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The use of deslorelin implants for the long-term contraception of lionesses and tigers

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Abstract. Contraception is an essential tool for controlling reproduction in captive and free-ranging lions. This paper describes the treatment and contraception of 23 captive and 40 free-ranging lionesses (*Panthera leo*) and four captive tigers (*Panthera tigris*) in South Africa using 3×4.7 mg, 2×4.7 mg, 9.4 mg or $4.7 + 9.4$ mg deslorelin implants. Thirty-one lionesses were treated more than once at 11- to 60-month intervals. In Brazil, two lionesses were treated with 9.4-mg implants and faecal progesterone and oestradiol concentrations were monitored for 920 days. All combinations of deslorelin showed the length of contraception to be around 30 months with one 3×4.7 mg treatment lasting 40 months in one captive lioness. The mean time taken to reconception was 30.1 months for the 3×4.7 mg combination. The faecal analyses of the lionesses in Brazil reflected quiescent ovarian activity for periods of 17 and 30 months, respectively, when small oestradiol peaks but no progesterone peaks started to appear. This confirmed the field observations in South Africa. No side effects occurred although several of the lionesses were treated repeatedly for up to 8 years. Deslorelin (Suprelorin) is a safe and effective means of controlling reproduction in captive or free-ranging populations of lions. Where contraception is to be maintained, the implementation of implants at 24-month intervals is recommended.

Introduction

Left unmanaged, discrete free-ranging lions on fenced game reserves reproduce at an alarming rate. This leads to rapid depletion of prey species, inbreeding and breakouts into neighbouring communities. Large carnivores like lions and tigers also breed exceptionally well under zoo conditions and, with limited possibilities to place captive lions, reproduction needs to be managed. Adult free-ranging lionesses under extensive conditions such as the Kruger National Park reconceive when the cubs are ~20 months old. Our experience in smaller fenced reserves is that litter intervals tend to be shorter. Compared with extensive conditions like in the Kruger National (50%) and Etosha National (40%) Parks, cub survival is close to 100% in smaller fenced reserves (Smithers 1983). This is most likely due to less competition from other lions with fewer or no pride take-overs and less cub predation by hyenas. In zoos it is common practice to remove cubs for hand raising or euthanasia soon after birth. As a result, female lions and tigers come on heat and reconceive much sooner and sometimes within the first month after parturition.

Previously we reported the use of long-acting biocompatible deslorelin implants to downregulate reproduction in a variety of carnivores, including African lionesses (Bertschinger *et al.* 2001, 2002). At the time, a limited number of lions had been treated but the data had shown that the implants were both safe and effective at controlling reproduction of the species. The deslorelin implants, marketed as Suprelorin (Peptech Animal Health, Sydney), have been specifically formulated to deliver long-term release of deslorelin, which is a gonadotrophin-releasing hormone (GnRH) agonist. Following initial stimulation, the release of both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) is downregulated. The overall result is downregulation of ovarian and testicular functions, although in some species it is not effective in males (Munson *et al.* 2001; Trigg *et al.* 2001; Wright *et al.* 2001; Junaidi *et al.* 2003).

The present paper describes the extensive use of deslorelin at various doses and intervals to manage reproduction in captive and free-ranging African lionesses and a few captive tigers during the period 1999 to April 2007. During this period, over 200 treatments on at least 80 lionesses and four female tigers were carried out. The present paper, however, only reports on 67 females where follow-up examinations or observations were possible. It also reports on the faecal steroid profiles of two captive African lionesses housed with a vasectomised male in a zoo in Brazil.

Materials and methods

Suprelorin and Suprelorin12 are imported from Peptech Animal Health, Sydney and used with permission from The Medicines Control Council, Republic of South Africa, under Section 21 of Act 101 of 1965 (authorisation number: SP/14/2006).

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Animals and behavioural observations

In South Africa, captive lionesses ($n = 23$; ages 18 months to 8.5 years) and female tigers ($n = 4$; ages 2 to 4 years) were housed in typical zoo enclosures, mostly with night rooms and ample space to exercise during the day in the presence of males. All of these animals were fed meat. Keepers saw the animals daily when any interactions between sexes would have been noted. Another 40 (ages 18 months to 13 years) free-ranging lionesses were treated on private fenced game reserves ranging from 1500 to 22 000 ha. These lions ranged the reserves with males and were seen on a regular basis (almost daily) by rangers on game drives or conservation business. For food they were reliant on hunting.

In Brazil, two captive African lionesses were housed together with a vasectomised male at the São Paulo Zoo for the past 3 years. The oestrous cycles of the females were monitored by means of observation (signs of oestrus and mating) and faecal oestradiol and progesterone profiling.

Immobilisation

Captive lionesses and tigers were immobilised in their enclosures or night rooms by means of darting using 2.5 to 3 mg kg^{-1} Zoletil 100 (Virbac, Halfway House, South Africa) for treatments and collection of blood samples and vaginal smears. Spontaneous recovery was allowed. Free-ranging lionesses were darted opportunistically but most were immobilised using bait. Immobilising drugs used were Zoletil, a combination of ketamine and medetomidine or a combination of Zoletil (60 mg total dose) and medetomidine (6 mg total dose). With the latter two combinations, the medetomidine was reversed with Antisedan (atipamizole HCl, Pfizer Animal Health, Sandton, South Africa), which is a major advantage when working with free-ranging lions.

Sample collections and examinations

Blood samples for serum progesterone concentration (SPC) were collected from all immobilised South African lionesses. Vaginal smears for cytology were then taken and most females were subjected to transrectal ultrasound examination using a 7.5-mHz linear probe (Aloka 900, Tokyo, Japan) fitted to a custom-made handle. This handle allowed the probe to be positioned over and to follow the uterine horns.

In Brazil, faecal samples were collected from each female from the night room every 1–7 days and an aliquot was stored at –20°C until extracted for assay. The period of observation started on Days –72 (Lioness Salita) and –45 (Lioness Zomba), respectively (Day 0 = day of deslorelin treatment), and continued until Day 920 (≈30 months).

Deslorelin treatment

Previously we reported on the use of 12-mg deslorelin (2 × 6 mg implants) to downregulate reproduction in African lionesses and where females ($n = 2$) were not retreated, reconception took place 29 months later (Bertschinger *et al.* 2002). However, these implants were discontinued, meaning that, at the beginning of this trial, there were no guidelines available on dose and frequency of treatment required with the newly manufactured 4.7-mg implants (Suprelorin, Peptech Animal Health, Sydney). As a result, the treatment regime has varied quite a bit. Initially, the dose of deslorelin used per treatment was 14.1 mg (3 × 4.7 mg; number of treatments = 43). In 2004, the 9.4-mg implants became available. At first we treated each female with 14.1 mg (1 × 9.4 plus 1 × 4.7; number of treatments = 23), but in 2004 and again in 2006 we treated several females (number of treatments = 50) with a single 9.4-mg implant (Suprelorin12, Peptech Animal Health, Sydney). Owing to unavailability of the 9.4-mg implants in 2006, some animals were treated with 2 × 4.7 mg (number of treatments = 10) implants. All females were implanted subcutaneously on the left or right side of the neck, which was noted. Thirty-six females (including two tigers) were treated once, 12 (including two tigers) twice (intervals of 14–60 months), 11 three times (intervals of 11–33 months), two four times (intervals of 17–49 months) and six females five times (intervals of 11–30 months). Lionesses previously treated once with 12 mg (2 × 6 mg; number of treatments = 5) were part of the group treated five times. The four longest periods during which individual lionesses have been subjected to continuous treatment with deslorelin was five ($n = 3$), six ($n = 2$), seven ($n = 2$) and eight ($n = 1$) years. The lionesses in Brazil were each given a single 9.4-mg implant on Day 0.

Abortion procedure

Females found to be pregnant were either left untreated, treated with deslorelin or aborted and treated with deslorelin. The prostaglandin dinoprost (Lutalyse, 7.5 mg; Pfizer Animal Health) was used on three consecutive or alternate days as an abortifacient. The second and third doses were administered by means of darting. Abortifacient treatment was delayed by 2 weeks in lionesses and tigers that were mated less than 2 weeks earlier, provided SPCs were raised above anoestrus or inter-oestrus levels.

Hormone assays

Faecal extractions (Brazil lions) were done according to Brown *et al*. (1993, 1994, 1996) and Brown and Wildt (1997). Samples were lyophilised and 0.2 g was extracted in 5 mL of 90% ethanol in water. Samples were vortex-mixed for 1 min and then placed in a boiling water bath for 25 min. Samples were then centrifuged for 15 min at 500*g* and the supernatant was recovered. The pellet was re-suspended in 5 mL of 90% ethanol and the process was repeated. The supernatants were combined and then dried under a flow of air. Samples were taken up in 1 mL of absolute methanol, vortex-mixed for a minute and transferred to an ultrasonic cleaner for 15 min. They were then diluted 1 : 40 with phosphate-buffered saline (PBS)–gelatine buffer for radioimmunoassay (RIA). Progesterone was assayed using a progesterone RIA (Progesterone DSL 3900, Diagnostics Systems Laboratories, Webster, TX, USA) that has been validated for the determination of feline faecal progesterone. The intra-assay and inter-assay coefficients of variation (CVs) of the progesterone assay were 7.43% and 3.11% respectively. Faecal oestradiol was analysed using RIA (Oestradiol Coat-a-Count, Diagnostic Products, Los Angeles, CA, USA). The intra-assay and inter-assay CVs of oestradiol assay were 7.43% and 3.60% respectively. Simple linear regression between the standard curve of the kit and the curve obtained by serial dilutions of the standard hormone in faecal matrix with very

low values were: progesterone $R^2 = 0.98$ and oestradiol $R^2 = 0.99$ (Viau *et al.* 2005).

SPC was determined on thawed serum samples using progesterone RIA kits (Progesterone Coat-a-Count, Diagnostic Products) as previously described (Bertschinger *et al.* 2001, 2002).

Results

In South Africa, the use of deslorelin implants at various doses was 100% successful at preventing pregnancy in 63 lions and four tigers. With the 2 × 4.7 implants, however, we have incomplete data, because the maximum interval from treatment in the nine females treated so far is only 9 months. During this period, no animals have shown heat. In Table 1, the anoestrus periods (≥20 months) following treatment are shown. The thirty-six remaining females had been treated at intervals of 11 to <20 months and were not included in Table 1. Although they were successfully downregulated until the next treatment, it provided insufficient time in our experience for reversal to take place. Reversibility could be shown in 8 of 14 lionesses treated with 3 × 4.7-mg deslorelin implants. Seven of the eight lionesses conceived and produced live cubs. The eighth female was re-implanted during her first post-treatment oestrus. The mean period until reconception in this group was 30.1 months. Reversibility was shown in one of the nine females given 9.4-mg deslorelin. Four (including the last-mentioned) of these were re-implanted whereas the others were in anoestrus with durations varying from 27 to 36 months. Four of the 6 females treated with the 4.7 + 9.4 mg combination were re-treated, with the two remaining being 23 months post treatment and in anoestrus. The mean SPC during anoestrus was 3.11 nmol L^{-1} (±s.d. 1.72; $n = 109$), whereas during oestrus it was 6.95 nmol L^{-1} (±s.d. 5.68; $n = 6$). During pregnancy, SPC ranged from 63.7 to 212.8 nmol L^{-1} (mean 135.38 ± s.d. 42.68 nmol L^{-1}).

Three captive lionesses and two tiger females were treated with dinoprost during various stages of pregnancy (Table 2). Each one of these females was also implanted with deslorelin on the day of pregnancy diagnosis. No cubs were seen as a result of these pregnancies.

Table 1. Summary of 30 lionesses and one female tiger treated with different doses of deslorelin
Only females that have reached anoestrus periods of 20 months or more are included

Dose of deslorelin	ID of female c = captive w = free-ranging	Anoestrous periods of females: (months) No heat Not retreated	No heat Retreated	1st heat Retreated	Interval until conception (months)
3 × 4.7 mg (14.1 mg)	Nischila (w)				15
	Dharma (w)	30			
	Tabby (w)				40
	Gertrud (w)	35			
	Elsa (w)				30
	Midget (w)				31
	One-Eye (w)				31
	Cora (w)				37
	Doris (w)		33		
	Nweti (w)			30	
	Dyason (w)				27
	Simone (c)		24		
	Kiara (c)		24		
	Shumba (c)		24		
	#60 (w)		23		
	#26 (w)		23		
	Mean				Mean = 30.1
9.4 mg	Sabre (c)		20		
	Amber (c)		30		
	IDA7 (w)	27			
	Begera (c)	36			
	Elsa (c)	36			
	Subadult (w)			30	
	Cayla (c)		30		
	Emma (c)	30			
	[A]Laya (c)	33			
4.7 + 9.4 mg (14.1 mg)	Niobe (c)		32		
	Jesse (w)	23			
	Scar (w)	23			
	7E34 (w)		27		
	CBD7 (w)		21		
	IDF3 (w)		21		

[A]Female tiger.

Table 2. Summary of lionesses and tiger females treated with the abortifacient dinoprost

Species	Female ID	SPC nmol L^{-1}	Approximate stage of gestation	Dinoprost treatment	Dose of deslorelin
Tiger	Olga	177.92	<3 weeks	7.5 mg on three alternate days 2 weeks later	9.4 mg
Tiger	Orcha	63.70	70 days	7.5 mg on three alternate days from PD	9.4 mg
Lion	Happy	212.80	80 days	7.5 mg on three consecutive days from PD	4.7 + 9.4 mg
Lion	Senanga	194.08	3 weeks	7.5 mg on three consecutive days from PD	4.7 + 9.4 mg
Lion	Diana	107.29	3 weeks	7.5 mg on three consecutive days from PD	4.7 + 9.4 mg

SPC = serum progesterone concentration, PD = pregnancy diagnosis.

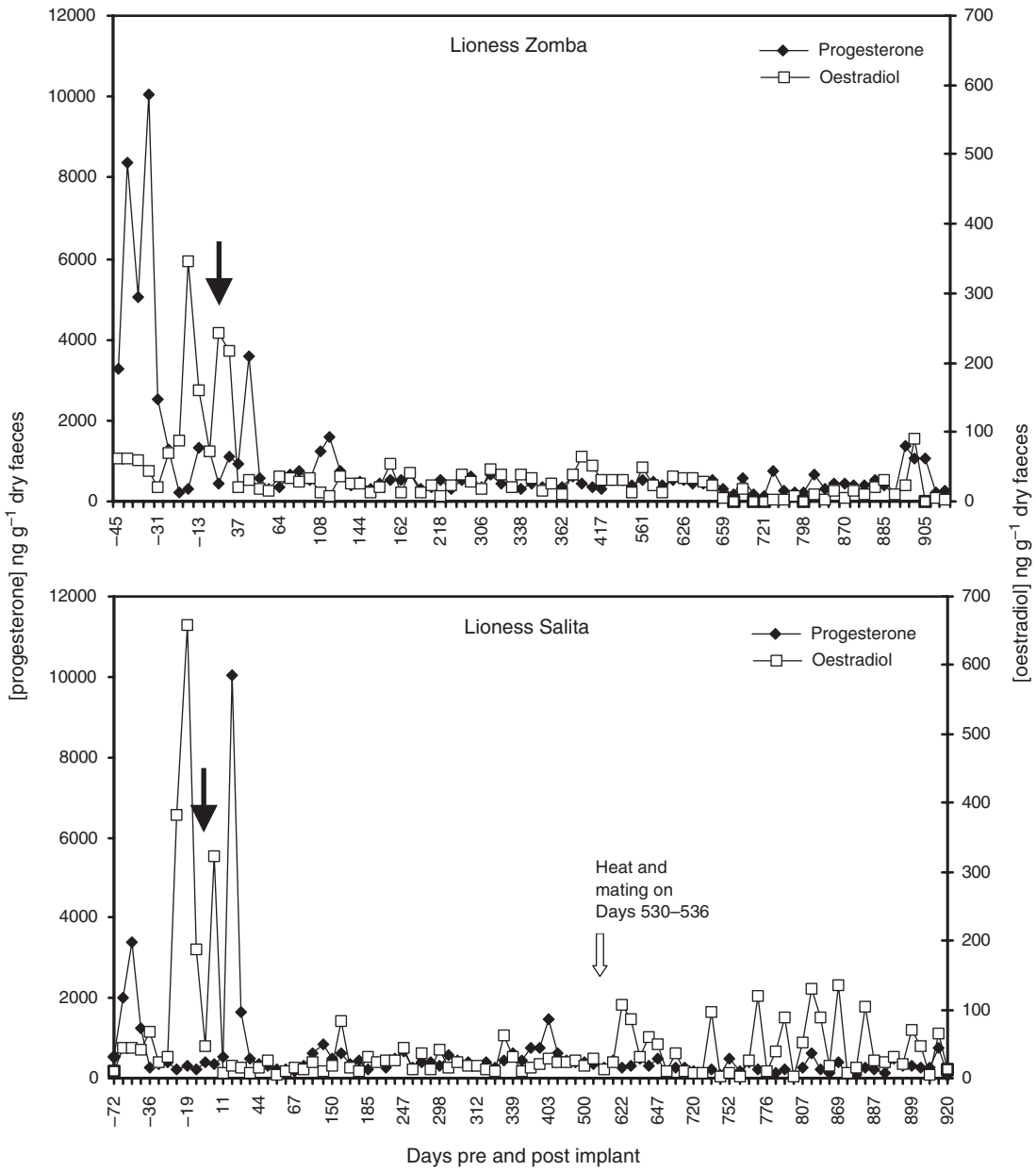

Fig. 1. Faecal oestradiol and progesterone metabolite profiles of two lionesses in Sao Paulo Zoo before and after treatment with 9.4 mg deslorelin on Day 0 (solid black arrows).

No side effects as a result of deslorelin treatment were observed in any females, with some having been treated four to five times over 5–8-year periods. Two captive lionesses were treated with 9.4-mg deslorelin during pregnancy (70 and 90 days of gestation, respectively) but cubs were never seen. It is not clear whether they aborted or lost the cubs postnatally.

The faecal progesterone and oestradiol concentrations of the two captive lionesses (Zomba and Salita) in Brazil are shown in Fig. 1. As can be seen from the profiles, both females had been cycling shortly before the treatment with deslorelin. In Zomba, there was an oestradiol peak on Day 4 following treatment and a progesterone peak from Days 37 to 43. Salita showed an oestradiol peak on Day 7 and progesterone on Days 16 to 17. Behavioural signs of heat and mating were not observed and the duration of progesterone peaks were short (~7 and 2 days respectively). By Day 920 post treatment, Zomba had shown no signs of oestrus despite minor oestradiol peaks towards the end of the observation period. Salita showed oestrus with mating on Days 530 to 536 post implantation and her faecal oestradiol concentrations were marginally higher during this period. However, no rise in faecal progesterone was seen. Following that, she remained in behavioural anoestrus until Day 920 but with periodic small peaks of oestradiol.

Discussion

The traditional approach to contraception of large felids has been the use of slow-release silicon implants impregnated with progestins like melengestrol acetate (MGA). Although highly effective as a contraceptive, the prolonged use of MGA is associated with serious side effects like cystic endometrial hyperplasia–pyometra and uterine, mammary and hepatic tumours. Another side effect is the development of virulism, particularly in lionesses. Obesity also seems to be a sequel to MGA treatment (Munson 2001; Munson *et al.* 2001, 2002, 2005).

Deslorelin as a long-acting implant (Suprelorin, 4.7 and Suprelorin12, 9.4 mg implants) was developed to downregulate reproduction and reproduction-related behaviour in female domestic dogs (Trigg *et al.* 2001; Wright *et al.* 2001), male domestic dogs (Trigg *et al.* 2001; Junaidi *et al.* 2003) and domestic female and male cats (Munson *et al.* 2001). The implants have been shown to be safe and downregulatory effects are completely reversible. Their use as contraceptives in wild carnivores dates back to 1998 (Bertschinger *et al.* 2001). The implants have also been used as a contraceptive in male carnivores, particularly cheetahs, many of which have been treated yearly for up to 7 years (Bertschinger *et al.* 2006).

This study demonstrated that deslorelin (Suprelorin/Suprelorin12) is a safe and effective means of controlling captive and free-ranging populations of lions as well as captive female tigers. The doses tested were 3×4.7 mg, 9.4 mg, $4.7 + 9.4$ mg and 2×4.7 mg. With the exception of the 2×4.7 mg dose all other regimens appear to be effective for ~30 months but possibly longer when the 9.4-mg implants are used. The pay-out of the 9.4-mg implants was designed to release deslorelin for a longer period than the 4.7-mg implants (T. E. Trigg, unpubl. data). We could show complete reversal in eight of 14 females treated with 3×4.7-mg implants, the mean time required until reconception being 30.1 months. In a previous study, two out of two lionesses conceived 29 months after treatment with 12 mg (2×6 mg) deslorelin (Bertschinger *et al.* 2002). The data for the 9.4 mg and 4.7 mg plus 9.4 mg are as yet incomplete, meaning that time taken to complete reversal is not available yet. The data in Table 1, however, provides useful information with regard to these two doses. At 27, 30, 33 and 36 months, one, three, one (tiger) and two lionesses were still in anoestrus respectively. One lioness treated with 9.4 mg showed her first heat 30 months after treatment. In the case of the combination treatment ($4.7 + 9.4$ mg) one female each were still in anoestrus after 27 and 32 months respectively. One female treated with the 3×4.7-mg dose recovered fertility much sooner at 15 months than the rest of the group. It is possible that this outlier may have been due to the earlier 4.7-mg implants as she was treated in 2000.

Neither of the two lionesses that were treated during pregnancy produced cubs. This may have been due to the effects of the implants but may also have been due to poor mothering ability and eating of the cubs at birth, which is common in wild captive felids. Previous experience in African wild dogs, however, showed that deslorelin implants do not cause abortion. Bitches gave birth to live puppies and were able to raise them unassisted (Bertschinger *et al.* 2001, 2002). Wright *et al.* (2001) found that use of deslorelin in two pregnant bitches (>5 pups and 2 pups, determined by ultrasound examination) resulted in failure of each pregnancy around Day 40 of gestation. Aborted puppies were found for one bitch, none found for the other.

As an additional tool to reproductive management of lions and tigers, we introduced the use of dinoprost as an abortifacient. Other than mild salivation, dinoprost produced no other side effects. The fact that females can be darted for follow-up treatment once recovered and mobile makes it a useful tool. It should be mentioned, however, that each one of these females was also implanted with deslorelin at the time of pregnancy diagnosis. Owing to the possible abortion induced by deslorelin in the two females described above, we cannot be absolutely sure that abortion was solely due to the prostaglandin. Previously, however, we have shown that dinoprost induces luteolysis in dioestrus non-pregnant cheetahs (H. J. Bertschinger, unpubl. data).

Following treatment, mating was seen in three lionesses (67 and 97 days, 12 months and 18 months respectively) during what was considered to be the post treatment anoestrus period. None of these females produced cubs that could be attributed to these observations. The female that was mated on Days 67 and 97 (3×4.7 mg) was immobilised and examined after each incident and found to have baseline SPC. The wild female mated after 12 months (9.4 mg) lay on her side instead of in the lordosis position during mating. The third lioness mated after 18 months (9.4 mg) also demonstrated a normal mating position. False mating or forced mating is known to occur in African lions and may be an expression of dominance by the male. Females will probably rather submit to the whims of the male than suffer the potentially dangerous consequences.

The data gained from the lions in Brazil is invaluable because they confirm the findings in the South African lions. Both lionesses show distinct oestradiol and progesterone profiles of cycling females before treatment with deslorelin. Following the implants, oestradiol peaks were seen in both females and a small

and transient rise in progesterone metabolites were also observed. This is consistent with our observations in wild lions where females appear to be attractive to males without allowing them to mate within the first week post implant. Certainly, although we advise female and male separation in captive lions during the first 3 weeks, we have never seen pregnancies as a result of this attractive period. According to the faecal ovarian steroids, Lioness Zomba remained quiescent until close to the end of the observation period around Day 900 (30 months) when South African lionesses show reversal. The other lioness, Salita started showing signs of recovery earlier as can be seen in Fig. 1. Small oestradiol peaks started to occur at around Day 550 (17.7 months) and continued to occur until the end of the observation period. She showed heat with mating at around the same time but neither this or the oestradiol peaks were associated with significant increases in progesterone. Perhaps it is these small oestradiol peaks that cause behavioural changes in our South African lionesses.

In conclusion, the use of deslorelin implants allows managers of game parks and zoos to control reproduction in prides selectively and thus slow down the rate of population growth to whatever their requirements may be (adaptive management). From our results it would seem that either 3×4.7 mg or 9.4 mg implants can be effectively used as contraception for lionesses and female tigers for a period of ~30 months or longer. Where contraception is to be maintained, we recommend the implementation of implants at 24-month intervals. Prolonged use of deslorelin implants for up to 8 years have produced no visible or measurable side effects.

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