

## Research & Investigation in Science – NSTA 7

**This assessment demonstrates the candidate’s ability to design and conduct open-ended investigations in a science discipline, requiring data processing and analysis, and reporting the results. This requirement can be met by completing an undergraduate or graduate course that requires original scientific research.**

NSTA Standards:

1.d Understand research and can successfully design, conduct, report and evaluate investigations in science.

1.e Understand and can successfully use mathematics to process and report data, and solve problems, in their field(s) of licensure.

I was extremely lucky to receive an internship and a \$1400 stipend to research with Dr. JoGayle Howard, Senior Scientist at the Smithsonian Institution. I spent 4.5 months at the Smithsonian’s Conservation and Research Center (CRC) located in Front Royal Virginia. Dr. Howard’s main project was trying to develop an artificial insemination protocol for the clouded leopard (*Neofelis nebulosa*). This is extremely important because the leopards are losing natural habitat and so their fate relies on captive breeding efforts. Clouded leopards bond-pair, and if it is not done from early childhood the male will kill the female upon introduction. It is vital for scientists like Dr. Howard to develop an IA protocol to take advantage of leopards that are not pair-bonded as adults, and increase the genetic diversity and health of a species.

I worked in the reproductive endocrinology lab on her project, which began before me, and continued after I left. When I arrived they had already tested out a protocol that had worked on cheetahs but was unsuccessful on clouded leopards. After investigating the possible explanations, it was found that clouded leopards are spontaneous ovulators, whereas cheetahs were induced ovulators. The cheetah protocol induced their “dormant” ovary and so the scientists were able to control their hormone cycle. In the leopard, the ovary is constantly in cycle and when the scientists induced estrus and ovulation, they had no way of knowing in which phase of the hormone cycle the cat was in. This produced extremely variable results.

To control the cyclicity of the cat, an ovary suppressant (altrenogest progesterone) was added to the protocol to make it resemble an induced ovulatory. Before using the newly

developed protocol, images were taken of the ovary using intrascopic technology which showed that the cat was ovulating. Unfortunately, no pregnancies resulted from the new protocol. It was decided that the protocol would be given to the cats again, but this time the hormone levels would be monitored to see what was actually happening.

Fecal samples were collected every day from a zoo keeper and quickly frozen to trap the hormones before they degraded. There were approximately 120 samples for each of the 20 cats located in zoos across the country and in Thailand. The Smithsonian has revolutionized hormone extraction to be non-invasive using this fecal extraction technique. It was my job to extract the hormones from the fecal samples, check the extraction efficiencies, and then enzymeimmunoassay the samples to determine the level of progesterone and estrogen in the samples.

The job required extreme organization, note-taking, and labeling since this extraction process and efficiency testing took most of the 4 months. I first had to freeze-dry the samples in a lyophilizer to extract all water. The samples were then smashed into a powder and placed into separate labeled test-tubes. As a control, the same amount of fecal powder was weighed out into each tube (approximately 0.2g). Then a radioactive tracer was added to each of the tubes (tritium) to be used later in the extraction efficiency. If 90% of the tracer was extracted during the extraction process, then it could be assumed that 90% of the hormones were also. Working with radioactive materials required me to get a certification in laboratory safety, which is an integral part of being a scientist.

The extraction process required using an array of laboratory equipment including pipettes, scales, fume hoods, boiling hoods, vortexes, centrifuges, rack shakers and dangerous chemicals like tritium and strong alcohols. Math was also required to make dilutions of methyl alcohol and ethyl alcohol, since bottles were used up every other day or so. The process took approximately 8 hours for 120 samples. A detailed protocol is also listed on the website under "Laboratory Manual Excerpts," which was provided to me by the Smithsonian Institution upon arrival.

The next step was to make sure I actually extracted the hormone metabolites. This required the reading of the radioactive tracer by a DPM Beta Counter machine (disintegrations

per minute). A fraction of the extracted hormone samples was placed into scintillation vials with a repeat pipette. The Beta Counter printed off a sheet that showed the percentage of the radioactive tracer in the sample by using the formula:

$$(\text{amount observed divided} / \text{amount expected}) * 100.$$

My extraction efficiency was always over 70%, which is great for a carnivorous animal.

The next step was to determine the amount of metabolites that were in the original samples via Enzyme Linked ImmunoSorbent Assay (enzymeimmunoassay). Because I was working on a side project with black-footed ferrets and going to the National Zoo on Friday's to work in the genetics lab, I only spent two weeks on this portion of the process. This is a very delicate technique requiring very precise pipetting skills and deep concentration. First a hormone-specific antibody was added to coat the wells of a polystyrene microtiter plate. Any unabsorbed antibody is washed away with distilled water. A fraction of the original fecal samples (that now contain hormone in alcohol) is added to the wells along with a labeled hormone (HPR-labeled). These two enzymes compete to bind to the antibodies coating the plate. When the labeled enzyme attaches to the antibody it creates a color change that the computer reads. The more hormone in the sample, the less the color change because that means there was more HPR-labeled enzymes reacting with the antibodies. Therefore, color change and hormone concentration are inversely proportional. Standards were also created on the plate to assure accurate and precise laboratory technique. In the two weeks that I performed this technique my standard curves were extremely accurate and precise. The fall intern would have taken over where I left off, continuing the EIA protocol.

As a side project I also worked in the black-footed ferret laboratory. In this lab we "flushed" females every 3 days, which includes squirting water into their vagina to collect epithelial cells. We then went through a staining process to determine the percent of keratinized cells. If the cells were hard, they would not soak up the pink dye and appear orange in color. We used a microscope to count the number of keratinized cells. If the ferret's cells were over 85% keratinized, they were chosen to be artificially inseminated. When I was there, we artificially inseminated a ferret with 8 year old frozen sperm!

There are no words to describe my experience with the Smithsonian. I was able to watch all sorts of animals be artificially inseminated, including pandas, ferrets, and cheetahs. I stayed up all night to watch a clouded leopard give birth to cubs. I even got to harvest bamboo! It was an amazing experience that I will never forget. I learned an incredible amount about the nature of science and laboratory work. In particular I learned that laboratory work can be extremely tedious and time consuming but the end result is worth the sore pipette thumbs and feces smell.

Research and investigation is vital for students to appreciate the discipline of science. It is important to test out theories and explore possibilities because nothing in science is a fact and there are many ways to investigate at the same problem. When students begin to realize that science is more like water than concrete, they become less afraid of “being wrong.” Through exploration and investigation they will find that the scientific method is full of dead-ends and twisty turns. Although nature may be beautiful, finding answers is not.