUC "GUIDELINES FOR THE USE OF EMBRYOS OF EGG-LAYING VERTEBRATE SPECIES IN RESEARCH & TEACHING," and will abide by it.
	Eggs will be destroyed prior to 80% of the embryonic period and are not expected to hatch.
	The IACUC and Attending Veterinarian will be informed if any eggs hatch.
	Eggs will only be shared with laboratories maintaining either an AUP or Notice of Intent to Live Embryos Form for the embryonic stage of this species.

PI Signature:

Date:
