

University of Louisville
Institutional Animal Care and Use Committee
Policies and Procedures

The Use of Chicken/Avian Embryos

Policy: Use of chicken embryos on or after embryonic developmental day 18 (72 hours prior to hatching) requires submission of a full IACUC *Proposal* form for review and approval of the IACUC. For procedures occurring on or before day 17, investigators must complete the “*Use of Avian Embryos in Research*” form for IACUC review and approval. This policy encompasses all use, including research, teaching, or testing.

Guidelines for other avian species should consider the developmental rate with adjustments based on *relative time to hatching* in accordance with those outlined within this policy for chicken embryos. It is the responsibility of the researcher to provide necessary information concerning the species that the proposed research will use.

Rationale: While avian embryos are not considered live animals under PHS policy, there is a consensus in the scientific community that beyond a critical point in development avian embryos are capable of experiencing pain. Chickens are precocial avian species and are more advanced morphologically during development than are altricial species. The vertebral column and nervous system start developing before the end of embryonic day one (E1). Since offspring are born fully functional, as evident by their ability to locomote, feed, and care for themselves, it is illogical to conclude that the neural capacity to experience pain is not also intact prior to hatching. The exact stage of development at which this capacity is sufficiently developed to warrant concern has not yet been determined. Both the Institute of Laboratory Animal Resources (ILAR) of the National Research Council and the AVMA recognize that a critical period in chick embryo development occurs 72 hours prior to hatching. At this time, the chick is completely developed with only the absorption of the yolk sac not complete. The NIH’s Animal Research Advisory Committee guidelines for the euthanasia of rodent feti states that at approximately 60% of the embryonic developmental period, the neural tube has developed into a functional brain and the likelihood that a fetus may perceive pain should be considered. Extrapolating these guidelines to a 21-day chicken developmental period would indicate that procedures beyond embryonic day 13 (E13) should give special consideration to the animal’s ability to perceive pain.

Procedures, Guidelines, and Exceptions:

1. Chick embryos younger than embryonic day 13 (E13) are assumed unable to experience pain. E13 and younger embryos may be euthanized by hypothermia (typically conducted by placing the eggs in a -20°C freezer or < 4°C for 4 hours) or via other means. Death should be confirmed by decapitation, membrane disruption, maceration, or other suitable method.
2. It should be assumed that chick embryos between E13 – E17 can experience pain and must be euthanized by decapitation or other rapid and humane method.

3. Chick embryos at or older than embryonic day 18 (E18) may be euthanized by methods such as CO₂, anesthetic agents, or decapitation. Because respiration begins during embryonic development, the unhatched chicken's environment may normally have a CO₂ concentration as high as 14%. Thus, CO₂ concentrations for euthanasia should be especially high. The *AVMA Guide for Euthanasia* reports a CO₂ concentration of 60% to 70% with a 5-minute exposure time as optimal while other sources recommend a 90% CO₂ for at least 20 minutes. *Dry ice is not acceptable as a source of CO₂ euthanasia.*
4. When utilizing chick embryos at day E18 or later, the *IACUC Proposal* must include methods for humane euthanasia of animals in the event of inadvertent hatching. Avian embryos that hatch are live vertebrate animals and are regulated by PHS Policy.
5. Any deviations from the guidelines and procedures outlined in this policy must reviewed and approved by the IACUC in the form of a full *Proposal*.

References:

- Avian Embryo Use. *IACUC Policies*. Case Western Reserve University, July 2008.
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- Darnell, Diana K., and Gary C. Schoenwolf. "The Chick Embryo as a Model System for Analyzing Mechanisms of Development." *Methods in Molecular Biology*. Vol. 135. Totowa, NJ: Humana. Print. Developmental Biology Protocols Vol.1.
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- Policy for Use of Avian Embryos. *Animal Care and Use Committee Guidelines*. Florida State University, Sept. 2005.
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- The Public Health Service Responds to Commonly Asked Questions." *ILAR News* 33.4 (1991): 68-70. *Office of Laboratory Animal Welfare*. <http://grants.nih.gov/grants/olaw/references/ilar91.htm>