Long-term effects of deslorelin implants on reproduction in the female tammar wallaby (*Macropus eugenii*)

C A Herbert, T E Trigg[1], M B Renfree[2], G Shaw[2], D C Eckery[3] and D W Cooper

Department of Biological Sciences, Macquarie University, North Ryde, New South Wales, 2109, Australia, [1]Peptech Animal Health, Locked Bag No. 2053, North Ryde, New South Wales, 2113, Australia, [2]Department of Zoology, University of Melbourne, Victoria, 3010, Australia and [3]AgResearch, Wallaceville Animal Research Centre, Private Bag 40063, Upper Hutt, New Zealand

Correspondence should be addressed to C A Herbert; Email: cherbert@rna.bio.mq.edu.au

Abstract

The contraceptive and endocrine effects of long-term treatment with implants containing the GnRH agonist deslorelin were investigated in female tammar wallabies (*Macropus eugenii*). Fertility was successfully inhibited for 515 ± 87 days after treatment with a 5 mg deslorelin implant (*n* = 7), while control animals gave birth to their first young 159 ± 47 days after placebo implant administration (*n* = 8). The duration of contraception was highly variable, ranging from 344 to 761 days. The strict reproductive seasonality in the tammar wallaby was maintained once the implant had expired. This inhibition of reproduction was associated with a significant reduction in basal LH concentrations and a cessation of oestrous cycles, as evidenced by low progesterone concentrations. There was evidence to suggest that some aspect of either blastocyst survival, luteal reactivation, pregnancy or birth may be affected by deslorelin treatment in some animals. These results show that long-term inhibition of fertility in the female tammar wallaby is possible using slow-release deslorelin implants. The effects of deslorelin treatment were fully reversible and there was no evidence of negative side effects. Slow-release GnRH agonist implants may represent a practicable method for reproductive management of captive and semi-wild populations of marsupials.
Reproduction (2005) **129** 361–369

Introduction

Overabundant marsupial populations are recognised as a challenging problem for wildlife managers in Australia. The iconic status of these animals often places socio-political constraints on management options, with lethal methods facing increasing opposition (Adderton Herbert 2004). This has resulted in research and development of new techniques to manage populations by reducing their fertility (fertility control), such as the use of synthetic progestins (Nave *et al.* 2000, 2002, Middleton *et al.* 2003) and gonadotrophin-releasing hormone (GnRH) agonists (Herbert *et al.* 2004a).

Treatment with the GnRH agonist deslorelin can inhibit reproduction for periods of ≥1 year in a range of eutherian species, including dogs (Trigg *et al.* 2001, Junaidi *et al.* 2003), cats (Munson *et al.* 2001) and heifers (D'Occhio *et al.* 1996, 2002). GnRH agonist-induced suppression of ovarian cycles is typically characterised by an inhibition of follicular development and a reduction in plasma oestradiol, progesterone and follicle-stimulating hormone (FSH) concentrations. In most species, GnRH agonist treatment results in an inhibition of pulsatile luteinising

hormone (LH) release. This may be accompanied by a reduction or maintenance of basal LH concentrations. Basal LH concentrations are reduced in women (Shaw *et al.* 1985) and macaques (Fraser & Sandow 1985), while in marmosets (Lunn *et al.* 1992) and heifers (Gong *et al.* 1995, Bergfeld *et al.* 1996) basal LH concentrations remain unchanged. Despite the variable effects on basal LH, the preovulatory LH surge appears to be universally inhibited, as is ovulation. Preliminary trials on a model macropodid species, the tammar wallaby (*Macropus eugenii*), have demonstrated that implants containing the GnRH agonist deslorelin inhibit follicular development and post-partum oestrus (Herbert *et al.* 2004a). The next phase of the investigation is to study the long-term effects on reproductive success and endocrine parameters.

The tammar wallaby is a monovular, polyoestrous species (Tyndale-Biscoe & Renfree 1987). Breeding is highly seasonal, with the majority of births occurring in late January and early February (Renfree & Tyndale-Biscoe 1973). Females mate within 1–2 h of giving birth (Rudd 1994) and the conceptus is held in embryonic diapause at the blastocyst stage. From the summer solstice to the winter solstice diapause is maintained by the suckling

stimulus of the pouch young (PY) (lactational quiescence) (Tyndale-Biscoe *et al.* 1974). If the young is lost or removed during this period the quiescent corpus luteum (CL) and blastocyst will reactivate (Renfree 1979, Renfree & Young 1979, Renfree *et al.* 1979) and birth and a new post-partum oestrus will occur approximately 26.4 ± 0.6 days later (Merchant 1979). If the young is removed or lost after the winter solstice (21 June in the Southern Hemisphere), reactivation of the CL and blastocyst is inhibited by photoperiod (seasonal quiescence) until after the summer solstice (Tyndale-Biscoe *et al.* 1974).

Hormonal profiles during pregnancy and the oestrous cycle have been well characterised in the tammar wallaby (Hinds & Tyndale-Biscoe 1982, Tyndale-Biscoe *et al.* 1983, Shaw & Renfree 1984). Basal progesterone concentrations during lactation and the first 5 days of the cycle are approximately 200 pg/ml. There is a transient peak of about 450 pg/ml lasting for 1–2 days between days 5 and 8, followed by a return to basal concentrations. From day 10 progesterone concentrations increase and remain around 500–800 pg/ml until the day of parturition, when concentrations return to basal levels (Hinds & Tyndale-Biscoe 1982, Harder *et al.* 1985) within 1–2 h of birth (Fletcher *et al.* 1990, Renfree *et al.* 1994, Shaw *et al.* 1996). The decline in plasma progesterone concentrations occurs more rapidly at the end of the pregnant cycle than the oestrous cycle (Tyndale-Biscoe *et al.* 1983, Hinds *et al.* 1990). Basal LH concentrations are low throughout the oestrous cycle with the exception of the preovulatory LH surge (Tyndale-Biscoe *et al.* 1983, Harder *et al.* 1985). Ovulation occurs approximately 24 h after the LH surge (Harder *et al.* 1985) or 43–60 h after birth (Renfree & Lewis 1996).

This study investigated the long-term effects of slow-release deslorelin implants on fertility and endocrine parameters in female tammar wallabies. The specific aims were to monitor LH and progesterone concentrations, duration of contraception and condition (weight) of animals during treatment.

Materials and Methods

Animals

The tammar wallabies used in this experiment were captured on Kangaroo Island, South Australia and transferred to the Macquarie University Fauna Park facility in August 1999. For the duration of the experiment they were held in grassed outdoor yards and fed specially formulated 'kangaroo' cereal pellets (Gordon Specialty Stock Feed, Yanderra, N.S.W, Australia) with water freely available. Experimental animals were held in two adjacent yards, with a ratio of one male to every four females during the breeding season, with at least one male present at all other times throughout the experiment. All females selected for this experiment were greater than 2 years of age with an average weight of 5.26 ± 0.16 kg. At the start

of the experiment all were nursing PY and were presumed to be carrying a dormant blastocyst from a post-partum mating. All experimental work was approved by the Macquarie University Animal Ethics Committee under approval Number 99009 and the animal handling and husbandry was conducted in accordance with National Health and Medical Research Council of Australia guidelines (National Health and Medical Research Council 1997).

Experimental design

This experiment was conducted over a period of 16 months from mid-April 2000 until August 2001. The animals were synchronised by removal of PY (RPY) on 3 May 2000 and were randomly assigned to one of two groups ($n = 8$/group). The control group received a placebo implant and the treatment group received one 5 mg deslorelin implant each at the time of RPY. The animals were monitored for the following 65 weeks. They were caught once every week during the breeding season (defined in this case as 1 January–31 June) and the pouch was examined for the presence of a neonate and the urogenital sinus for a copulatory plug. Any PY that were found were removed so that the treated animals were compared with control animals that were cycling rather than in lactational quiescence. Blood samples were collected weekly during the breeding season and fortnightly during the non-breeding season for measurement of progesterone and LH concentrations. Three blood samples were collected at weekly intervals before implant administration. Animals were weighed on each sampling occasion using hanging scales (Salter model 235 6S; max. = 10 kg, d = 0.05 kg).

During the third breeding season (January–June 2002) pouches were checked for the presence of young once every 2–4 weeks to determine the duration of contraception and the reversibility of treatment. The age of any PY found during this period was determined by measuring the head length with vernier callipers and calculating the age from tammar wallaby PY growth tables (Poole *et al.* 1991).

GnRH agonist implant

The GnRH agonist deslorelin (D-Trp[6]-Pro[9]-des-gly[10]-GnRH ethylamide) was formulated into implants that contained 5 mg deslorelin (Suprelorin, Batch DR027A; Peptech Animal Health Pty Ltd, North Ryde, Australia) as previously described (Trigg *et al.* 2001). In a real-time dissolution system the release of deslorelin was >1 μg/day for periods of >1 year (Trigg *et al.* 2001). The *in vivo* release rate in tammar wallabies has not been determined. This dose was previously found to be effective at suppressing post-partum mating and follicular development in tammar wallabies (Herbert *et al.* 2004a). Implants were placed s.c. between the shoulder blades using a single-use commercial implanting device sterilised by e-beam radiation. The

injection site was sealed with a veterinary tissue adhesive (Vetbond; 3M Animal Care Products, St Paul, MN, USA). The dimensions of a 5 mg implant were 2.3 mm in width and 12.5 mm in length.

Blood sampling

Blood was collected from the lateral tail vein of conscious animals between 07:30 and 10:00 h using a 21 gauge winged infusion set (Surflo; Terumo Corporation Macquarie Park, N.S.W., Australia) and a 5 ml syringe. Blood was transferred immediately into heparinised blood collection tubes (Vacuette, lithium heparin; Greiner Labortechnik, Kremsmuenter, Austria) and held on ice until centrifugation. The plasma was separated and stored in duplicate aliquots at $-20\,^\circ$C until assayed to determine the concentrations of LH and progesterone.

Hormone assays

LH assay

Plasma LH concentrations were determined using the method of Moore *et al.* (1997) validated for the tammar wallaby (Herbert *et al.* 2004*b*). The assay used antiserum raised in rabbits against ovine LH (Wa-R oLH) and purified possum LH as the standard (AgResearch, Wallaceville, New Zealand). There was no cross-reactivity with possum FSH (Moore *et al.* 1997). All samples from individual animals were run in the same assay to reduce variability. The assay sensitivity was 0.1 ng possum LH/ml plasma. The inter-assay coefficients of variation calculated for three quality control pools containing 0.26 ± 0.02, 1.01 ± 0.06 and 5.61 ± 0.56 ng/ml (means±S.D.) were 9.0, 6.3 and 10.1% respectively. The intra-assay coefficients of variation for the same pools were 7.7, 4.4 and 8.7% respectively.

Progesterone assay

Plasma progesterone concentrations were determined using the method of Renfree *et al.* (1994). Plasma samples (800 µl) were extracted using ethyl acetate (8 ml). The assay used antiserum No. 9817 (Bioquest, North Ryde, NSW, Australia) raised in sheep against progesterone-11α-hemisuccinate conjugated to human serum albumin. All samples from individual animals were run in the same assay to reduce variability. The efficiency of extraction was 81% and the assay sensitivity was 20 pg progesterone/tube. The intra-assay coefficients of variation calculated for two quality control pools containing 278.5 ± 29.4 and 873.5 ± 71.8 pg/ml (means±S.D.) were 10.5 and 8.2% respectively. The inter-assay coefficients of variation for two pools containing 262.2 ± 46.3 and 608.3 ± 103.6 pg/ml were 17.6 and 17.0% respectively.

Statistical analyses

Data for animal weight and LH concentrations over time were analysed by ANOVA using the general linear model repeated-measures procedure of SPSS, the model being $y =$ treatment, time, treatment × time interaction, with time as the repeated factor. Plasma progesterone concentrations ≥ 500 pg/ml were taken as indicative of a functional CL (Hinds & Tyndale-Biscoe 1982, Tyndale-Biscoe *et al.* 1983, Renfree *et al.* 1994, Shaw *et al.* 1996). Progesterone concentrations were used to determine the number of oestrous/pregnant cycles for each animal (following Hinds & Tyndale-Biscoe 1982, Renfree *et al.* 1994). The duration of contraception and the number of oestrous cycles and PY was compared for treated and control animals using Student's *t*-tests. Where necessary, data were transformed to $\log_{10}$ before analysis to improve homogeneity of variance. Results are presented as untransformed arithmetic means ± S.E.M. and reported as significant at $P < 0.05$.

Results

Live weight

The weights for each group were similar at the onset of treatment (control, 5.36 ± 0.22 kg; treated, 5.16 ± 0.23 kg; $P > 0.05$). There was no significant difference in weight between the two groups during the treatment period ($P > 0.05$). There was a significant change in weight over time ($P < 0.001$) but there was no significant treatment × time interaction ($P > 0.05$), suggesting that any changes over time were not significantly different between the two groups and were unlikely to be the result of treatment.

Mortality

Throughout the course of the experiment both control and treated animals appeared to maintain good health, based on the weights of the animals discussed above. During the first 15 months one treated and one control animal died 90 and 354 days after treatment respectively. Both animals had infections consistent with a common ailment in macropodid marsupials referred to as 'lumpy jaw'. Lumpy jaw is usually a fatal disease of captive macropodids and is thought to be caused by infection of jaw and mouth lesions with *Fusobacterium necrophorum* (Blanden *et al.* 1987). The death of the treated animal did not appear to be related to deslorelin treatment in any way. Results from this animal have not been included in the analyses because of the short time it was in the study and the unknown period of illness before symptoms were recognised. Results from the control animal have not been included in long-term repeated-measures ANOVA procedures due to the requirement for an equal number of observations for all animals. However, the data on number of PY have been included in the analysis because the animal was breeding until close to the time of death and data had been collected for a significant period of time.

Births

All control and treated females were synchronised by RPY at the time of implant administration, thus demonstrating their fertility at the start of the experiment. The first PY were observed in control and treated animals 159 ± 47 and 515 ± 87 days after implant administration and RPY respectively (Table 1). Four control females gave birth during the remainder of the 2000 breeding season and one female had an oestrous cycle. The remaining three control animals showed no evidence of pregnancy/oestrous cycles during this period. No treated animals gave birth during the remainder of the 2000 breeding season, although two animals underwent a pregnancy/oestrous cycle but no PY were detected.

In the 2001 breeding season control animals gave birth to significantly more young than treated animals. At the beginning of this season, seven of eight control animals gave birth within the expected time-frame (i.e. before mid-March). One control female (C2) did not give birth but underwent one oestrous cycle within this period (Table 1). Conversely, only one treated animal (T1) gave birth within the expected time-frame, but two other females cycled within this period. The timing of birth for animal T1 coincided with the first birth of the 2001 breeding season for five control animals (Table 1), presumably as a result of the reactivation of blastocysts at the start of the breeding season. After RPY and administration of a second implant, this female did not give birth again until the 2002 breeding season (6 March 2002).

Contraception was reversible in six of the seven treated animals, with PY born between 344 and 761 days post-treatment (Table 1). In one animal (T3) this birth was preceded by evidence of two matings (copulatory plugs), approximately 1 and 2 months before the first observation of a PY. There was also evidence to suggest that another animal (T2) had undergone more than one cycle before conceiving and giving birth.

Evidence of blastocyst reactivation

After RPY at the beginning of May, it is expected that females should subsequently give birth within approximately 26 days, or at the beginning of the next breeding season (late January/early February). Based on progesterone concentrations and the detection of neonates (Table 1), there was evidence of successful blastocyst reactivation and subsequent birth in at least five of eight control animals (C1, C2, C3, C5 and C6). Two of the remaining animals (C7 and C8) underwent non-pregnant cycles, one at the end of 2000 breeding season and the other at the beginning of the 2001 breeding season, while the remaining animal (C4) either gave birth after a longer pregnancy following blastocyst reactivation or had one non-pregnant cycle before pregnancy and birth in June 2000 (Table 1).

Only one treated animal (T1, discussed above) showed evidence of successful blastocyst reactivation. Two treated animals (T4 and T6) showed evidence of a non-pregnant

cycle after treatment in 2000 and two females (T2 and T3) had a non-pregnant cycle at the beginning of the 2001 breeding season. The remaining two females showed no evidence of cycles during the period of expected blastocyst reactivation.

Plasma LH and progesterone

Mean plasma LH concentrations

Before the onset of treatment there were no significant differences in the concentrations of LH between the two groups ($P > 0.05$), although the concentrations tended to be higher in treated than control animals (control, 0.23 ± 0.05 ng/ml; treated, 0.55 ± 0.11 ng/ml). During the non-breeding season, from week 5 to week 30 post-treatment, plasma LH concentrations were significantly higher in control animals (control, 0.37 ± 0.03 ng/ml; treated, 0.19 ± 0.01 ng/ml; $P < 0.05$ for treatment and time), although there was no significant treatment × time interaction ($P > 0.05$, Fig. 1). In addition there appeared to be seasonal fluctuations in control animals, with plasma LH concentrations significantly higher during the non-breeding season (non-breeding season, 0.40 ± 0.03 ng/ml; breeding season, 0.19 ± 0.02 ng/ml; $P < 0.01$).

Plasma progesterone concentrations

Mean concentrations of progesterone were not significantly different between treated and control animals during the period of lactational quiescence before treatment (control, 320 ± 17 pg/ml; treated, 307 ± 24 pg/ml; $P > 0.05$). These concentrations were slightly higher than those measured in two ovariectomised animals (252 and 262 pg/ml). Progesterone concentrations remained low in treated animals, as reflected in the observation that control animals had significantly more pregnancies/oestrous cycles than treated animals (Table 1).

Discussion

This study has demonstrated that treatment with a slow-release deslorelin implant can inhibit reproduction for extended periods of time in female tammar wallabies. In six out of seven animals there was no evidence of successful reproduction for at least 344 days after implant administration. This minimum duration of contraception was very similar to the duration of 340 and 359 days previously recorded for two animals in a smaller trial (Herbert *et al.* 2004a). Inhibition of reproductive activity in the current study was associated with a reduction in peripheral plasma LH and progesterone concentrations. These effects were fully reversible. The general health of tammars was maintained during treatment, with no negative side effects apparent.

LH concentrations in treated animals were lower and less variable than in control animals for a period of 30 weeks. The sustained decline in plasma LH concentrations suggests that desensitisation of pituitary gonadotrophs,

Table 1 Summary of reproductive parameters for control and deslorelin-treated female tammar wallabies during the year 2000 and 2001 breeding seasons after RPY and treatment on 3 May 2000.

	Year 2000 breeding season				Year 2001 breeding season				Birth within expected range[^]	Time to first detected PY
	1st PY	1st cycle[#]	No. PY	No. cycles[#]	1st PY	1st cycle[#]	No. PY	No. cycles[#]		
Control										
C1	30 May	30 May	1	1	3 Mar	1 Feb	5	5	Y	27
C2	30 May	30 May	1	1	—	14 Mar	0	1	N	27
C3	6 Jun	6 Jun	2	2	31 Jan	31 Jan	3	5	Y	34
C4	19 Jun	19 Jun	2	2	1 Feb	1 Feb	4	5	Y	47
C5	—	—	0	0	31 Jan	31 Jan	3	4	Y	273
C6	—	—	0	0	1 Feb	1 Feb	4	5	Y	274
C7	—	5 Jul	0	1	7 Feb	7 Feb	2	3	Y	280
C8	—	—	0	0	5 Mar	1 Feb	2	4	Y	306
Mean			0.75 ± 0.31	1.00 ± 0.27			2.88 ± 0.55	4.0 ± 0.5		159 ± 47
Treated										
T1	—	—	0	0	1 Feb	1 Feb	1	1	Y	274, 398[^A]
T2	—	—	0	0	12 Apr	7 Feb	3	5	N	344
T3	—	—	0	0	25 Apr	28 Feb	1	3	N	357
T4	—	6 Jun	0	1	2 May	2 May	1	1	N	364
T5	—	—	0	0	—	—	0	0	N	705
T6	—	6 Jun	0	1	—	20 Jun	0	1	N	761
T7	—	—	0	0	—	—	0	0	N	>802[^B]
Mean			0*	0.29 ± 0.18*			0.86 ± 0.40***	1.57 ± 0.67*		515 ± 87**

[^A]Days after 2nd implant; [^B]the animal died at this time; *$P < 0.05$; **$P < 0.01$; ***$P < 0.005$.
[#] Number of pregnancies or oestrous cycles defined by elevated progesterone concentrations. The date given represents the end of the oestrous/pregnant cycle.
[^] The expected range is within a 2 week period at the end of January and beginning of February, or, if a blastocyst is not present the animal will undergo a non-pregnant cycle and then give birth by mid-March.
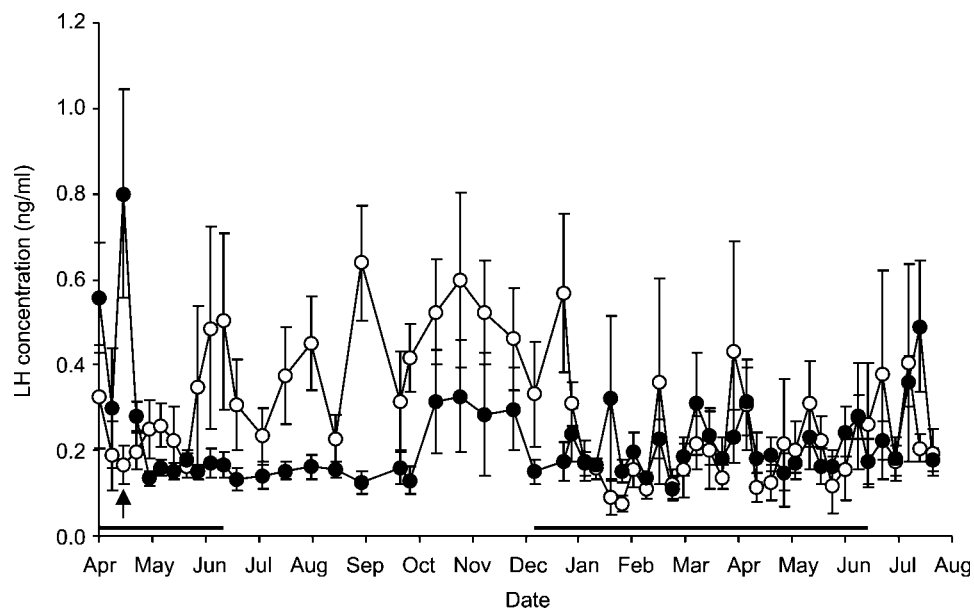
Figure 1 Plasma concentrations of LH (ng possum LH/ml) in control (○, *n* = 8) and deslorelin-treated (●, *n* = 7) female tammar wallabies. ↑, time of implant administration. Horizontal bar represents the period of the breeding season. Values are means± s.e.m.

with a resultant reduction in LH release, is a primary mechanism of action of deslorelin in the female tammar. The lower variation in mean LH concentrations in treated animals suggests there may be an absence of pulsatile LH release, as has been demonstrated in the ewe (McNeilly & Fraser 1987) and heifer (Gong *et al.* 1996). In women, GnRH agonist treatment is associated with a reduction in LH pulse amplitude and frequency (Shaw *et al.* 1985).

In control animals there was a significant rise in basal LH concentrations during the non-breeding season. A similar observation was made by Horn and Tyndale-Biscoe (unpublished observations in Tyndale-Biscoe & Renfree 1987). This seasonal variation is apparently not related to any change in the pituitary sensitivity to GnRH, as observed in the ewe (Goodman *et al.* 1982), or to storage of LH in the pituitary as the duration and amplitude of LH response to GnRH does not vary throughout the year (Tyndale-Biscoe & Renfree 1987). Numerous studies have also demonstrated that there is no seasonal change in ovarian–pituitary feedback effects in response to oestradiol treatment (Horn *et al.* 1985) or ovariectomy (Evans *et al.* 1980, Tyndale-Biscoe & Hearn 1981, Horn *et al.* 1985, Hinds *et al.* 1992). Therefore, the seasonal variations in basal LH concentrations do not appear to be the result of a change in the pituitary's sensitivity to GnRH or oestradiol, nor in its storage of LH. The elevated LH concentrations in the non-breeding season may be the result of other factors acting on the pituitary, perhaps a pineal influence, but they do not appear to be important in the control of seasonal breeding (Tyndale-Biscoe & Renfree 1987).

Deslorelin treatment resulted in a cessation of oestrous cycles during treatment, as indicated by basal progesterone concentrations. This is supported by previous observations that treatment with deslorelin inhibited follicular development and oestrus in the female tammar (Herbert *et al.* 2004a). During deslorelin treatment progesterone

concentrations were only slightly higher than those measured in two ovariectomised animals. These basal progesterone concentrations were presumably of adrenal, rather than ovarian, origin, as proposed by Sernia *et al.* (1980) for ovariectomised animals. Extended periods of acyclicity during long-term GnRH agonist treatment have previously been reported in marmosets (Lunn *et al.* 1992) and heifers (D'Occhio *et al.* 1996). In marmosets, busere-lin treatment resulted in a gradual decline in progesterone concentrations, which then remained at low, follicular phase concentrations until 136 ± 18 days after treatment was initiated (Lunn *et al.* 1992). In heifers, progesterone concentrations were maintained at basal levels for 203 ± 28 and 170 ± 28 days during treatment with one or two deslorelin implants respectively, thus indicating the absence of a functional CL (D'Occhio *et al.* 1996). The inhibition of ovarian cycles, as evidenced by a reduction in plasma progesterone concentrations, therefore appears to be a common response to long-term GnRH agonist treatment. This is presumably the result of down-regulation of GnRH receptors on pituitary gonadotrophs and desensitisation of the pituitary gland to endogenous GnRH (D'Occhio *et al.* 2000). These processes may suppress FSH concentrations below the threshold required for follicular development (Fraser 1993). If FSH concentrations are sufficient to maintain some degree of follicular development, as has been suggested in heifers, the pituitary appears to be desensitised to the extent that it cannot respond to increasing oestradiol concentrations with a preovulatory LH surge (D'Occhio *et al.* 1996). The end result is a reduction in plasma progesterone concentrations, due to the absence of a functional CL.

The tammar wallaby CL appears to be autonomous once formed (Tyndale-Biscoe *et al.* 1974). Total hypophysectomy does not inhibit the normal growth and secretory functions of an already formed CL (Hearn 1974) and pas-

sive immunisation against GnRH does not affect CL and blastocyst reactivation after RPY, nor does it affect birth (Short *et al.* 1985). Given these findings it was expected that a large proportion of treated animals, which were presumed to be carrying dormant blastocysts, would give birth after RPY and deslorelin treatment. Because treatment occurred late in the breeding season, birth would be expected either within approximately 26 days or at the beginning of the next breeding season if the female had already become seasonally quiescent. Indeed, as reported in Herbert *et al.* (2004*a*), four out of five treated animals successfully gave birth after deslorelin treatment coupled with RPY in March (although one PY died). In the present study, quiescent blastocysts reactivated and resulted in successful birth in at least five control animals. The proportion of control animals that apparently did not have blastocysts (25 or 37.5%) was similar to previously reported figs of 17% for wild animals (Renfree & Tyndale-Biscoe 1973) and 23% for captive animals (Harder *et al.* 1984). In contrast to the results for control animals, successful blastocyst reactivation and birth appeared to occur in only one treated animal. Four other treated animals appeared to have one cycle immediately after deslorelin administration and RPY or at the beginning of the next breeding season, but no PY were detected. These animals either had a non-pregnant cycle or lost their young. As there is little difference between progesterone concentrations during the pregnant and non-pregnant (oestrous) cycle (Hinds & Tyndale-Biscoe 1982) it is impossible to discriminate between the two hypotheses with the sampling regime employed.

These results suggest that long-term GnRH agonist treatment may affect some aspect of either blastocyst survival, luteal reactivation, pregnancy or birth in the female tammar wallaby. There may be a difference in the effects of the agonist on luteal reactivation at different times of the year, as there was no significant difference in the number of treated vs control animals giving birth in a previous short-term trial (Herbert *et al.* 2004*a*). This may be similar to the seasonal variation observed after progesterone treatment in the tammar. The CL and blastocyst may reactivate when progesterone is administered in December, but this is not observed at other times of year (Renfree & Tyndale-Biscoe 1973). As animals in this study were treated late in the breeding season the blastocysts may have been exposed to deslorelin for periods in excess of 7 months. Therefore it is hard to distinguish between the potential effects of the duration of treatment and the time of year. It is also possible that blastocyst reactivation was delayed until after the inhibitory effects of deslorelin treatment had subsided, but in some animals there was evidence of at least one oestrous cycle before pregnancy. Treatment with deslorelin has previously been found to have a negative effect on pregnancy in dogs. Failure of pregnancies at about day 40 of gestation in two bitches was associated with low plasma progesterone concentrations, possibly as a result of regression of the CL due to low plasma concen-

trations of LH (Wright *et al.* 2001). However, LH receptors have not been detected on the tammar CL (Stewart & Tyndale-Biscoe 1982), and so the lower LH concentrations resulting from deslorelin treatment should have no effect. Currently we do not have sufficient data to confirm whether there is a relationship between deslorelin treatment and blastocyst survival/reactivation in the tammar wallaby. This requires further investigation.

One treated female (T1) gave birth at the beginning of the 2001 breeding season at the same time as many of the control animals, 70 days earlier than any of the other treated animals. There are three possible explanations for this female's response to treatment: (i) she may be a genetic non-responder, (ii) this birth may be the result of reactivation of a dormant blastocyst and not actually represent a return to fertility, as defined by follicular development, or (iii) she may have returned to fertility earlier than other animals. The first is unlikely because when this animal was given a second deslorelin implant there was a sustained decline in progesterone concentrations and the animal did not breed again until 398 days later. Given the timing of birth, the first young may have been a blastocyst reactivating at the start of the breeding season and may not have been the result of a fertile mating after deslorelin administration.

The duration of temporary infertility varied widely between animals, with the first observation of successful birth occurring between 274 and >802 days after deslorelin administration. Similar individual variation in response to the same deslorelin implants has been reported for domestic cats (Munson *et al.* 2001) and heifers (D'Occhio *et al.* 1996). D'Occhio *et al.* (1996) attributed this variation to variability among implants in the duration and amount of agonist released, as the return to oestrus was much less variable when implants were removed at 28 or 56 days after administration. However, they also pointed out that differences among individuals in sensitivity to the agonist could not be excluded. It is possible that the dose administered to tammar wallabies in the current trial was close to the threshold required to inhibit reproduction, resulting in variable responses between individual animals. The duration between treatment and the resumption of breeding, defined as the time to the first successful birth after treatment, is the time-frame which is of most interest to wildlife managers, as they are principally concerned with reducing the reproductive output of a population. However, it was interesting to note that in at least two animals there was evidence of multiple cycles before they gave birth after treatment. This may be the case in other animals, but may not have been detected because they did not breed until after the cessation of blood sampling. This suggests that some animals may undergo a number of unsuccessful cycles before they are capable of successfully breeding post-deslorelin treatment.

In summary, this study has demonstrated that long-term contraception can be achieved in female tammar wallabies using deslorelin implants. Fertility was inhibited for a

period of time equivalent to at least one breeding season in this group of animals with no apparent negative side effects. This demonstrates the potential of these implants to reversibly inhibit reproduction in macropodid marsupials. Best results in the field would be achieved if treatment was timed to coincide with the period of anoestrus in this species, and this is probably true of other marsupial species. The practicability of using these implants to control fertility of captive and semi-wild macropodid populations should now be tested. Further trials are warranted on overabundant species which require management, such as the eastern grey kangaroo, *M. giganteus*, and the koala, *Phascolarctos cinereus*, to determine if the results obtained in this 'model' marsupial reflect what happens in other species.

Acknowledgements

References

Adderton Herbert C 2004 Long-acting contraceptives: a new tool to manage overabundant kangaroo populations in nature reserves and urban areas. *Australian Mammalogy* **26** 67–74.

Bergfeld EG, **D'Occhio MJ & Kinder JE** 1996 Pituitary function, ovarian follicular growth, and plasma concentrations of 17 beta-estradiol and progesterone in prepubertal heifers during and after treatment with the luteinizing hormone-releasing hormone agonist deslorelin. *Biology of Reproduction* **54** 776–782.

Blanden DR, **Lewis PR & Ferrier GR** 1987 Vaccination against lumpy jaw and measurement of antibody response in wallabies (*Macropus eugenii*). *Veterinary Record* **121** 60–62.

D'Occhio MJ, **Aspden WJ & Whyte TR** 1996 Controlled, reversible suppression of estrous cycles in beef heifers and cows using agonists of gonadotrophin-releasing hormone. *Journal of Animal Science* **74** 218–225.

D'Occhio MJ, **Fordyce G**, **Whyte TR**, **Aspden WJ & Trigg TE** 2000 Reproductive responses of cattle to GnRH agonists. *Animal Reproduction Science* **60–61** 433–442.

D'Occhio MJ, **Fordyce G**, **Whyte TR**, **Jubb TF**, **Fitzpatrick LA & Cooper NJ** *et al.* 2002 Use of GnRH agonist implants for long-term suppression of fertility in extensively managed heifers and cows. *Animal Reproduction Science* **74** 151–162.

Evans SM, **Tyndale-Biscoe CH & Sutherland RL** 1980 Control of gonadotrophin secretion in the female tammar wallaby (*Macropus eugenii*). *Journal of Endocrinology* **86** 13–23.

Fletcher TP, **Shaw G & Renfree MB** 1990 Effects of bromocriptine at parturition in the tammar wallaby, *Macropus eugenii*. *Reproduction, Fertility, and Development* **2** 79–88.

Fraser HM 1993 GnRH analogues for contraception. *British Medical Bulletin* **49** 62–72.

Fraser HM & Sandow J 1985 Suppression of follicular maturation by infusion of a luteinizing hormone-releasing hormone agonist starting during the late luteal phase in the stumptailed macaque monkey. *Journal of Clinical Endocrinology and Metabolism* **60** 579–584.

Gong JG, **Bramley TA**, **Gutierrez CG**, **Peters AR & Webb R** 1995 Effects of chronic treatment with a gonadotrophin-releasing hormone agonist on peripheral concentrations of FSH and LH, and ovarian function in heifers. *Journal of Reproduction and Fertility* **105** 263–270.

Gong JG, **Campbell BK**, **Bramley TA**, **Gutierrez CG**, **Peters AR & Webb R** 1996 Suppression in the secretion of follicle-stimulating hormone and luteinizing hormone, and ovarian follicle development in heifers continuously infused with a gonadotropin-releasing hormone agonist. *Biology of Reproduction* **55** 68–74.

Goodman RL, **Bittman EL**, **Foster DL & Karsch FJ** 1982 Alterations in the control of luteinizing hormone pulse frequency underlie the seasonal variation in estradiol negative feedback in the ewe. *Biology of Reproduction* **27** 580–589.

Harder JD, **Hinds LA**, **Horn CA & Tyndale-Biscoe CH** 1984 Oestradiol in follicular fluid and in utero-ovarian venous and peripheral plasma during parturition and postpartum oestrus in the tammar, *Macropus eugenii*. *Journal of Reproduction and Fertility* **72** 551–558.

Harder JD, **Hinds LA**, **Horn CA & Tyndale-Biscoe CH** 1985 Effects of removal in late pregnancy of the corpus luteum, Graafian follicle or ovaries on plasma progesterone, oestradiol, LH, parturition and post-partum oestrus in the tammar wallaby, *Macropus eugenii*. *Journal of Reproduction and Fertility* **75** 449–459.

Hearn JP 1974 The pituitary gland and implantation in the tammar wallaby, *Macropus eugenii*. *Journal of Reproduction and Fertility* **39** 235–241.

Herbert CA, **Trigg TE & Cooper DW** 2004*a* Effect of deslorelin implants on follicular development, parturition and post-partum oestrus in the tammar wallaby (*Macropus eugenii*). *Reproduction* **127** 265–273.

Herbert CA, **Trigg TE**, **Renfree MB**, **Shaw G**, **Eckery DC & Cooper DW** 2004*b* Effects of a gonadotropin-releasing hormone agonist implant on reproduction in a male marsupial, *Macropus eugenii*. *Biology of Reproduction* **70** 1836–1842.

Hinds LA & Tyndale-Biscoe CH 1982 Plasma progesterone levels in the pregnant and non-pregnant tammar, *Macropus eugenii*. *Journal of Endocrinology* **93** 99–107.

Hinds LA, **Tyndale-Biscoe CH**, **Shaw G**, **Fletcher TP & Renfree MB** 1990 Effects of prostaglandin and prolactin on luteolysis and parturient behaviour in the non-pregnant tammar, *Macropus eugenii*. *Journal of Reproduction and Fertility* **88** 323–333.

Hinds LA, **Diggle PJ & Tyndale-Biscoe CH** 1992 Effects of the ovary, sucking stimulus and season on the pattern of LH and FSH release in the female tammar, *Macropus eugenii*. *Reproduction, Fertility, and Development* **4** 25–34.

Horn CA, **Fletcher TP & Carpenter S** 1985 Effects of oestradiol-17β on peripheral plasma concentrations of LH and FSH in ovariectomized tammars (*Macropus eugenii*). *Journal of Reproduction and Fertility* **73** 585–592.

Junaidi A, **Williamson PE**, **Cummins JM**, **Martin GB**, **Blackberry MA & Trigg TE** 2003 Use of a new drug delivery formulation of the gonadotrophin-releasing hormone analogue deslorelin for reversible long-term contraception in male dogs. *Reproduction Fertility, and Development* **15** 317–322.

Lunn SF, **Cowen GM**, **Morris KD & Fraser HM** 1992 Influence of the gonad on the degree of suppression induced by an LHRH agonist implant in the marmoset monkey. *Journal of Endocrinology* **132** 217–224.

McNeilly AS & Fraser HM 1987 Effect of gonadotrophin-releasing hormone agonist-induced suppression of LH and FSH on follicle growth and corpus luteum function in the ewe. *Journal of Endocrinology* **115** 273–282.

Merchant JC 1979 The effect of pregnancy on the interval between one oestrus and the next in the tammar wallaby, *Macropus eugenii. Journal of Reproduction and Fertility* **56** 459–463.

Middleton DR, **Walters B**, **Menkhorst P & Wright P** 2003 Fertility control in the koala, *Phascolarctos cinereus*: the impact of slow-release implants containing levonorgestrel or oestradiol on the production of pouch young. *Wildlife Research* **30** 207–212.

Moore LG, **Ng Chi W**, **Lun S**, **Lawrence SB**, **Heath DA & McNatty KP** 1997 Isolation, characterisation and radioimmunoassay of luteinizing hormone in the brushtail possum. *Reproduction, Fertility, and Development* **9** 419–425.

Munson L, **Bauman CS**, **Asa CS**, **Jochle W & Trigg TE** 2001 Efficacy of the GnRH analogue deslorelin for suppression of oestrous cycles in cats. *Journal of Reproduction and Fertility Supplement* **57** 269–273.

National Health and Medical Research Council 1997 *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes*, edn 6. Canberra, ACT: Australian Government Publishing Service.

Nave CD, **Shaw G**, **Short RV & Renfree MB** 2000 Contraceptive effects of levonorgestrel implants in a marsupial. *Reproduction, Fertility, and Development* **12** 81–86.

Nave CD, **Coulson G**, **Poiani A**, **Shaw G & Renfree MB** 2002 Fertility control in the eastern grey kangaroo using levonorgestrel implants. *Journal of Wildlife Management* **66** 470–477.

Poole WE, **Simms NG**, **Wood JT & Lubulwa M** 1991 *Tables for Age Determination of the Kangaroo Island Wallaby (Tammar)*, Macropus eugenii, *from Body Measurements. Technical Memorandum No. 32, 37*. Canberra, ACT: CSIRO Division of Wildlife and Ecology.

Renfree MB 1979 Initiation of development of diapausing embryo by mammary denervation during lactation in a marsupial. *Nature* **278** 549–551.

Renfree MB & Lewis AM 1996 Cleavage *in vivo* and *in vitro* in the marsupial *Macropus eugenii. Reproduction, Fertility, and Development* **8** 725–742.

Renfree MB & Tyndale-Biscoe CH 1973 Intrauterine development after diapause in the marsupial *Macropus eugenii. Developmental Biology* **32** 28–40.

Renfree MB & Young IR 1979 Steroids in pregnancy and parturition in the marsupial, *Macropus eugenii. Journal of Steroid Biochemistry* **11** 515–522.

Renfree MB, **Green SW & Young IR** 1979 Growth of the corpus luteum and its progesterone content during pregnancy in the tammar wallaby, *Macropus eugenii. Journal of Reproduction and Fertility* **57** 131–136.

Renfree MB, **Shaw G & Fletcher TP** 1994 Evidence for the essential role of prostaglandins for parturition in a marsupial, *Macropus eugenii. Journal of Reproduction and Fertility* **102** 433–446.

Rudd CD 1994 Sexual behaviour of male and female tammar wallabies (*Macropus eugenii*) at post-partum oestrus. *Journal of Zoology (London)* **232** 151–162.

Sernia C, **Hinds L & Tyndale-Biscoe CH** 1980 Progesterone metabolism during embryonic diapause in the tammar wallaby, *Macropus eugenii. Journal of Reproduction and Fertility* **60** 139–147.

Shaw G & Renfree MB 1984 Concentrations of oestradiol-17 beta in plasma and corpora lutea throughout pregnancy in the tammar, *Macropus eugenii. Journal of Reproduction and Fertility* **72** 29–37.

Shaw G, **Renfree MB & Fletcher TP** 1996 A role for glucocorticoids in parturition in a marsupial, *Macropus eugenii. Biology of Reproduction* **54** 728–733.

Shaw RW, **Kerr-Wilson RH**, **Fraser HM**, **McNeilly AS**, **Howie PW & Sandow J** 1985 Effect of an intranasal LHRH agonist on gonadotrophins and hot flushes in post-menopausal women. *Maturitas* **7** 161–167.

Short RV, **Flint AP & Renfree MB** 1985 Influence of passive immunization against GnRH on pregnancy and parturition in the tammar wallaby, *Macropus eugenii. Journal of Reproduction and Fertility* **75** 567–575.

Stewart F & Tyndale-Biscoe CH 1982 Prolactin and luteinizing hormone receptors in marsupial corpora lutea: relationship to control of luteal function. *Journal of Endocrinology* **92** 63–72.

Trigg TE, **Wright PJ**, **Armour AF**, **Williamson PE**, **Junaidi A & Martin GB** *et al.* 2001 Use of a GnRH analogue implant to produce reversible long-term suppression of reproductive function in male and female domestic dogs. *Journal of Reproduction and Fertility Supplement* **57** 255–261.

Tyndale-Biscoe CH & Hearn JP 1981 Pituitary and ovarian factors associated with seasonal quiescence of the tammar wallaby, *Macropus eugenii. Journal of Reproduction and Fertility* **63** 225–230.

Tyndale-Biscoe CH & Renfree MB 1987 *Reproductive Physiology of Marsupials*,Cambridge: Cambridge University Press.

Tyndale-Biscoe CH, **Hearn JP & Renfree MB** 1974 Control of reproduction in macropodid marsupials. *Journal of Endocrinology* **63** 589–614.

Tyndale-Biscoe CH, **Hinds LA**, **Horn CA & Jenkin G** 1983 Hormonal changes at oestrus, parturition and post-partum oestrus in the tammar wallaby (*Macropus eugenii*). *Journal of Endocrinology* **96** 155–161.

Wright PJ, **Verstegen JP**, **Onclin K**, **Jochle W**, **Armour AF & Martin GB** *et al.* 2001 Suppression of the oestrous responses of bitches to the GnRH analogue deslorelin by progestin. *Journal of Reproduction and Fertility Supplement* **57** 263–268.