Noninvasive analysis of faecal reproductive hormone (progesterone and 17β-estradiol) metabolites trend in captive bred female ball pythons (P. regius)

Mara Bertocchi,Igor Pelizzone,Enrico Parmigiani,Patrizia Ponzio,Elisabetta Macchi,Federico Righi,Nicola Di Girolamo,Enrico Bigliardi,Laura Denti,Carla Bresciani,Francesco Di Ianni

Abstract

The royal python (*Python regius*) is commonly bred in captivity. To have a successful breeding, accurate monitoring of the reproductive activity is necessary. The use of non-invasive monitoring methods in exotics is important in order to minimize stress. For this purpose, ultrasound has been anecdotally used to monitor royal python reproductive activity. However, there is limited information regarding the reproductive cycle of this species. The aim of the present study is to monitor the female reproductive cycle of the royal python using ultrasonography and gonadal steroid metabolite measurements in the faeces. The reproductive activity of one hundred twenty-nine adult female *P. regius* was examined during two consecutive years. We performed brief scans on non-anaesthetized snakes using a portable ultrasound system and a 10-12 MHz linear array transducer (MyLab™ 30 Gold, Esaote). Ultrasound features, dimension and echogenicity of the reproductive structures were determined. During the second reproductive cycle, the hormonal profiles of 30 animals were also evaluated, with a monthly collection of faecal samples. These samples were classified according to reproductive stage, and the mean faecal progesterone and 17β-estradiol levels were calculated using the results from an enzyme-linked immunosorbent assay (ELISA). Progesterone levels increased during the reproductive cycle. estradiol levels showed greater variability, although they appeared to increase before coupling when compared to the levels between coupling and egg laying. The present study suggests that it is possible to identify different phases in the female royal python reproductive cycle: anovulatory phase, transition, folliculogenesis and embryogenesis. Ultrasound is also useful for identifying follicular regression or slugs. Gonadal steroid metabolite measurements from the faeces could help integrate the reproductive information. The use of ultrasonography in addition with the steroid metabolite measurement in the faeces gives an accurate picture of ovarian activity in captive adult female royal pythons.

Citation: Mara Bertocchi,Igor Pelizzone,Enrico Parmigiani,Patrizia Ponzio,Elisabetta Macchi,Federico Righi,Nicola Di Girolamo,Enrico Bigliardi,Laura Denti,Carla Bresciani,Francesco Di Ianni Noninvasive analysis of faecal reproductive hormone (progesterone and 17β-estradiol) metabolites trend in captive bred female ball pythons (P. regius). **protocols.io**

dx.doi.org/10.17504/protocols.io.kttcwnn

Published: 15 Nov 2017

Protocol

Faecal sample collection

Step 1.

Monthly faecal samples were collected fresh (within few hours after defecation), placed in plastic bags and stored at -20°C until analysis. Each of these samples was valid as associated with a particular stage of the reproductive cycle by performing an ultrasound scan on the same day as the sample collection. Each sample was labelled with the animal's code and date of collection. The hormone assay was performed within six months of the sample collection.

Faecal Steroid Hormone extraction

Step 2.

Faecal samples were lyophilized, weighed, and crushed, and then two aliquots of the sample (0.25 g each) were placed into extraction tubes, sealed with a Teflon cap and stored at −20°C. Each aliquot was thoroughly mixed for 30 min using a multivortex with one mL of 80% methanol (Sigma Aldrich, St. Louis, MO, USA). The suspension was then centrifuged at 500 g for 20 min and the supernatant was recovered. An aliquot (0.5 mL) of the supernatant was transferred into a new vial and evaporated at 50°C for 14 h. After evaporation, the dried extracts were stored at room temperature in dark boxes for 15 days and then kept at −80°C until they were assayed. One day before the SSFM analyses, the dried extracts were re-diluted in 0.5 mL of 80% methanol. An aliquot of the extract was diluted to 1:10 in the assay buffer (Arbor Assay®, Ann Arbor, MI, USA). The mixture was then vortexed and left to rest for 5 min twice to ensure complete steroid solubility.

Faecal Steroid Hormone analysis

Step 3.

Faecal immunoreactive progestogen (FPM) and oestrogen (FEM) metabolite concentrations were determined using two multi-species progesterone and estradiol enzyme immunoassay kits (K025-H5, K030-H5; Arbor Assay, DetectX®, Ann Arbor, Michigan, USA) to determine steroids on different biological matrices such as faeces and urine. All analyses were repeated twice. The concentration of FPM and FEM was expressed as ng/g and pg/g of faeces dry matter.

LINK:

http://www.arborassays.com/documentation/inserts/K030-H.pdf

Clinical validation

Step 4.

The hormonal response was validated through a clinical validation (reproductive and status behavior) and the correlation of hormonal values with the ultrasound assessments.

Clinical validation was performed on the animals' reproductive behaviours including acceptance of the male, mating and depositions, which were observed and recorded daily. The animal's reproductive status was clinically identified through ultrasonography. The follicular stages of development and degrees of calcification were observed from the ultrasound recording, and an absence of follicles indicates reproductive inactivity.