

Deslorelin implants in free-ranging female eastern grey kangaroos (*Macropus giganteus*): mechanism of action and contraceptive efficacy

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Abstract

Context. Fertility control offers a non-lethal management technique for iconic yet overabundant wildlife. Slow-release hormonal implants containing deslorelin show promise for managing free-ranging populations, particularly in peri-urban reserves, but most studies have been limited to captivity.

Aims. We investigated the efficacy and mechanism of deslorelin implants in free-ranging female eastern grey kangaroos (*Macropus giganteus*) under realistic management conditions.

Methods. We assigned females to a deslorelin (9.4 mg, $n = 53$) or placebo ($n = 56$) group at three peri-urban sites in Victoria, Australia, and monitored reproductive success for 3 years by observing young in the pouch. We tested the plasma LH response of control and treated females to exogenous GnRH, and compared the size of ovarian follicles between the two groups.

Key results. Deslorelin implants reduced fertility at all three sites. No deslorelin-treated females bred in Year 1 at Anglesea and Serendip versus 42% and 44% of control females respectively. At Plenty Gorge, 60% of deslorelin-treated females bred in Year 1 versus 100% of control females. In Year 2, between 11% and 39% of the treated females bred versus between 82% and 100% of control females at all sites. The contraceptive efficacy reduced by Year 3 when between 43% and 57% of the treated females bred versus between 85% and 100% of controls. A GnRH challenge elicited higher plasma LH concentrations in control than in treated females, and unlike untreated females, treated females lacked ovarian follicles >2 mm.

Conclusions. Deslorelin implants reduced fertility in free-ranging female eastern grey kangaroos over three successive breeding seasons. Chronic exposure to deslorelin desensitised the pituitary gland to GnRH and suppressed follicular development, but did not inhibit the development of a blastocyst, pregnancy or lactation in at least some females that had conceived before treatment.

Implications. Effective population management using deslorelin implants will require females to be re-treated on multiple occasions because the contraceptive effect lasts only a portion of their reproductive life. This would be practical only at sites where kangaroos are relatively easy to capture. The timing of treatment is also important in a species that undergoes embryonic diapause, particularly at sites providing high-quality habitat.

Additional keywords: fertility control, GnRH agonist, hormonal contraception, marsupial, population control, wildlife management.

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Introduction

The management of overabundant, and often charismatic, native species worldwide is a challenging issue. Large herbivores such as elephants (*Loxodonta africana*; Whyte *et al.* 1998), feral horses (*Equus caballus*; Symanski 1996) and deer (Côté *et al.* 2004) can become locally overabundant, causing damage to habitat, conflict with humans and concerns for animal welfare. Management actions applied to these species attract intense public attention. Lethal control is becoming publicly unacceptable, so there is increasing demand for non-lethal management techniques such

as fertility control (Garrott 1995). Although there have been major advances in fertility-control techniques in the past two decades (Kirkpatrick and Turner 2008), much of the research has been focussed on captive animals. The performance of these techniques in wild populations requires more attention.

The eastern grey kangaroo (*Macropus giganteus*) is an iconic Australian marsupial. It often reaches high densities in peri-urban parks and reserves, leading to overgrazing that threatens the environmental values of the area (Coulson 2001). The close proximity of humans and kangaroos also increases the risk of

vehicle collisions and attacks by kangaroos on humans and dogs (Coulson 2001). Lethal control of eastern grey kangaroos in peri-urban sites is frequently rejected as a management option because of widespread public opposition and human safety concerns (Adderton Herbert 2004). Fertility control of females using long-acting hormonal implants offers a non-lethal management alternative, which can be applied on a population scale (Adderton Herbert 2004). One such implant containing the gonadotrophin-releasing hormone (GnRH) agonist deslorelin shows promise for managing these populations (Herbert *et al.* 2005, 2006; Eymann *et al.* 2007; Lohr *et al.* 2009).

The eastern grey kangaroo is a large herbivore (adult females from 20 to 35 kg, males from 40 to 85 kg) with an extensive range in eastern Australia, from Tasmania to much of Queensland (Poole 1983). It can breed throughout the year, but births peak between December and March (Poole 1983). Females are polyoestrous, monovular and gestation is ~37 days; the highly altricial young are suckled in the pouch for between 280 and 320 days, and then for up to 6 months after pouch exit (Poole 1975). If environmental conditions are suitable, females may enter oestrus and mate later in lactation when the pouch young is at least 100 days old; any resulting conceptus enters diapause at the blastocyst stage, and remains dormant until the current young permanently exits the pouch (Clark and Poole 1967). The ability of eastern grey kangaroos to produce a young usually every year (Poole 1975) for between 8 and 10 years (Quin 1989) makes fertility control a useful technique for limiting population growth.

Implants containing deslorelin, a GnRH agonist, were developed for use in domestic animals (Trigg *et al.* 2001). They have more recently been applied to wildlife such as exotic carnivores (Bertschinger *et al.* 2001, 2002). Current knowledge of the contraceptive action in marsupials is based on tammar

wallabies (*Macropus eugenii*). As in heifers (D'Occhio *et al.* 2000), continuous exposure to deslorelin desensitises the pituitary gland of tammar wallabies to GnRH, reducing the secretion of follicle stimulating hormone (FSH) and luteinising hormone (LH), so that follicular development and ovulation are suppressed (Herbert *et al.* 2004). Deslorelin implants inhibited reproduction for between 1 and 2 years in captive tammar wallabies (Herbert *et al.* 2005), and in a small sample of captive eastern grey kangaroos one implant inhibited reproduction for approximately 2 years (Herbert *et al.* 2010). However, females in these captive trials were offered food, water and shelter *ad libitum* (Herbert *et al.* 2006; Woodward *et al.* 2006). Under natural conditions, seasonal food limitation and occasional drought reduce fecundity in wild populations of western grey kangaroos (*Macropus fuliginosus*) and red kangaroos (*Macropus rufus*; Shepherd 1987), and similar effects could be expected in eastern grey kangaroos. Thus, it is important to test deslorelin implants under natural conditions, where the contraceptive effect may be longer than it is in captivity.

The aim of the present study was to test the efficacy of deslorelin implants in free-ranging eastern grey kangaroos under realistic management conditions. Specifically, we investigated the contraceptive success, longevity and mechanism of deslorelin implants under varying environmental conditions.

Materials and methods

Study sites

The three peri-urban study sites (Table 1) near Melbourne, Australia, range in size from 73 to 1355 ha. The climate at all sites is temperate, and average annual rainfall ranges from

Table 1. Characteristics of the study sites in Victoria, Australia

Kangaroo density was calculated from total counts at Anglesea Golf Club in summer (2004) and winter (2006, 2008, 2010; Inwood *et al.* 2008; M. Wilson and J. Cripps, unpubl. data), from vantage-point surveys at Serendip Sanctuary by Parks Victoria since 1995, and from faecal-pellet and vantage-point surveys at Plenty Gorge Parklands in Winter and Spring 2007 (Bone 2007). Pasture biomass was measured from 2006 until 2008 at Serendip Sanctuary (Garnick *et al.* 2010; Borland *et al.* 2011; M. Wilson and J. Cripps, unpubl. data) and in 2008 and 2009 at Plenty Gorge Parklands (M. Wilson and J. Cripps, unpubl. data). Pasture biomass was not measured at Anglesea Golf Club

Characteristic	Anglesea Golf Club	Serendip Sanctuary	Plenty Gorge Parklands
Location	38°40'S, 144°19'E	38°00'S, 144°24'E	37°65'S, 145°9'E
Size (ha)	73	250	1355
Mean max. temp (°C)			
Winter	14	15	14
Summer	22	26	26
Mean ann. rainfall (mm)	814	447	652
Kangaroo density (kangaroos ha ⁻¹)	1.9–4.1	0.9–4.5	0.6
Winter pasture (g m ⁻² dry weight)		20–36	87–90
Key habitats	Golf course greens and fairways, remnant woodland	Grassy paddocks, open woodland, wetland	Grassy paddocks, grassy woodland, riparian woodland, wetland
Main management issue	Wildlife–human conflict	Animal welfare	Habitat degradation
Causes of mortality in study animals (% individuals affected per site)	Vehicle collision (9%), suspected dog attack (2%), unknown causes (6%)	Vehicle collision (13%), emaciation from lumpy jaw (5%), shot in a cull (5%), drowning (3%), injury then euthanasia (3%), unknown causes (8%)	Vehicle collision (9%), suspected <i>Phalaris</i> poisoning (4%)

447 mm at Serendip Sanctuary to 814 mm at Anglesea Golf Club, with peak rainfall in October at each site.

Anglesea Golf Club, Anglesea, is 100 km south-west of Melbourne on the southern coast of Victoria. Residential properties bound the southern and eastern sides of the golf course, and coastal shrubland and heath woodland bound the northern and western sides. Irrigated fairways, a permanent water supply and strips of woodland providing shelter have enabled kangaroos to reach densities of up to 4.1 ha⁻¹ (Table 1). The kangaroos often move into residential areas, resulting in a high incidence of kangaroo–vehicle collisions and other human–wildlife interactions.

Serendip Sanctuary is located 60 km south-west of Melbourne. The site is bounded by small rural residential properties and large farming properties. Serendip was once a sheep farm and, consequently, has large areas of cleared pasture, remnant patches of woodland and stock troughs that provide kangaroos with a permanent water supply (Table 1). Serendip receives the lowest rainfall of the study sites and has only one-third of the winter pasture available at the third study site, Plenty Gorge Parklands (Table 1). Kangaroos were implanted at Serendip at the end of a population irruption; population density reached 4.5 ha⁻¹ in 2006, then crashed to 2.6 ha⁻¹ in just 6 months (M. Smith, Parks Victoria, pers. comm.).

Plenty Gorge Parklands, 20 km north-east of Melbourne, is adjacent to intensive urban development. This linear parkland is the largest of the study sites and consists of dissected landscape, with a habitat mosaic of improved pasture, grassy woodlands, foothill forest, remnant box–ironbark forest, wetlands and a wooded gorge (Table 1). Abundant food and a permanent water supply from the Plenty River, ephemeral wetlands and an adjoining golf course support ~700 kangaroos (Table 1). This is the lowest kangaroo density (0.6 ha⁻¹) of the study sites, but the population is still thought to be damaging habitat for other species in the park (T. Varcoe, Parks Victoria, pers. comm.).

Experimental design

We assessed the effect of deslorelin implants on reproduction of adult female eastern grey kangaroos at the three sites. When possible, we captured females with small (<100 days old) pouch young to confirm they were fertile, but would not be pregnant or in diapause, because in this kangaroo species lactational suppression of oestrus lasts at least 100 days postpartum (Clark and Poole 1967). Once we had the animal in the hand, we removed any young <210 days old from all treated and control females, and inserted the implants. This is likely to be the strategy used in any management program aiming to prevent population increase, given the effort involved in capture. Young older than 210 days were near pouch exit, at which time the mother will mate or a blastocyst will be reactivated, so we did not remove these young from the pouch. Successful reproduction in Year 1 implied that the female either conceived again after her young was removed or had an embryo in diapause at the time of treatment. We treated females only when they were first captured. We did this over 2 years (2007 and 2008) at Serendip and over 1 year at Anglesea (2008) and Plenty Gorge (summer 2007/08; Table 2). From then on, we monitored their

reproductive state after each breeding season, with each breeding season classified as 1 year.

We chose capture techniques to suit the environmental conditions and behaviour of kangaroos at each site. We used draw-string traps (Coulson *et al.* 2003) and tranquilising darts to catch kangaroos at Serendip Sanctuary and Plenty Gorge Parklands, and a pole syringe (King *et al.* 2011) at Anglesea Golf Club. We physically restrained kangaroos in draw-string traps, then immobilised them with Zoletil 100 (i.m., 5 mg kg⁻¹, 1 : 1 of Zolezapam and Tiletamine; Virbac, Milperra, NSW, Australia). We darted kangaroos in the thigh muscle from the back of a vehicle from 30 m at night, with the aid of a spotlight. At Serendip, Zoletil 100 (as above) was delivered by an injection arrow fired from a crossbow (Pro-medic; Wildvet, Melbourne, Vic., Australia), and at Plenty Gorge it was delivered by a self-injecting dart (Pneu-Dart, Williamsport, PA, USA) fired from a CO₂-powered rifle (Montech 2; Montech, Melbourne, Vic., Australia). Kangaroos at Anglesea were habituated and readily approached on foot during daylight, so we captured them using a 1.4- or 4.5-m pole syringe containing Zoletil 100 (as above).

We randomly assigned adult females to a treatment or control group at each site (Table 2). We conducted a general health examination and measured body mass, crus (lower leg) and pes (foot) length. We recorded pouch condition and teat state; we classified females with everted teats as adults (Poole and Catling 1974). We removed any pouch young as described above, and recorded its sex and measured its head, crus and pes length. To identify kangaroos from a distance, we marked them with a unique combination of coloured, reflective ear-tags (Allflex

Table 2. Summary of treatment groups

Female eastern grey kangaroos were treated at first capture, and then recaptured in subsequent years at Anglesea Golf Club and Serendip Sanctuary to confirm observations of reproductive state. The number of females subject to a GnRH challenge is given in parentheses. The number of mortalities in each treatment group, across the three sites, is included

Study site	Control	Deslorelin (9.4 mg)
Anglesea Golf Club		
Capture and treatment Feb.–Aug. 2008	23	24
Recapture		
Feb.–June 2009	10 (5)	11 (11)
Sep. 2009–Jan. 2010	4	10 (2)
Serendip Sanctuary		
Capture and treatment		
May–Aug. 2007	11	15
Jan.–July 2008	10	3
Recapture		
Jan.–July 2008	6	9 (5)
Jan.–July 2009	10 (3)	8
Plenty Gorge Parklands		
Capture and treatment		
Oct. 2007–Feb. 2008	12	11
Total		
Initial captures	56	53
Recaptures	30 (8)	38 (18)
Mortality	8	14

Australia, Brisbane, Qld, Australia) and a coloured PVC collar (Innova International, Melbourne, Vic., Australia).

We evaluated the contraceptive effect of the implants by recording the reproductive state of females in winter and spring until 2011. Because most young are born in summer, they cause a noticeable bulge in the pouch area by winter and are often directly visible through the pouch opening in spring. Our observations were opportunistic (on any particular day, ~70% of treated females could be observed) and we conducted them using binoculars (12 × 32; Saxon, Melbourne, Vic., Australia) or a telescope (20–60 X; Kowa, Japan).

We recaptured as many females at Anglesea and Serendip in subsequent years as possible (Table 2). We administered a GnRH challenge (see below) to these females, to test whether chronic exposure to deslorelin desensitises the pituitary gland to GnRH (Scheele *et al.* 1996). We palpated the implants to confirm their presence, and validated observations of reproductive state by inspecting the pouch. If a young was present, we took the body measurements described above to estimate date of birth (Poole *et al.* 1982). We did not attempt to recapture females at Plenty Gorge where, because of the terrain, they were very difficult to capture.

Implants

Females in the deslorelin group received subcutaneous implants once between the shoulder blades as previously described (Herbert *et al.* 2004). The implants contained 4.7 mg deslorelin ($(D-Trp^6-Pro^9-des-gly^{10}-GnRH$ ethylamide) and measured 12.5 × 2.3 mm (Suprelorin, batch SBL035B; Peptech Animal Health, Sydney, NSW, Australia), with a release rate of 1 $\mu g day^{-1}$ for at least 1 year (Trigg *et al.* 2001). We inserted two implants (total dose 9.4 mg) into each female assigned to the deslorelin group, by using a preloaded sterile hypodermic implanter with a 13-gauge needle, and carried out the same procedure for control females but without an implant. We closed the skin opening made by the implanter with veterinary skin adhesive (Vetbond; Therapon, Melbourne, Vic., Australia), dusted the implant site with antibiotic powder (Tricin; Jurox, Hunter Valley, NSW, Australia), and gave each female a prophylactic dose of antibiotic (Duplocillin, i.m., 0.1 mg kg⁻¹; Intervet, Bendigo, Vic., Australia) to reduce the risk of infection.

GnRH challenge

We conducted a GnRH challenge on eight control and 18 treated females between 6 and 23 months post-treatment at Anglesea and Serendip (Table 2). All control females except one had a pouch young at the time of challenge. In the deslorelin group, four females from Anglesea and one from Serendip had pouch young when they were challenged. Young of treated females were born 8.8 ± 1.2 (mean \pm s.e.) months post-treatment (Season 2 post-treatment) so were the result of the treatment waning, and the GnRH challenges were conducted 3.4 ± 1.1 (mean \pm s.e.) months after their birth.

We collected blood (2 mL) from the lateral tail vein by using a 21-gauge needle and 5-mL syringe while the female was immobilised with Zoletil 100 (see above). We collected the first sample at Time 0, removed the syringe containing the blood, left the needle in place, and injected 2 $\mu g kg^{-1}$ synthetic

GnRH (Fertagyl; Intervet, Sydney, NSW, Australia) in 0.9% sterile saline. We collected subsequent samples 15 and 30 min after GnRH injection. We immediately transferred blood to 5-mL heparinised vacutainer tubes (Becton Dickinson, Sydney, NSW, Australia), separated the plasma by centrifugation (1850g, 15 min), then stored the samples at $-20^{\circ}C$ until assayed.

LH assay

We measured plasma concentrations of LH by using a heterologous enzyme immunoassay developed for African elephants (*Loxodonta africana*; Graham *et al.* 2002). The assay used an anti-bovine LH monoclonal antibody (1:400 000, #518-B7; supplied by J. Roser, University of California, Davis, CA, USA), biotinylated ovine LH label (1:200 000; supplied by A. F. Parlow, NIDDK National Hormone and Peptide Program, Harbour UCLA Medical Centre, CA, USA) and NIH-bovine standards (1.95–1000 pg per 50 μL ; supplied by A. F. Parlow, NIDDK National Hormone and Peptide Program, Harbour UCLA Medical Centre, CA, USA). We assayed all samples and standards in duplicate.

We validated the enzyme immunoassay for eastern grey kangaroos in terms of the bovine standard protein by demonstrating parallelism between serially diluted samples and the respective standard curve. The sensitivity of the assay was 0.08 ng mL⁻¹. We determined the recovery by measuring LH from pooled samples spiked with known concentrations of exogenous bovine LH that was 97% ($n=4$). The inter-assay coefficient of variation measured from pooled samples with high (4.21 ng mL⁻¹) and low (0.56 ng mL⁻¹) LH concentrations was 6.9% ($n=4$). The intra-assay variation calculated from three pooled samples (0.35 ng mL⁻¹, 0.52 ng mL⁻¹, 0.71 ng mL⁻¹) run three times (first two samples) or six times (third sample) on the same plate was 2.8%.

Reproductive tracts

We examined the reproductive tract of six treated females that died during the study from a range of causes, including vehicle collision (3), drowning (1), shot in a cull (1) and euthanasia as a result of injury (1). For comparison, we collected reproductive tracts from three untreated females with no pouch young in the breeding season (December 2008) at Woodlands Historic Park, 24 km from Plenty Gorge, and from three females with pouch young in the non-breeding season (May 2009) at Mt Rothwell Nature Reserve, 17 km from Serendip. We dissected the uteri, examined each one for presence of a fetus, but did not flush for blastocysts if no fetus was present because of the logistical constraints of the fieldwork. We fixed the ovaries in 10% neutral-buffered formalin that were then embedded in paraffin wax, serially sectioned at 6 μm , and stained with Lillie-Mayer haematoxylin (Cat # AHLM; Australian Biostain, Traralgon, Vic., Australia) and eosin Y (1% solution, pH 5.3; Cat # AEY; Australian Biostain) for histological examination. We measured the diameter of the largest follicle in each ovary by using a microscope and compared the size of follicles in treated and untreated females.

Data analysis

We tested for differences in the body mass and crus length of females at the time of treatment among sites by using a one-way ANOVA, followed by a *post hoc* comparison using Tukey's HSD test. We used an independent samples *t*-test to test for differences in body mass and crus length between the groups (control, deslorelin) at each site. We performed a chi-square test for independence (with Yates' continuity correction) to compare the frequency of females carrying pouch young at the time of treatment among sites and then between the groups within sites. We calculated the birth date of pouch young from the mean of three estimates from head, crus and pes length measured using the growth curves from Poole *et al.* (1982), then quantified date of birth as the number of days from the first month of births (1 July = Day 1). We examined differences in birth dates among sites at the time of treatment by using one-way ANOVA followed by Tukey's HSD *post hoc* tests, and between groups at each site by using independent samples *t*-tests. We also compared the birth date of young from treated females that had resumed breeding with those born to control females using an independent-samples *t*-test.

A generalised linear mixed model with kangaroo ID as a random effect would have been optimal to test the effect of deslorelin, time since treatment and study site on reproduction, because it accounts for repeated measures. However, small sample sizes and values of 0 and 100% reproductive success in treated females at Anglesea and Serendip in Year 1, and in control females at Plenty Gorge, respectively, resulted in large parameter estimates with even larger standard errors in the output of the model. Therefore, we analysed the effects of deslorelin and study site by using logistic regression separately for each year to avoid pseudoreplication. Year of treatment was standardised so that all females were treated in Year 1. We then tested the effect of time since treatment on reproductive success, by using a generalised linear mixed model with a binomial error distribution and kangaroo ID as a random effect, excluding control females. We excluded control females because the strong effect of treatment had already been established (analysis above) and to remove the values of 100% reproductive success, which affected the fit of the model. We also excluded females at Plenty Gorge in the first year because the analysis above demonstrated that the group was an outlier and problems were encountered (large parameter estimates and standard errors) when trying to fit models with these data included.

We tested for a difference in the plasma LH concentration of control and treated females at each sampling period (0, 15, 30 min) of a GnRH challenge by using a Kruskal–Wallis test. We then compared the LH concentrations of treated females with and without pouch young, using a Kruskal–Wallis test. We carried out all statistical analyses using SPSS version 17.0 and R Version 2.13.0 and report mean \pm standard error.

Results

Animals at time of treatment

Body mass of females differed among sites at the time of treatment ($F_{2,89} = 15.35$, $P < 0.01$); females were heavier at

Plenty Gorge (31.9 ± 0.8 kg) than at Anglesea (26.1 ± 0.7 kg) and Serendip (28.0 ± 0.6 kg; Tukey's HSD, $P < 0.05$), but there was no difference in crus (lower leg) length among the sites ($F_{2,89} = 0.26$, $P = 0.77$). There was no difference in body mass between the control and deslorelin groups at any site (Anglesea: $t_{36} = -0.02$, $P = 0.99$; Serendip: $t_{30} = -0.48$, $P = 0.64$; Plenty Gorge: $t_{18} = -1.25$, $P = 0.16$). There was no difference in crus length between the control and deslorelin groups at Anglesea and Serendip (Anglesea: $t_{36} = -0.64$, $P = 0.53$; Serendip: $t_{30} = -1.82$, $P = 0.08$), but deslorelin-treated females at Plenty Gorge had a longer leg than did control females ($t_{18} = -2.24$, $P = 0.04$).

At the time of treatment, most selected females at Anglesea (97%) and Plenty Gorge (95%) had pouch young, but there were fewer (49%) females with pouch young at Serendip at the start of the experiment ($\chi^2_1 = 29.96$, $P < 0.01$). There was no difference in the proportion of females with pouch young between control and deslorelin groups at any site (Anglesea: $\chi^2_1 = 1.08$, $P = 0.30$; Serendip: $\chi^2_1 = 2.53$, $P = 0.11$; Plenty Gorge: $\chi^2_1 = 1.05$, $P = 0.31$). Birth dates of young present at the start of treatment differed among sites ($F_{2,64} = 11.89$, $P \leq 0.01$); births occurred earlier in the season at Plenty Gorge (July to February, mean = 27 October, $n = 18$) than at Anglesea (October to May, mean = 5 January, $n = 35$) and Serendip (September to March, mean = 13 January, $n = 14$; Tukey's HSD, $P < 0.05$). There was no difference in birth date of young carried at the time of treatment between the groups (Anglesea: $t_{33} = 0.89$, $P = 0.39$; Serendip: $t_{12} = -0.44$, $P = 0.67$; Plenty Gorge: $t_{16} = 0.82$, $P = 0.42$). The age of young at the time of treatment did not differ among sites ($F_{2,65} = 3.14$, $P = 0.05$) because we captured females at Plenty Gorge earlier than at Anglesea and Serendip (Table 2). The age of young at treatment also did not differ between control and deslorelin groups at each site (Anglesea: $t_{33} = 0.33$, $P = 0.74$; Serendip: $t_{12} = -0.46$, $P = 0.65$; Plenty Gorge: $t_{16} = 0.73$, $P = 0.48$).

In total, 22 females died during the study (Tables 1, 2), including eight at Anglesea (control $n = 2$; deslorelin $n = 6$), 11 at Serendip (control $n = 6$; deslorelin: $n = 5$) and three at Plenty Gorge (all deslorelin). In addition, one treated female at Plenty Gorge disappeared in November 2008.

Reproductive success

In the first year, reproductive success was higher at Plenty Gorge than at Anglesea and Serendip, irrespective of the treatment group (Table 3); all control females had young at Plenty Gorge compared with 42% to 44% at Anglesea and Serendip, respectively, and 60% of treated females at Plenty Gorge had young in contrast to 0% at Anglesea and Serendip (Fig. 1). In the second and third years, reproductive success of control females was high and did not differ among sites, with 82–100% of females having pouch young (Table 3, Fig. 1). Females treated with deslorelin had lower reproductive success than did control females at all three sites in all 3 years after treatment (Table 3, Fig. 1), but the contraceptive effect decreased over time (Table 4); 43–57% of treated females were fertile in the third year at all of the sites (Fig. 1).

Table 3. Multiple logistic regressions indicating the effect of deslorelin treatment and study site on reproductive success of female eastern grey kangaroos by year post-treatment

Treatment (deslorelin, control); study site (Anglesea Golf Club, Serendip Sanctuary, Plenty Gorge Parklands). Reproductive success was measured by the presence of a pouch young. Testing the treatment × site interaction is equivalent to testing the adequacy of fit of the strictly additive model

Year (n)	Predictor	Deviance	d.f.	P (χ^2 -test)	Odds ratio: deslorelin vs control ^A
1 (92)	Treatment	32.10	1	<0.001	0.00
	Site	35.10	2	<0.001	
	Treatment × site	0	2	1.0	
2 (82)	Treatment	42.63	1	<0.001	0.02
	Site	3.57	2	0.17	
	Treatment × site	3.10	2	0.21	
3 (66)	Treatment	16.75	1	<0.001	0.08
	Site	1.42	2	0.49	
	Treatment × site	1.2	2	0.53	

^AEstimate of the odds ratio for having young, deslorelin versus control, from the strictly additive logistic regression model: $\text{logit}[\text{Pr}(\text{pouch young})] = \text{treatment} + \text{site}$.

Six treated females gave birth in the first year of treatment at Plenty Gorge (Fig. 1). At the time of treatment, these females had pouch young between 86 and 274 days old, which we removed, except one that was near the time of pouch exit. Thus, these females reproduced after their existing young were removed at treatment and the young born post-treatment survived to permanent pouch exit. Of these six females, one (ID 2) reproduced each year post-treatment, whereas the other five females did not reproduce in the second year of treatment.

Most (seven) of the nine treated females (seven at Anglesea, two at Serendip) that were contracepted in the first year of treatment, but had a pouch young in the second year, also had a young in the third year. The five treated females recaptured between February and June at Anglesea in the second year of treatment gave birth between December and March, similar to 10 control females (deslorelin mean: 5 January; control mean: 4 January; $t_{13} = -0.032$, $P = 0.10$).

GnRH challenge

There was no difference in LH concentration of control ($\chi^2_1 = 2.31$, $P = 0.13$) or treated females ($\chi^2_1 = 1.46$, $P = 0.23$) between the study sites across all sampling periods, so we pooled data from both study sites for further analyses. There was no difference in LH concentration of eight control and 18 treated females at Time 0, before injection of GnRH ($\chi^2_1 = 0.00$, $P = 1.00$; control: 0.544 ± 0.075 ng NIH-bovine LH mL⁻¹, deslorelin: 0.666 ± 0.127 ng mL⁻¹). Unlike treated females, control females responded to the GnRH injection with an increase in LH, peaking 15 min post-injection (Fig. 2). LH concentrations were higher in control than treated females both 15 min ($\chi^2_1 = 7.72$, $P < 0.01$; control: 0.952 ± 0.044 ng mL⁻¹, deslorelin: 0.714 ± 0.125 ng mL⁻¹) and 30 min ($\chi^2_1 = 6.53$, $P = 0.01$; control: 0.864 ± 0.049 ng mL⁻¹, deslorelin: 0.676 ± 0.119 ng mL⁻¹) post-

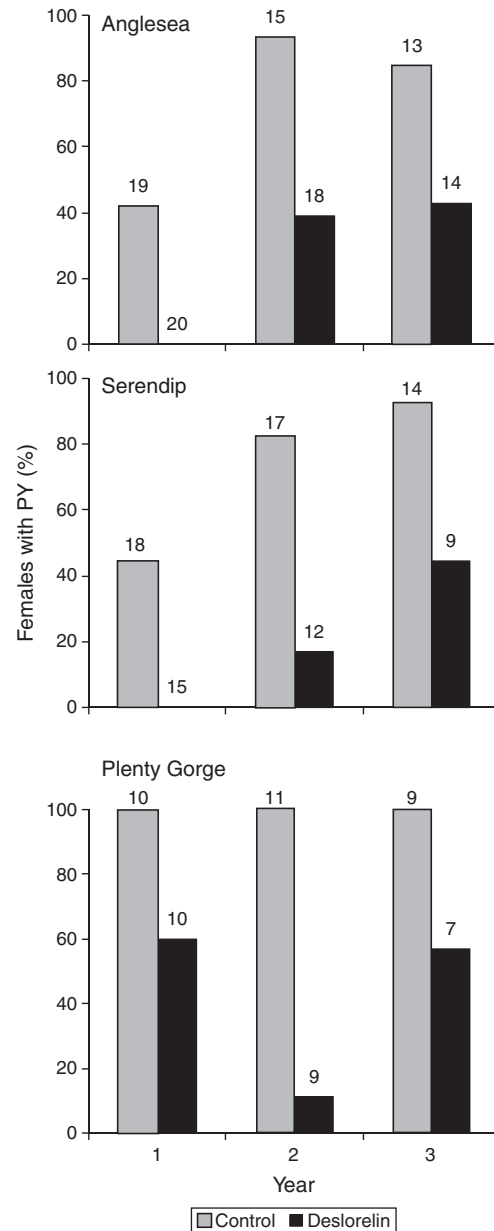


Fig. 1. Percentage of control and deslorelin-treated adult female eastern grey kangaroos observed with pouch young (PY) by year at Anglesea Golf Club, Serendip Sanctuary and Plenty Gorge Parklands in Victoria. All females were treated in Year 1 and small young were removed. To be successful in Year 1, the female had to conceive again after her young was removed at treatment or have an embryo in diapause at the time of treatment. It was not possible to observe all females each year; n values are given above the bars.

injection. There was no difference in LH concentration between treated females with and those without pouch young across all sampling periods (Time 0: $\chi^2_1 = 0.06$, $P = 0.81$; Time 15: $\chi^2_1 = 0.88$, $P = 0.35$; Time 30: $\chi^2_1 = 0.55$, $P = 0.46$). However, one of these five treated females with a pouch young responded to GnRH with an increase in LH similar to control females; LH concentration increased by 131% 15 min post-injection (Fig. 2).

Table 4. Generalised linear mixed model indicating the effect of time since deslorelin-treatment and study site on reproductive success of female eastern grey kangaroos

Kangaroo ID was included as a random effect. Study site (Anglesea Golf Club, Serendip Sanctuary, Plenty Gorge Parklands). Reproductive success was measured by the presence of a pouch young. Data from the first year at Plenty Gorge have been omitted from the model. Anglesea Golf Club is the reference level for study site. $n = 44$

Response variable	Predictor	Estimate	s.e.	d.f.	<i>P</i>
Females with pouch young	Intercept	-7.53	1.15	59	<0.001
	Site: Plenty Gorge	-0.56	1.27	51	0.66
	Site: Serendip	-0.99	1.17	51	0.40
	Year	2.68	0.40	59	<0.001

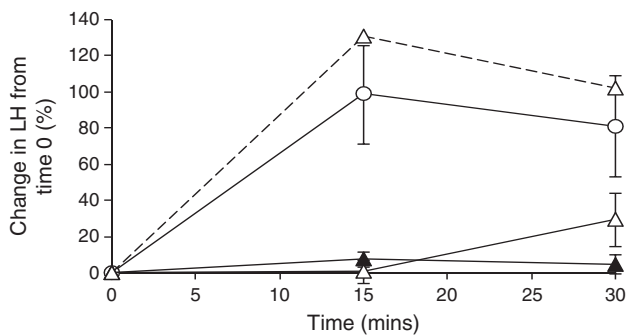


Fig. 2. Percentage change (mean \pm s.e.) in the concentration of plasma luteinising hormone (LH) from the time of injection of synthetic gonadotrophin-releasing hormone (GnRH; Time 0). Legend: control females (open circles, solid line; $n = 8$), deslorelin-treated females with pouch young (open triangles, solid line; $n = 4$), deslorelin-treated females without pouch young (solid triangles, solid line; $n = 13$). A single treated female with a pouch young that responded to the GnRH challenge is plotted separately (open triangles, dashed line). The GnRH challenges were conducted between 6 and 23 months post-implantation at Anglesea Golf Club and Serendip Sanctuary. All LH values were above the sensitivity of the assay (0.08 ng mL^{-1}).

This female was challenged 13.5 months post-treatment, which was midway in the range of time females were challenged post-treatment.

Follicular development

Of the three untreated females sampled in the breeding season, two had large follicles (diameter $>4 \text{ mm}$). The three untreated females sampled outside the breeding season had early antral follicles (diameter range $1.0\text{--}2.6 \text{ mm}$) and one female had a large follicle (diameter = 4 mm). The dominant follicles of untreated females appeared normal histologically, regardless of when they were sampled. In contrast, none of the 6 treated females had follicles $>2 \text{ mm}$ in diameter, and there was no evidence of an active corpus luteum. The 2-mm follicles in treated females showed the following signs of atresia: the granulosa cells had pulled away from the theca matrix, cellular debris and macrophages were in the antral space, there were few mitotic cells and more pycnotic nuclei than normal.

Discussion

Fertility control using long-acting deslorelin implants offers a non-lethal alternative for management of overabundant eastern grey kangaroos. These implants inhibited reproduction by desensitising the pituitary gland to GnRH, whereas they did not inhibit development of a blastocyst in diapause, pregnancy or lactation in females that conceived before treatment. Once the effect of deslorelin waned, treated females had the same reproductive rate as did control females, despite evidence of a continued depression of the hypothalamic–pituitary–gonadal axis.

Deslorelin implants significantly reduced reproductive output over three breeding seasons, but their efficacy decreased with time. The contraceptive effect of the implants lasted longer in the present study of free-ranging kangaroos, which showed a more gradual return to fertility, than in previous captive trials where fertility rose from 30% in the second year of treatment to 88% in the third year (Herbert *et al.* 2010). In contrast, fertility rose by only 26% on average over the three sites between the second and third years of treatment in the present field trial. Only between 43% and 57% of females had pouch young in the third year (Fig. 1). The largest increase in the proportion of fertile females between the second and third year of treatment (11–57%) was at Plenty Gorge, the site with environmental conditions most resembling optimal captive conditions. It appears that the environmental pressures experienced by free-ranging animals, compared with captive animals, reduce the number of females capable of breeding in a given year, thus extending the contraceptive effect of the implant.

At Plenty Gorge, six of the 10 treated females had pouch young in the first year of treatment. One continued to breed throughout the study, suggesting that her implant was lost or defective, but we were unable to recapture her to confirm whether the implant had stayed in place. Alternatively, she may have been genetically non-responsive to deslorelin (Herbert *et al.* 2006; Lohr *et al.* 2009). The remaining five females did not have young in the second year of the study, so it is most likely that the births in Year 1 arose from a blastocyst in diapause conceived before treatment. Female eastern grey kangaroos have been reported to enter oestrus and mate when the current pouch young is at least 100 days old (Clark and Poole 1967). With pouch young estimated between 86 and 274 days old, the females at Plenty Gorge may have had a quiescent blastocyst when treated, which was then reactivated by removing the current young. A lack of effect of deslorelin implants on reactivation of the blastocyst, or pregnancy or birth, is consistent with previous studies showing that gonadotrophins are not needed for reactivation from diapause or maintenance of pregnancy in tamar wallabies (reviewed in Tyndale-Biscoe and Renfree 1987). Moreover, most (5 out of 6) young born to treated females at Plenty Gorge survived to pouch exit, suggesting that deslorelin implants do not affect lactation or maternal behaviour required to rear young to pouch exit. The alternative is that their births might have resulted from a mating within the acute phase of treatment, when the pituitary gland had not yet been desensitised to GnRH, and LH and FSH are elevated (Gong *et al.* 1996). Female eastern grey kangaroos can enter oestrus between 6 and 16 days after the removal of pouch young (Poole and Catling 1974). However, Woodward *et al.* (2006) and Herbert *et al.*

(2006) found that female eastern grey kangaroos exhibited behavioural oestrus after treatment with deslorelin and removal of their young, but their matings were unsuccessful. Furthermore, the acute phase of GnRH agonist treatment is between 4 and 7 days in other species (Fraser and Lunn 1989; Winslow *et al.* 1992; D'Occhio *et al.* 1996), suggesting that induced gonadotrophin surge after treatment would be too soon for sufficient follicular development, causing luteinisation without ovulation (Macmillan and Thatcher 1991; Woodward *et al.* 2006).

Two lines of evidence suggest that continual treatment with deslorelin desensitises the pituitary gland to GnRH, thus suppressing the release of gonadotrophins (FSH and LH) in eastern grey kangaroos, as it does in other species. First, females implanted with deslorelin did not respond to exogenous GnRH with an LH surge, unlike control females did. Second, follicular growth was restricted to ≤ 2 mm in all six implanted females autopsied, and follicles that reached 2 mm in diameter showed signs of atresia, unlike for untreated females. Chronic exposure to a GnRH agonist reduced LH concentrations to basal levels in female tammar wallabies (Herbert *et al.* 2004, 2005), cows (Gong *et al.* 1996) and marmosets (*Callithrix jacchus jacchus*; Lunn *et al.* 1992), and this was coupled with suppressed follicular development. Initial follicular growth but a lack of maturation in these species, and in studies in which the pituitary gland is removed (Hearn 1974; Panyaniti *et al.* 1985; Driancourt *et al.* 1987), suggest that follicular growth is initially independent of gonadotrophins, whereas the final stages of development require FSH and LH.

As the effect of deslorelin waned, females appeared to resume breeding as normal; seven of nine implanted females that had a pouch young in the second year of the study also reproduced in the third year. There was also no difference in the timing of birth between control and implanted females. However, some depression of the hypothalamic–pituitary–gonadal axis continued after the contraceptive effect of deslorelin diminished. Deslorelin inhibited the response of four out of five females to a GnRH challenge, despite their mating and giving birth months after treatment. GnRH agonists act by suppressing the release of gonadotrophins rather than downregulating the ovaries, as D'Occhio *et al.* (1997) demonstrated by inducing follicular development and ovulation in deslorelin-treated heifers with exogenous FSH and LH. Therefore, sufficient FSH and LH concentrations circulating in some individuals may support ovarian activity in deslorelin-treated females, even though the pituitary gland cannot respond to GnRH. Further investigation is required to determine the nature and duration of this effect.

Management implications

There are many contexts in which hormonal contraception via slow-release implants can be used to manage kangaroos. To achieve an acceptable level of population control, managers must determine the appropriate proportion of females to treat (Todd *et al.* 2008). They must also consider four context-dependent factors when selecting an implant. First, the implant must have an appropriate duration of efficacy. Although some

small populations or captive-breeding facilities may require implants with a relatively short duration to maximise effective population size (Lande and Barrowclough 1987), most management scenarios require long-lasting contraceptives. Female eastern grey kangaroos typically breed annually over a 10-year period (Quin 1989), so must be infertile for a large proportion of this time for effective population control (Coulson *et al.* 2008). Therefore, population management in peri-urban areas, such as Plenty Gorge and Serendip, with large populations of kangaroos that are difficult to capture demands a long-lasting contraceptive implant.

Second, the mode of implant delivery is critical to the efficiency of a management program; acceptable population control often requires large numbers of females to be treated and costs for personnel limit the time that can be spent on a program. Inserting deslorelin implants with a hypodermic implanter is rapid, requires minimal training and can be performed at the point of capture. The small size of deslorelin implants also increases the potential for remote delivery by darting or pole syringe, which would eliminate the stress of animal capture and reduce management time (Kirkpatrick *et al.* 1990). At sites with a resident population of kangaroos that are readily approached by humans, such as Anglesea Golf Club, females could be implanted with a pole syringe every 2 or 3 years.

Third, the timing of implant placement is critical for maximising the duration of contraception. Since deslorelin does not inhibit pregnancy or reactivation of a blastocyst in diapause, fertilisation before treatment will result in birth in the first year. Eastern grey kangaroos have a peak in births from December to March (Poole 1983), so between April and May would be the optimal time to implant females. At this time, most females will have given birth, minimising the probability of pregnancy at the time of treatment. The young will also be small (mostly <100 days), reducing the likelihood that females mated and have a blastocyst in diapause. This is particularly important at sites that provide high-quality habitat because eastern grey kangaroos in good environmental conditions are more likely to mate earlier in lactation (Poole 1973); we observed a higher prevalence of diapause at Plenty Gorge, which had the highest pasture biomass, lowest kangaroo density and heaviest females of the three study sites (Table 1).

Fourth, the side-effects of hormonal implants must also be considered (Nettles 1997; Gray and Cameron 2010). Deslorelin does not appear to interrupt lactation, so is safe for females that are pregnant or carrying a dormant blastocyst. There was also no change in the timing of births in treated females that resumed breeding in the second and third years of the study, so treatment is unlikely to alter survival of young by shifting the timing of peak energetic demand, at the end of pouch life (Cripps *et al.* 2011), to less productive seasons.

In terms of the management requirements for eastern grey kangaroos in peri-urban areas, deslorelin has a short duration of efficacy; it does not control fertility for the length of a female's reproductive life. However, the ease of implant insertion and potential for remote delivery may outweigh its abbreviated efficacy at sites where kangaroos can be readily approached by humans and re-treated. The timing of implantation is critical for deslorelin, particularly at sites that provide high-quality habitat,

because it does not inhibit diapause, pregnancy or lactation. Finally, the absence of any adverse responses to deslorelin suggests that it is safe to use in a management context; however, further research is required to confirm a lack of pathological effects or behavioural impacts in free-ranging populations.

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