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

by

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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.

Signature:

Date:

Abstract

This thesis documents the annual profiles of the primary reproductive steroids testosterone (T), 17β-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 ± 3.00 ng ml^{-1}) and E2 (778.0 ± 120.00 pg ml^{-1}) concentrations occur coincident with late spermatogenesis and observations of mating, respectively. Plasma P4 concentrations remain basal (< 1.2 ng ml^{-1}) throughout the annual reproductive cycle. In females, increasing plasma E2 concentrations (275.2 ± 33.87 pg ml^{-1} – 715.1 ± 106.68 pg ml^{-1}) are associated with vitellogenesis and plasma T peaks (6.3 ± 0.63 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 ± 1.27 ng ml^{-1} and falling rapidly prior to parturition. Concurrently, plasma P4 concentrations in non-reproductively active adult females remain basal (1 – 2 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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Table of contents

Abbreviations used in the text

Animals
SVL snout-vent length

Chemicals
DCM dichloromethane
DEE diethylether
EtOH ethanol
MeOH methanol
$ChCl_3$ chloroform
UV ultraviolet

Methods
GSI gonadosomatic index
HPLC high performance liquid chromatography
RIA radioimmunoassay
Rf 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
17α-OH-P4 17-alpha-hydroxyprogesterone
17α-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