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 gestation 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 gestation day 17, investigators must complete the one-page “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 gestation 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 gestation 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/Animal Research Advisory Committee guidelines for the euthanasia of rodent feti states that at approximately 60% of the gestation 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 gestation 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:
http://casemed.case.edu/ora/iacuc/forms/Policy%20for%20Use%20of%20Avian%20Embryos.pdf
http://www.avma.org/issues/animal_welfare/euthanasia.pdf
"Guidelines for the Euthanasia of Rodent Feti and Neonates." ARAC Guidelines. NIH OACU.  
http://oacu.od.nih.gov/ARAC/
http://msucares.com/poultry/reproductions/poultry_chicks_embryos.html