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Repeated use of the GnRH analogue deslorelin to down-regulate reproduction in male cheetahs (*Acinonyx jubatus*)

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Abstract

The GnRH analogue deslorelin, as a subcutaneous implant, was initially developed in Australia as an ovulation-inducing agent in mares. Its uses, for the suppression of reproduction in the domestic dog and cat and in other species, including humans, have been developed subsequently. Such implants have been used as a contraceptive modality in a variety of wild carnivores, both males and females. This paper describes the use of deslorelin implants as a contraceptive agent for cheetah males maintained in a semi-captive environment and housed in various camps together with females. Annually, male cheetahs were treated for 1 ($n = 2$), 2 ($n = 7$), 3 ($n = 9$), 4 ($n = 3$) or 5 ($n = 1$) consecutive years with an implant containing 4.7, 5.0 or 6.0 mg of deslorelin. On the first day of treatment and then on an annual basis, blood testosterone concentrations were analysed, testicular measurements were taken, appearance of penile spikes was determined, and semen was collected and evaluated. Pregnancy rates of mated or inseminated females were determined. A dose of 6 mg of deslorelin suppressed reproduction for at least 1 year, whereas with 4.7 and 5 mg of deslorelin, 3 of 17 males had a few non-motile spermatozoa in their ejaculates. All testosterone concentrations were basal at 1 year post-implant and no side effects were observed. We concluded that deslorelin implantation, at a dose of 6 mg, was a safe and reliable method of annual contraception in male cheetahs.

Keywords: Deslorelin; GnRH; Down-regulation; Contraception; Cheetah

1. Introduction

Population control by means of contraception has become an important tool in the management of wild carnivores in southern Africa. In most cases, especially with endangered species like the cheetah and African wild dog, a reversible method is required. The GnRH analogue, deslorelin, in a long-acting biocompatible subcutaneous implant (Peptech Animal Health, Sydney), was initially developed in Australia as an ovulation-inducing agent in mares. Its uses, for the control of reproduction in the domestic dog and cat and in other species, including humans, have been developed subsequently [1,2]. It has also been used as a contraceptive agent in a variety of wild carnivores, both males and females [3,4]. Contrary to the side effects reported in some female carnivores treated with gestagen implants [5], no adverse side effects have

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Table 1
Twelve cheetahs treated with 5 mg (2001) or 4.7 mg (2002–2004) deslorelin implants annually for 3 years

Observation	Cheetah ID and age during first examination in 2001											
	AJ3, 9 years	AJ132, 5 years	AJ133, 5 years	AJ138, 8.5 years	AJ187, 5 years	AJ225, 3.5 years	AJ226, 3.5 years	AJ227, 3.5 years	AJ255, 3.5 years	AJ256, 3 years	AJ259, 9 years	AJ261, 5 years
February 2001, Time 0												
R testis (mm)	25 × 19	27 × 19	27 × 17	26 × 19	25 × 18	27 × 19	30 × 20	25 × 20	27 × 19	23 × 16	29 × 19	27 × 19
L testis (mm)	27 × 19	27 × 19	27 × 18	26 × 18	29 × 18	31 × 18	29 × 21	27 × 20	26 × 18	24 × 17	24 × 20	28 × 17
Testosterone (nmol/L)	3.43	5.37	8.81	3.5	6.45	0	9.66	4.01	0.36	6.86	7.45	11.68
Penile spikes	3	3	3	3	3	3	3	3	3	3	3	3
Sperm	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Few[a]	Yes	Yes	Yes	Yes
Body weight (kg)	31	43	40	56	55	48	47	47	40	36	50	45
March 2002, Time 1												
R testis mm	24 × 16	25 × 18	24 × 17	24 × 19	25 × 17	24 × 18	26 × 15	22 × 14	24 × 19	17 × 11	22 × 17	24 × 18
L testis mm	24 × 16	26 × 18	25 × 17	24 × 19	24 × 18	23 × 17	24 × 14	22 × 14	23 × 19	17 × 12	21 × 17	23 × 17
Testosterone (nmol/L)	0	0.47	0	0.67	0	0	0.96	0	0	0	1.32	0
Penile spikes	2	3	2	2	2	2	2	1	2	1	2	2
Sperm	Few	Yes	None	None	None	Few[a]	Few[a]	None	Yes	None	None	Yes
Body weight (kg)	32	41	38	54	55	45.5	45	44	37	34	43	41
February 2003, Time 2												
R testis mm	21 × 16	21 × 12	23 × 12	20 × 13	20 × 12	21 × 12	18 × 13	19 × 16	20 × 13	18 × 11	16 × 14	18 × 12
L testis mm	22 × 16	20 × 12	21 × 13	20 × 14	22 × 12	20 × 12	20 × 14	19 × 14	19 × 12	17 × 12	17 × 14	17 × 12
Testosterone (nmol/L)	0.06	0	0	0	0	0	0	0	0	0	0	0
Penile spikes	2	1	1	2	1	1	1	1	2	2	1	1
Sperm	None	None	None	None	None	None	None	None	None	None	None	None
Body weight (kg)	31	40	38	54	57	47.5	46.5	46	38	35	44	45
March 2004, Time 3												
R testis mm	20 × 17	17 × 12	16 × 12	20 × 13	19 × 13	24 × 18	21 × 15	19 × 15	20 × 13	18 × 13	18 × 13	18 × 14
L testis mm	19 × 17	17 × 12	17 × 12	20 × 14	18 × 13	21 × 15	20 × 14	20 × 15	19 × 13	19 × 13	19 × 14	18 × 14
Testosterone (nmol/L)	0	0	0	0	0	0	0	0	0	0	0	3.27
Penile spikes	2	1	1	1	1	1	1	1	1	1	1	2
Sperm	Debris	None	None	None	None	None	None	None	None	None	None	None
Body weight (kg)	35	42	39	50.5	52	48	46	45	39.5	37	45	46

Penile spikes: 1 = poorly developed, 2 = moderately developed, 3 = prominent. Note: penile spikes cannot disappear once they have been formed, their size is regulated by androgens. Yes = presence of normal sperm cells.

[a] Dead sperm.

been observed with deslorelin. In most species, the continuous release of deslorelin from the implant down-regulates FSH and LH release, thereby controlling gonadal activity in both males and females. Previously, deslorelin (6 or 12 mg) was used once in each of six cheetah males. Animals examined 45 days after implant had undetectable blood testosterone concentrations, but their semen samples had high concentrations of spermatozoa. By 3 months, ejaculates from two males were azoospermic, the others becoming azoospermic later following treatment administration. All males remained azoospermic for at least 21 (12 mg) and 12 (6 mg) months after a single treatment [3,4]. The present paper describes the repeated use of deslorelin implants of various doses and release rates, on an annual basis, as a contraceptive agent in male cheetahs in a semi-captive environment.

2. Materials and methods

The cheetahs used in the present study were housed in enclosures (size, 10–1500 ha) in mixed sexes. Annually from 1999 to 2004, male cheetahs were treated for 1 ($n = 2$), 2 ($n = 7$), 3 ($n = 9$), 4 ($n = 3$) or 5 ($n = 1$) consecutive years with an implant containing 4.7, 5.0 or 6.0 mg of deslorelin. GnRH analogue implant dose was 12 mg (1999), 6 mg (2000), 5 mg (2001) and 4.7 mg (2002–2004). Data collected on the first day of contraception and on an annual basis at 12–14-month intervals were: blood testosterone concentration, testicular measurements (from 2001), appearance of penile spikes, evaluation of semen collected by electro-stimulation [6], and pregnancy rates. Blood testosterone concentrations were determined by radioimmunoassay using a commercial kit (Coat-A-Count total testosterone kit; Diagnostic Products Corporation, Los Angeles, CA, USA) [4]. The presence of viable spermatozoa was used as an indicator of potential fertility [4]. Penile spikes were evaluated subjectively according to prominence on a scale of 1–3. Testicular measurements (length and width) were carried out using vernier callipers. In the group treated twice, one male showing aberrant sexual behavior towards humans was given 9.4 mg deslorelin each year to correct behavioral abnormalities. Animal AJ138 (Table 1) died 2 days after it was immobilized for examination in 2004, 13 months after the last implant. Testes were fixed in 10% buffered formalin and prepared for histological examination.

A mixed model was used to determine the effects of cheetah, time and side (left or right testis) on the length and width of the testes. Cheetah was considered a random effect, whereas time and side of testis (left or right) were fixed. Times 0, 1, 2 and 3 were the times immediately before first, second and third contraceptive treatments, and 13 months after the third contraceptive treatment, respectively. Side of testis had no effect on either response variable and was removed from the model. A two-tailed Mann–Whitney test was used to compare the length of the testes of the younger animals versus the older animals, and a one-tailed Mann–Whitney test was used to compare the width of the testes over time or between young and old animals. For all statistical tests, $P < 0.05$ was considered significant.

3. Results

A dose of 6 mg suppressed reproduction for at least 1 year, whereas with 4.7 or 5 mg, 3 of 17 males had a few spermatozoa in the ejaculate, most of which were dead (Table 1) and 3 had normal ejaculates. Table 1 shows the results from 12 of the 14 cheetahs treated annually for 3 years in which a complete data set was available. Testosterone concentrations were reduced to basal concentrations at 1 year in eight animals, whereas four were at values still over the detection limit of the assay. The pattern in the remaining cheetahs treated from one to five times was comparable. Among the males with spermatozoa, three also had secondary signs of androgenic activity, as demonstrated by the prominence of penile spikes. During the second and third years of initial treatment, with the exception of sperm debris in one male, no additional ejaculates with spermatozoa present were seen. Testosterone concentrations were consistently basal and penile spikes were evaluated as either poorly or moderately developed in appearance from the second year of treatment.

There were differences among cheetahs ($P < 0.001$) for testicular length and width. However, neither testicular length nor width before onset of contraception was correlated with age or body mass. Both the length and width of the testes differed among periods ($P < 0.001$). Length, as well as width, decreased from Time 0 to 1, with a further decrease to Time 2, but thereafter remained constant (Fig. 1). Cheetah differed in the testicular size response to repeated deslorelin treatments ($P < 0.001$); after the third treatment, the five younger animals (3–3.5 years at the beginning of the study) appeared to respond differently than the older animals (5–9 years at the beginning of the study). The length of the testes of the younger animals remained the same (number of testes = 6) or increased by 1–3 mm ($n = 4$) between Times 2 and 3, whereas the testicular length in the older animals ($n = 7$) remained the same (number of testes = 4), decreased by 1–7 mm ($n = 8$)

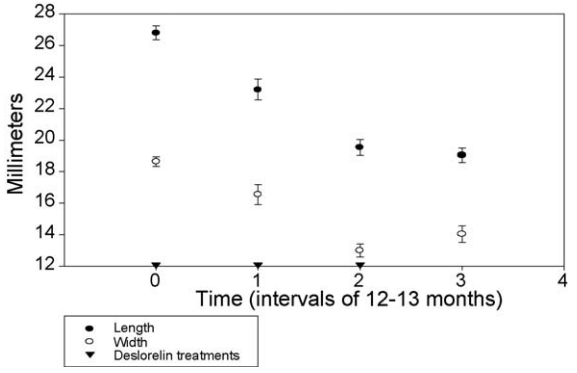

Fig. 1. Testicular dimensions of 13 cheetahs (*Acynonyx jubatus*) before and following three annual treatments of deslorelin (vertical bars indicate standard errors).

and increased by 2 mm in two testes ($P < 0.05$). The width of the testes remained constant or increased by 1–6 mm during Times 2 and 3. The testes of the five young animals tended to increase more in width than those of the seven older animals ($P = 0.07$).

The quality of the histological sections from animal AJ138 was suboptimal because the animal was found several hours after he died. The testicular sections showed distinct suppression of spermatogenesis. The tubules contained mainly spermatogonia with occasional presence of spermatocytes and spermatids. No spermatozoa were visible in the tubules (Figs. 2 and 3).

4. Discussion

The contraceptive efficacy of deslorelin in male cheetahs described previously [3,4] was confirmed in this extended study. Furthermore, between February 1999 and May 2004, no pregnancies were recorded. Deslorelin administered once a year at the doses described was effective at down-regulating testicular function in all males for a minimum period of 1 year. The down-regulation was reflected in basal or lowered blood testosterone concentrations and the absence of spermatozoa in most ejaculates 1 year after implant. The ejaculates observed in some males after 1 year of treatment were abnormal in sperm numbers and quality, suggesting that these males were infertile. It is not known if these abnormalities were the product of renewed spermatogenesis or remnants in the epididymis after cessation of sperm production. Given that most

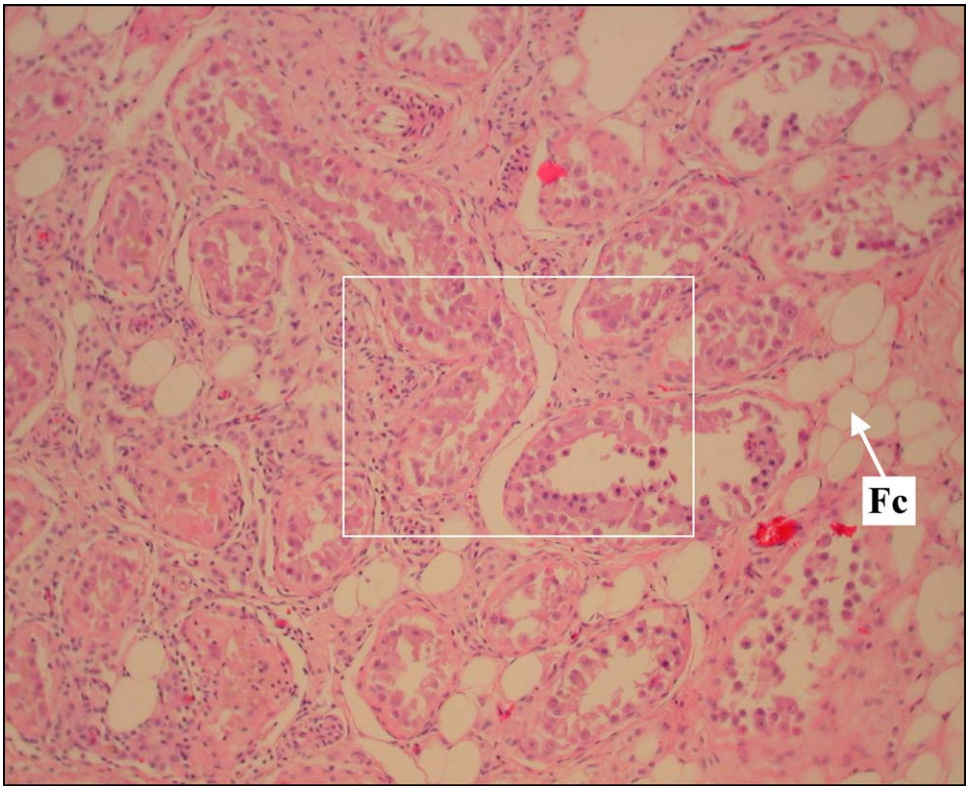

Fig. 2. Histological section (H&E; x100) of the testis of Cheetah AJ138 exposed to three annual deslorelin implants. The last implant was administered 12 months prior to death of the animal. The rectangular area is enlarged in Fig. 3. Fc = possible fat cells.

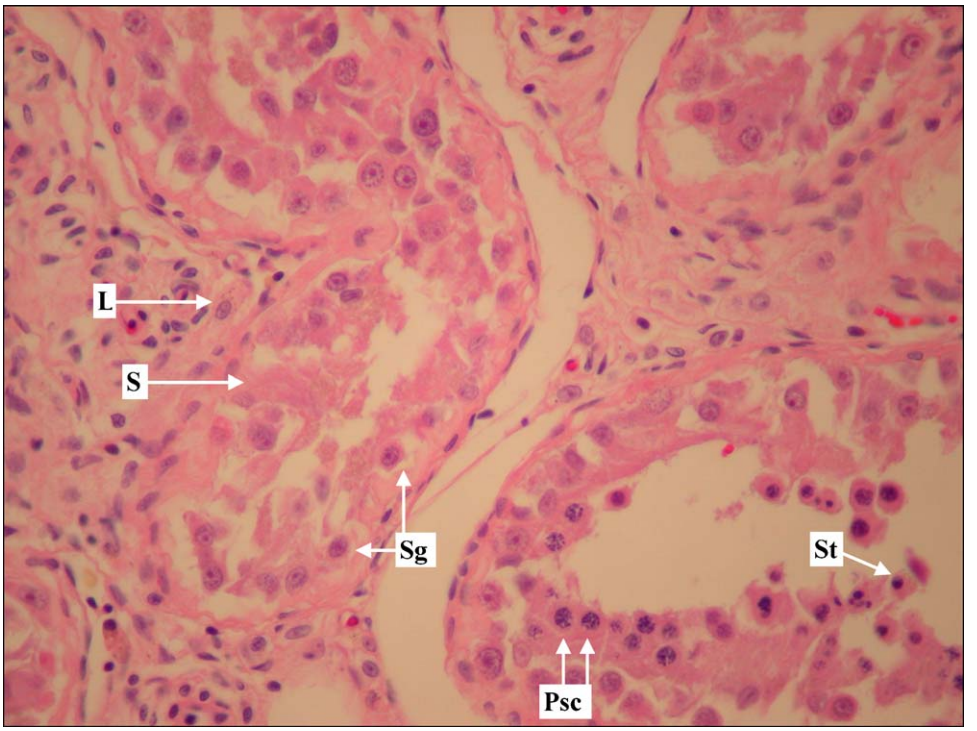

Fig. 3. Histological section (H&E; x400) of the testis of Cheetah AJ138. L = Leydig cell; Psc = primary spermatocytes; S = Sertoli cell; Sg = spermatogonia; St = spermatid.

spermatozoa were non-motile or sperm debris (including epithelial cells, sperm heads, sperm tails) we believe the latter to be true. Furthermore, if testicular size is an indicator of seminiferous tubule activity as in other species, the further reduction in size observed between the first and second year of treatment suggests a further decline in spermatogenesis. Perhaps spermatogenesis was either further down-regulated by the treatment or that the inactive cell stages of spermatogenesis take a long time to be totally inhibited. One year after the third implant, testicular size reached the minimum and no further changes were observed, except in the younger males where testicular size increased slightly. As shown in Table 1, testicular size increased without a corresponding increase in body weight. The testes histology realized from the tissues of the animal that accidentally died confirmed the previous observation of arrest of spermatogenesis.

Deslorelin effectively suppressed sexual behavior (no males were observed attempting to mate) and inhibited penis spike development. The latter parameter was considered a good indicator of treatment efficacy by reflecting the suppressive effect on testosterone synthesis. No side effects, including significant changes in body weight, were seen in any of the males.

In conclusion, deslorelin was a safe and reliable method to inhibit, through pituitary GnRH receptor down-regulation, sexual function in male cheetahs. The optimal annual dose for male cheetahs was approximately 6 mg/animal.

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