

Supplementary material for

The effect of testosterone suppression on health and parasite burden in male eastern grey kangaroos (*Macropus giganteus*)

Maquel E. Brandimarti^{A,E}, Rachael Gray^B, Zoe J. Hilton^A, Tamara Keeley^C, 'Kangaroo' Phil Murray^D and Catherine A. Herbert^A

^ASchool of Life and Environmental Science, The University of Sydney, JD Stewart building, Camperdown, NSW 2006, Australia.

^BSydney School of Veterinary Science, The University of Sydney, McMaster building, Camperdown, NSW 2006, Australia.

^CSchool of Agriculture and Food Sciences, The University of Queensland, Building 8117A, Ground Floor, Gatton, Qld 4343, Australia.

^DNelson Bay Golf Club, 57 Dowling St, Nelson Bay, NSW 2315, Australia.

^ECorresponding author. Email: maquel.brandimarti@live.com.au

Appendix 1

Table A1. Pilot study (n=2) serum testosterone concentration (nmol/L) and mean (\pm s.e.m.) testis measurements (mm) *Before* and *After* treatment of adult male eastern grey kangaroos (*Macropus giganteus*) with Bopriva; a short term testosterone suppressing vaccination. The impact of two types of injection on effectiveness of Bopriva in suppressing testosterone was trialled. The testosterone concentration of a female is also included for comparison. The pilot study was undertaken from September 2018 to February 2019 at Nelson Bay Golf Course, Port Stephens, New South Wales, Australia.

Animal identification		013	144	015 (female)
Injection type		Subcutaneous	Intramuscular	NA
Injection site reaction	Primary injection	No	No	NA
	Booster injection	No	No	NA
Testosterone nmol/L	<i>Before</i>	44.03 [†]	20.3	0.45
	<i>After</i>	1.74	0.45	NA
Testis breadth (mm)	<i>Before</i> (n=2)	30.885 (0.92)	32.485 (0.12)	NA
	<i>After</i> (n=2)	29.785 (0.99)	28.4*	NA
Testis width (mm)	<i>Before</i> (n=2)	63.27 (0.93)	66.1 (0.5)	NA
	<i>After</i> (n=2)	56.755 (1.75)	52.6*	NA
Testis length (mm)	<i>Before</i> (n=2)	42.43 (0.03)	44.62 (1.42)	NA
	<i>After</i> (n=2)	37.815 (3.92)	32.8*	NA

[†]serum collected in 2017

*raw value presented as n=1

A2. Pilot study methodology for determining serum testosterone concentration

Serum testosterone analyses were conducted at the Royal North Shore Hospital, St Leonards, Sydney. Historic serum samples from pilot animals (animal identification 013 and 144) in 2017 were analysed in addition to serum samples taken at four weeks (booster injection) and/or eight weeks after the primary injection (Fig. 1). Thawed serum testosterone was analysed using the chemiluminescent microparticle immunoassay (ARCHITECT 2nd Generation Testosterone, Abbott Laboratories, Wiesbaden, Germany). The assay has been validated for use in kangaroos (Miller et al. 2010), however a serum sample from a female was included for comparison. Serum samples were initially diluted 1:3 with testosterone assay specific diluent (ARCHITECT 2nd Generation Testosterone kit). However, samples were reanalysed 'neat' when results were <0.45 nanomoles (nmol)/litre (L) or at a 1:4 dilution when >35 nmol/L.

Table A3. Explanatory variable distribution and model selection for determining the effect of treatment on testosterone concentration and health parameters in adult male eastern grey kangaroos (*Macropus giganteus*) at Nelson Bay Golf Course, Port Stephens, New South Wales.

Variable	Distribution	Model
Testosterone ¹	Normal	LMM
Ticks	Neg binomial	GLMM
EPG	Neg binomial	GLMM
Weight	Neg binomial	GLMM
Teste width	Normal	LMM
Teste breadth	Normal	LMM
Teste length	Normal	LMM
Glucose ¹	Normal	LMM
WBC	Normal	LMM
Neutrophils	Poisson	GLMM
Lymphocytes	Normal	LMM
Eosinophils ¹	Normal	LMM

Monocytes	Poisson	GLMM
Basophils	Poisson	GLMM
RBC	Normal	LMM
HGB	Normal	LMM
HCT	Normal	LMM
MCV	Neg binomial	GLMM
MCH	Neg binomial	GLMM
MCHC	Neg binomial	GLMM
PLT ¹	Normal	LMM
NRBC	Poisson	GLMM
Albumin	Normal	LMM
TP	Normal	LMM
Globulin	Normal	LMM

EPG, worm eggs per gram faeces; WBC, white blood cell count; RBC, red blood cell count; HGB, haemoglobin concentration; HCT, haematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; PLT, platelets; NRBC, nucleated red blood cell count; TP, total protein

¹Cube root transformation applied