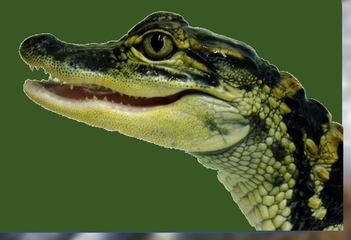
Effects of *in ovo* exogenous estrogen exposure on 5-months-old *Alligator mississippiensis* ovarian folliculogenesis

Tenleigh Ellis[1], Nicole L.B. Principato[2], Brandon Moore[1,3]
Department of Biomedical Science, University of Missouri CVM, Columbia, MO[1], Organon, Lansdale, PA[2], School of Health Sciences, Stephens College, Columbia MO[3]



Temperature Dependent Sex Determination and Developmental Estrogenic Exposure

Estrogenic environmental contamination is becoming more common in endemic species habitats. Environmental estrogen exposure compromises reproductive health among vertebrates and, more specifically, can override crocodilian temperature dependent sex determination (TSD) producing female incubated at male-producing temperatures (Fig. 1 top). However, less is known how developmental exogenous estrogen exposure may affect crocodilian ovary formation. This study investigated the morphological effects of developmental 17ß-estradiol exposure on the folliculogenesis in *Alligator Mississippians* ovaries (Fig. 1 bottom). Specifically, we are investigating ovarian cortex size and the quantity of enlarged, stage 3 follicles in diplotene arrest in yearling females.

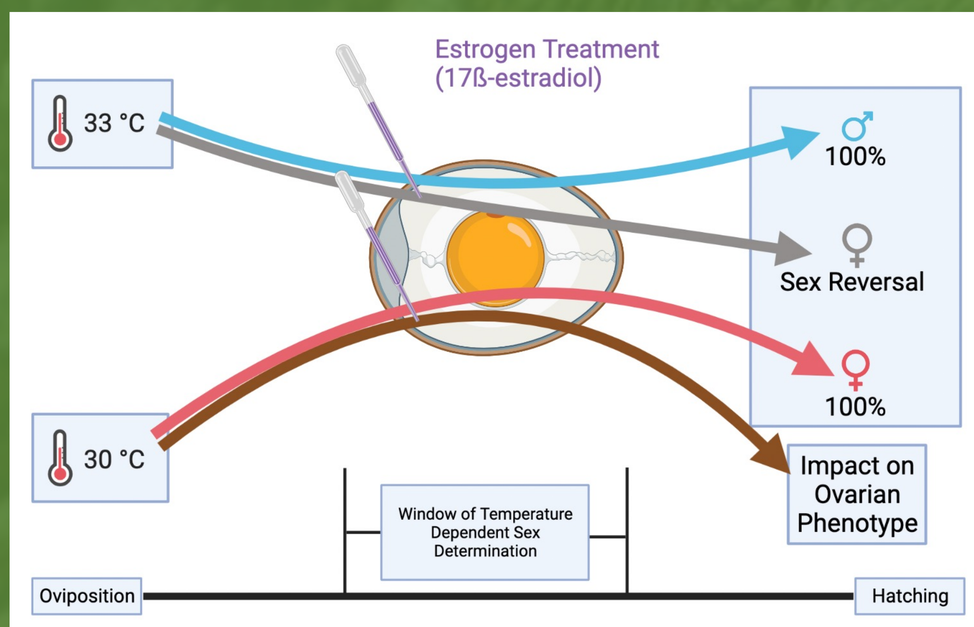


Figure 1: Schematic demonstrating the effects of exogenous estrogens on American alligator development.

Ovarian Analysis Methods

- American alligator eggs were collected from Lake Woodruff, Florida soon after oviposition and incubated at a female-producing 30°C.
- At developmental stage 24 eggs were treated with 50ng/egg 17ß-estradiol in ethanol (n=8) or vehicle alone (n=7) and raised until 5 months old when ovaries were collected at necropsy and divided into three zones (cranial, medial, and caudal).
- Each zone was formalin fixed, transverse paraffin sectioned, and H&E stained.
- Per ovary zone, two random images were selected for analysis
- Photos of the samples were taken using PixeLINK on a Accu-scope with the 10x objective. If the whole sample could not fit in the frame, the photos were then stitched using the Fiji plugin, pairwise stitching. The fusion method was linear blending with 30 check peaks, computed overlap, and subpixel accuracy.
- Area of the cortex and medulla will be calculated using the freehand tool in Fiji.
- Stage three follicles will be counted using the multipoint tool in Fiji.
- The number of follicles then will be divided by the area of the cortex to get a relative density for that tissue section.
- Density counts of the three sections of ovary will be averaged to characterize that ovary.
- Data will be analyzed via two-way ANOVA test to determine estrogen differences between treated and control, and the ovarian region.

Tissue Morphology and Hypotheses

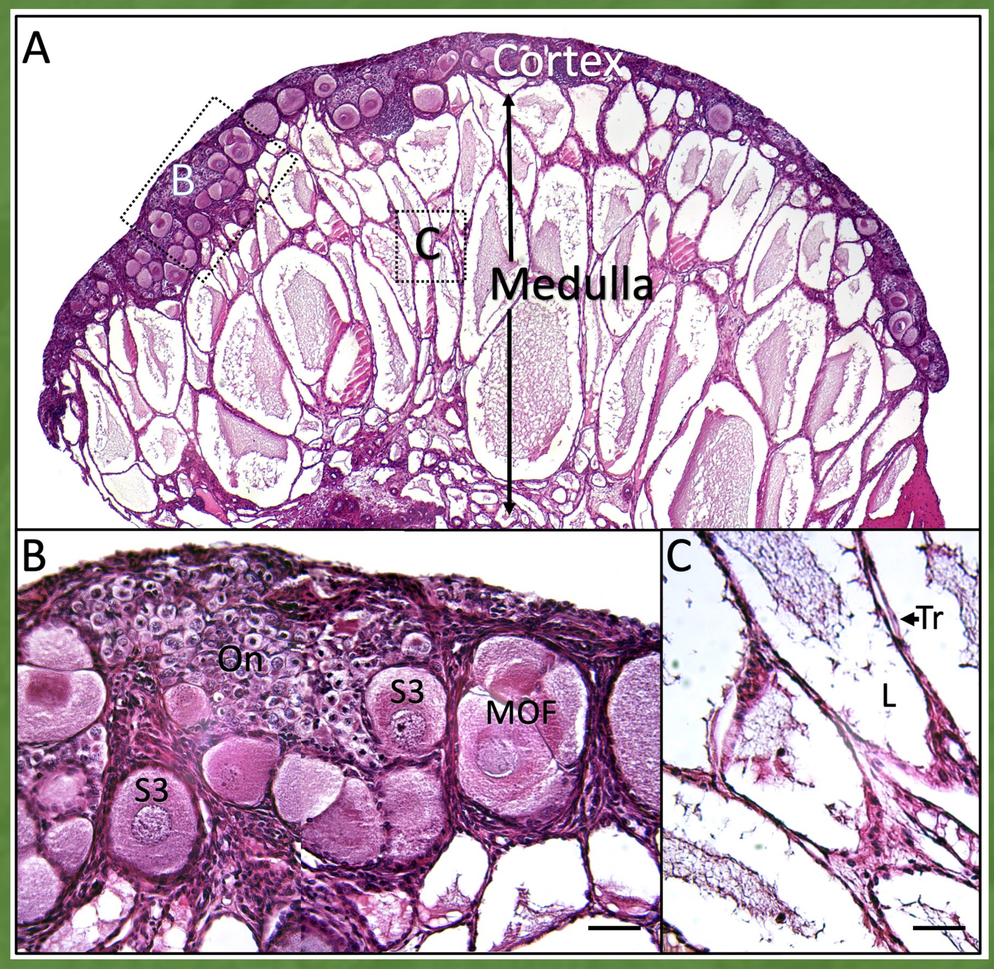


Figure 2: Five-month-old alligator ovary histological sections (H&E). A) Transverse section showing apical germinal cortex and underlying medullary lacunae. B) Magnification of inset box B in A. C) Magnification of inset box C in A. S3=stage three follicle, On = oogonial nest, MOF= multioocytic follicle, L= lacuna, and Tr=trabecula. Scale bars = 100 µm.

Hypothesized Results:

H_0: Estrogen treatment given at female producing temperature has *no effect* on ovarian development and subsequent morphology.

H_{a1}: Estrogen treatment given at female producing temperature stimulates ovary development resulting in *more* stage three follicles.

H_{a2}: Estrogen treatment given at female producing temperature results in negative paracrine/endocrine feedback and resultingly *fewer* stage three follicles produced.

Acknowledgements

Veterinary Research Scholars Program
University of Missouri

Stipend for Tenleigh Ellis is supported by an endowment established by IDEXX-BioAnalytics.

Thank you to VRSP instructors and Stephens College for their support.