ADVANCES IN OESTROUS SYNCHRONISATION TECHNOLOGY FOR ASSISTED BREEDING IN MARSUPIALS

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Declaration

I hereby certify that the work embodied in this thesis is my own work, conducted under normal supervision and is presented in the form of a series of papers.

The thesis contains published scholarly work of which I am the lead author. For such work a written statement, endorsed by the other authors and the Faculty Assistant Dean (Research Training), attesting to my contribution to the joint work has been included.

The thesis contains no material which has been accepted, or is being examined, for the award of any other degree or diploma in any university or 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 reference has been made in the text. I give consent to the final version of my thesis being made available worldwide when deposited in the University’s Digital Repository, subject to the provisions of the Copyright Act 1968 and any approved embargo.

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Dedications

It is with great love and affection that I dedicate this thesis to my wife Erin and my family. Your constant encouragement, support and love got me over the line. Thank you!

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‘The fact is that no species has ever had such wholesale control over everything on earth, living or dead, as we now have. That lays upon us, whether we like it or not, an awesome responsibility. In our hands now lies not only our future, but that of all other living creatures with whom we share the earth’.

– Sir David Attenborough, Life on Earth –
Preliminary pages

I. Publications included as part of the thesis

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

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II. Statement of contribution

By signing below, I confirm that Ryan Robert Witt contributed upward of 60% to the research, experimental design, data collection, analysis of data, development of figures and tables, and manuscript preparation for all publications in this thesis for which I am a co-author.

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III. Conference proceedings

Presentation


Poster

Table of Contents

Declaration .......................................................................................................................... ii
Acknowledgements .......................................................................................................... iii
Dedications ........................................................................................................................ v
Preliminary pages:
   I. Publications included as part of the thesis ................................................................. vii
   II. Statement of contribution ...................................................................................... viii
   III. Conference proceedings ..................................................................................... ix
Abstract ........................................................................................................................... xi
Overview .......................................................................................................................... xiii

Introduction and Literature Review:
   Chapter 1: GnRH agonist downregulation of the hypothalamic-pituitary-gonadal axis may offer a strategy to control and synchronise oestrous in marsupials .......................................................................................................................... 1
   Chapter 2: Recent advances in tools and technologies for monitoring and controlling ovarian activity in marsupials ........................................................................................................... 25

Research Chapters:
   Chapter 3: Ovarian suppression in a marsupial following single treatment with a gonadotrophin-releasing hormone agonist in microspheres ........................................ 38
   Chapter 4: Breeding in the fat-tailed dunnart following ovarian suppression with the gonadotrophin-releasing hormone agonist Lucrin® Depot ........................................... 49
   Chapter 5: Delayed return to oestrus following treatment with the gonadotrophin-releasing hormone agonist, Lucrin® Depot, in the tammar wallaby .......................................................... 62
   Chapter 6: Induction of synchronous oestrous after pre-treatment with the GnRH agonist, Lucrin® Depot, in the tammar wallaby ............................................................. 72

Conclusion:
   Chapter 7: Final Discussion ........................................................................................ 89
Abstract

Oestrous synchronisation technology has the capacity to advance genetic conservation outcomes for threatened marsupials by making use of selected spermatozoa in artificial insemination (AI). A technique capable of precise control of oestrus and ovulation in marsupials, of which most are spontaneous ovulators, remains the key limiting factor in developing practical AI programs. The major impediment is the corpus luteum (CL) which in marsupials becomes independent of hypothalamic-pituitary support after formation and persists in both pregnant and non-pregnant cycles. For this reason, eutherian synchronisation techniques that rely on targeting CL life have failed to induce luteolysis in marsupials.

The aim of this thesis was to investigate the potential to circumvent the marsupial CL and synchronise oestrous by targeting the hypothalamic-pituitary gonadal (HPG) axis with a gonadotrophin-releasing hormone (GnRH) agonist. GnRH agonists are small molecules rapidly removed from the body and thus are traditionally delivered as multiple injections or in slow release implants not suitable for assisted breeding applications. In contrast, Lucrin® Depot (AbbVie), a GnRH agonist in microspheres effects a one-month suppression of pituitary function after a single injection.

In this thesis, I detail the potential for Lucrin Depot to synchronise oestrous in two taxonomically distinct marsupials with alternative reproductive strategies, a dasyurid, the fat-tailed dunnart (*Sminthopsis crassicaudata*) and a macropod, the tammar wallaby (*Notamacropus eugenii*). In the fat-tailed dunnart, a dose of 5 mg kg\(^{-1}\) or 10 mg kg\(^{-1}\) of Lucrin Depot, resulted in reproductive suppression for 4 to 8 weeks, a return to reproductive activity at 8 to 12 weeks, and a complete return to cycling at 16 weeks. Following Lucrin-induced suppression, female dunnarts were fertile and conceived as early as 8 weeks after receiving 5 mg kg\(^{-1}\) but did not conceive until 14 or 15 weeks after receiving 10 mg kg\(^{-1}\). In tammar wallabies that underwent removal of pouch young (Day 0 RPY), Lucrin Depot inhibited the growth of pre-ovulatory follicles (all follicles <2mm, Day 31 RPY). An effective dose, 1.25 mg kg\(^{-1}\), delays oestrus until between Day 39-66 RPY if Lucrin-treated at the time of RPY, and between Day 43-71 RPY if Lucrin-treated on Day 10 RPY. The outcome of work in both the fat-tailed dunnart and tammar wallaby resolved that Lucrin Depot has the capacity to inhibit ovarian follicular activity, but alone does not synchronise oestrous to a degree needed for assisted breeding.

In the final research chapter, I show Lucrin Depot can be combined with exogenous gonadotrophins for ovarian stimulation and synchronisation. Pre-treatment of tammar wallabies with 1.25 mg kg\(^{-1}\) of Lucrin Depot on Day 0 RPY prior to single
doses of 20IU of PMSG on Day 20 RPY and 500IU of hCG on Day 23 RPY, synchronised oestrus and copulation to within 1 day (Day 26±0.1 RPY, n= 5 of 6). At autopsy and follow-up ovarian histology, it was determined that 20IU of PMSG adequately stimulates the healthy growth of pre-ovulatory follicles (around 10+ follicles >3mm per ovary Day 31 RPY). However, both the control superovulation group, and the Lucrin Depot-superovulation group did not ovulate in response to a single 500IU injection of hCG. Together the data presented in this thesis confirm that Lucrin Depot can form the basis of an oestrous synchronisation strategy in marsupials, and with further work to resolve the optimum ovulation treatment, it can be expected to become a practical assisted breeding tool for the recovery of threatened marsupials.
Overview

This thesis is presented as a collection of published articles (Chapter 2-5), and a prepared manuscript (Chapter 6). The papers presented in this thesis all relate to the development of oestrous synchronisation technology and assisted reproductive technology for marsupials to support the conservation of threatened species.

Chapter 1, the introduction, is the unpublished section of the literature review component, and details the essential background information required to appreciate the thesis objectives and research chapters. This includes a review of folliculogenesis in marsupials; reproductive patterns in the fat-tailed dunnart and tammar wallaby as model species; GnRH and the regulation of oestrous in marsupials; and introduces Lucrin Depot as the potential GnRH agonist for oestrous synchronisation as a standalone treatment, and in combination with exogenous gonadotrophins.

Chapter 2, is a published review that relates to the current status and limitations of applying assisted reproductive technology to female marsupials, and comprehensively evaluates the tools and technologies available to control female cycling for assisted breeding. Chapter 2 briefly highlights research completed in Chapter 3, 4 and 5 of the current thesis.

The following Chapters (3-6) directly relate to the development of a Lucrin Depot based oestrous synchronisation technology for marsupials. Chapters 3 and 4, cover developments of this technology in the small dasyurid, the fat-tailed dunnart, and Chapters 5 and 6, cover developments of this technology in a macropod, the tammar wallaby. Chapters 3-5, show promise for a hypothalamic-pituitary, GnRH agonist down-regulation approach for female synchronisation technology in marsupials. The research indicative that Lucrin Depot has the capacity to offer a level of oestrous control, and breeding, conception and birth of healthy young is possible in both species. Chapter 6 takes the technology further and combines the Lucrin Depot approach with a superovulation protocol (PMSG followed by hCG), with the aim of instigating a highly synchronous response.

Finally, Chapter 7 presents the holistic conclusions of the thesis and research findings, and provides final assessment on the viability of a Lucrin Depot-based synchronisation strategy for marsupials.