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**Steroids and Reproductive
Biology in the Blotched
Blue-tongued Lizard,
*Tiliqua nigrolutea***

by

Ashley Edwards BSc (Hons)

**Submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy, School of Zoology, University of Tasmania
(July, 1999)**

Declaration

This thesis contains no material which has previously been accepted for a degree or diploma by the University of Tasmania or any other tertiary institution and to the best of my knowledge and belief, contains no material previously published or written by another person, except where due acknowledgment is made in the text.

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Abstract

This thesis documents the annual profiles of the primary reproductive steroids testosterone (T), 17 β -oestradiol (E2) and progesterone (P4), in the reproductive cycles of male and female blue-tongued lizards, *Tiliqua nigrolutea*. Data collected from a large captive population over three consecutive reproductive seasons are included. Reproductive cycles are discussed in the context of other viviparous squamate reptiles, while a broader comparative approach is used to consider patterns of steroid biosynthesis and peripheral metabolism.

The annual patterns of circulating concentrations of T, E2 and P4 have been characterised for both sexes. In males, peak plasma T ($10.9 \pm 3.00 \text{ ng ml}^{-1}$) and E2 ($778.0 \pm 120.00 \text{ pg ml}^{-1}$) concentrations occur coincident with late spermatogenesis and observations of mating, respectively. Plasma P4 concentrations remain basal ($< 1.2 \text{ ng ml}^{-1}$) throughout the annual reproductive cycle. In females, increasing plasma E2 concentrations ($275.2 \pm 33.87 \text{ pg ml}^{-1} - 715.1 \pm 106.68 \text{ pg ml}^{-1}$) are associated with vitellogenesis and plasma T peaks ($6.3 \pm 0.63 \text{ ng ml}^{-1}$) in the mating and peri-ovulatory period. In pregnant females, plasma P4 concentrations are elevated for the first two thirds of gestation, peaking in the second trimester at $12.7 \pm 1.27 \text{ ng ml}^{-1}$ and falling rapidly prior to parturition. Concurrently, plasma P4 concentrations in non-reproductively active adult females remain basal ($1 - 2 \text{ ng ml}^{-1}$) throughout the year.

There is good circumstantial evidence for a multiennial reproductive cycle in females. Parturition occurs late in the active season, presumably leaving little time for females to store sufficient fat reserves to become vitellogenic in the following spring: reproductive opportunities are effectively missed in at least one year following a reproductive effort. Observed reproductive behaviours, including agonistic male – male interactions, mating, and parturition, are documented.

An investigation of gonadal steroid biosynthetic pathways in this viviparous squamate is presented. This compares variation in the relative contributions of the delta-4 and delta-5 steroidogenic pathways according to sex and reproductive condition. The delta-4 pathway

predominates in both sexes, aligning this species phylogenetically with other reptiles. However, there are clear differences between sexes and with changing reproductive condition in the patterns of production of pathway intermediates and end-products. Additionally, detection of a possibly novel polar steroid as a major end-product of steroid biosynthesis in both sexes is reported.

Peripheral (extragonadal) metabolism of T and E2 in a number of reproductively relevant steroid target tissues is compared at times of year chosen to represent three clearly distinctive reproductive conditions in each sex. There are differences both between sexes, between tissue types and with changing reproductive condition in the relative proportions of steroid conjugates and non-conjugated derivatives produced. Biosynthetic pathway activity and peripheral steroid metabolism both appear to be plastic in response to changing reproductive condition in *Tiliqua nigrolutea*.

With a comprehensive database of information about the reproductive endocrinology and physiology of *Tiliqua nigrolutea*, this species is now available as a model to further examine selected aspects of the steroid hormone control of reproductive physiology and behaviour in a cool temperate, viviparous reptile.

Ethics permits

This project was conducted with the approval of the University of Tasmania animal ethics committee under ethics permit numbers 95046 and 98015.

No permit or licence is required in Tasmania to collect (with the exception of collection from national parks) or keep reptiles.

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Abbreviations used in the text

Animals

SVL snout-vent length

Chemicals

DCM dichloromethane

DEE diethylether

EtOH ethanol

MeOH methanol

ChCl₃ chloroform

UV ultraviolet

Methods

GSI gonadosomatic index

HPLC high performance liquid chromatography

RIA radioimmunoassay

R_f elution coefficient

TLC thin layer chromatography

RCM relative clutch mass

GC-MS gas chromatography-mass spectroscopy

Statistics

ANOVA analysis of variance

(M)ANOVA repeated measures analysis of variance

Steroids

AD androstenedione

Δ4/4-ene delta 4 pathway

Δ5/5-ene delta 5 pathway

DHA dehydroepiandrosterone

E1 oestrone

E2 17-beta-oestradiol

E3 oestriol

EpiT epitestosterone

³[H] tritiated

P4 progesterone

P5 pregnenolone

T testosterone

3β-HSD 3-beta-hydroxysteroid dehydrogenase

17β-HSD 17-beta-hydroxysteroiddehydrogenase

5α-DHT 5-alpha-dihydrotestosterone

6α-OH-E2 6-alpha-hydroxyoestradiol

6β-OH-E2 6-beta-hydroxyoestradiol

11β-HSD 11-beta-hydroxysteroid dehydrogenase

11β-OH-T 11-beta-hydroxytestosterone

11-KT 11-ketotestosterone
17 α -OH-P4 17-alpha-hydroxyprogesterone
17 α -OH-P5 17-alpha-hydroxypregnenolone

Tissues

AR androgen receptor
CE columnar epithelium
CL corpus luteum
E epithelium
ER oestrogen receptor
HPG hypothalamic-pituitary-gonadal axis
L lumen
PR progesterone receptor
SER smooth endoplasmic reticulum
SS renal sexual segment
ZP zona pellucida
ZR zona radiata