

**Proposal # AWE-2005-56 – Matthew B. Lovern**  
Development and Validation of a Fecal Steroid Assay to Non-invasively Measure  
Hormone Levels  
PROGRESS SUMMARY  
6 February 2006

NOTE: Described below is the progress we have made to date in completing this work. I anticipate that we will be able to provide a Final Report by 15 April 2006, with our results submitted for publication shortly thereafter.

**Activities completed to date:**

□ Collection of all samples. We successfully established and maintained individual housing for 23 adult male and 17 female green anole lizards, *Anolis carolinensis*. Lizards were cared for and handled as described in the proposal, resulting in a total of 16 plasma and 440 fecal samples collected, providing us with all of the samples necessary to develop and validate our fecal steroid radioimmunoassay (RIA).

○ Briefly, all lizards were housed individually in the lab under conditions that simulated natural environmental temperature, humidity, and photoperiod. For 14-21 days, we checked at least daily (typically several times/day) for fecal samples from undisturbed lizards. All samples were collected, weighed to the nearest 0.1 mg, and stored individually at -70 C. This protocol provided us with the bulk of our fecal samples, the majority of which are being used in the development and validation of the RIA. Following this collection period, all animals were submitted to a capture restraint protocol (placement in a small plastic container) designed to induce moderate stress. After 20 minutes, blood samples were collected from eight males and eight females via the post-orbital sinus; the remaining animals were returned to their original cages immediately, without sampling. We then collected fecal samples for two days following the captive restraint. Once we have the RIA validated, we will be able to compare fecal steroid levels before and after capture restraint, and we will be able to compare steroid values obtained from blood to those obtained from feces.

□ Initial work on the RIA. Thus far, we have successfully extracted and measured androgen and glucocorticoid, but not estrogen, from the fecal samples. We have had moderate success with our ether-ethanol-hexane extraction, which provided on average 30% recovery efficiency. We are currently working on an alternative extraction technique (methanol-based, which has been used with high success now by a number of labs) which we hope will improve our recoveries, and in turn help us to measure estrogen if it is present. We have high hopes that given our initial success, this fecal RIA will produce results comparable to those obtained via more invasive measures.

□ Training of one graduate and one undergraduate student. With my training and supervision, the majority of the work performed thus far (husbandry, data collection, and RIAs) has been carried out by one M.S. student and one undergraduate research assistant. This award has thus had a considerable, positive impact on student training.

**Activities to be completed:**

□ Finalize RIA protocol. As mentioned above, we are working on an alternative extraction procedure to improve our recovery efficiency of steroids from the fecal samples. Additionally, we are still having some interference with our RIA following extraction, resulting in a lack of parallelism between serially diluted sample and the standard curve. We expect that this will be corrected with our new extraction procedure; if not, additional clean-up steps can be added to isolate the steroids. Once we have demonstrated that our RIA is working, we will run the “real” samples – the fecal samples collected two days before and after the capture restraint protocol and the plasma samples collected immediately after restraint, the RIA for which has already been validated.

□ Complete student training. Corina, my M.S. student, is still being trained on the fecal RIA, and she will be the primary author on the manuscript that we submit for publication. Thus, from design to presentation, she will have received a complete training and research experience with this project. We also have a new undergraduate assistant who is helping us (the first undergraduate is currently studying abroad), and who is thus getting some research experience because of this project.