Theriogenology 231 (2025) 190–196

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Original Research Article

Duration of efficacy and effect of implant location in adult queens treated with a 9.4 mg deslorelin subcutaneous implant

A. Baldan [a], M. Pereira [b,*], V. Pedrotti [a], S. Ferro [c], D. Gelli [b], C. Milani [b], Mollo A [b], C. Fontaine [d], G.M. De Benedictis [b], C. Stelletta [b], S. Romagnoli [b]

[a] Private Practitioner, Padova, Italy
[b] Department of Animal Medicine, Production and Health of the University of Padova, Italy
[c] Department of Comparative Biomedicine and Food Science of the University of Padova, Italy
[d] Companion Animal Medical Department, Virbac, Carros, France

ARTICLE INFO

Keywords:
9.4 mg deslorelin
queen
Periumbilical
Reproduction control

ABSTRACT

Deslorelin is a GnRH-agonist used off-label for contraception in female cats. Little is known about duration and safety of the 9.4 mg subcutaneous implant in the queen as well as its efficacy when placed periumbilically. Fourteen female cats were administered the 9.4 mg deslorelin implant (during interestrus or anestrus) either in interscapular (N = 8) or periumbilical (N = 6) sites, following general and reproductive examination, vaginal cytology, hematology, biochemistry and progesterone assay to ensure health status. All above procedures (except for progesterone assay) were repeated weekly during the first month, then every 2 months until 6 months and then every 6 months until treated cats regained full reproductive function. No side effects were observed in any treated queen. Post implantation estrus occurred in 40 % of the subjects. A significant increase in body weight was observed during treatment (12/14 queens gained weight), particularly at the end of the study. At the end of the study some queens mated, conceived and kittened, proving reversibility of the treatment. The average duration of action of the 9.4 mg deslorelin implant was 790 ± 155 days (range 525–1140 days) with no significant difference in duration or efficacy depending on implantation sites. The 9.4 mg deslorelin implant causes pharmacological sterilization for about 2 years in female cats, is fully reversible and caused no clinically relevant side effects when administered at both interscapular and periumbilical sites.

1. Introduction

Controlling reproduction in queens is becoming relevant presenting complaint for small animal veterinarians working in private practice or in shelters due to the increased interest in cat breeding as well as the unresolved issue of unowned, free-roaming and/or feral cats posing threats to animal as well as human health in many areas of the world. A new treatment option to approach these problems is offered by long-acting agonists of gonadotropin releasing hormone (GnRH) such as deslorelin [1]. Deslorelin is a long-acting GnRH agonist marketed in Europe with the indication of reversible medical sterilization in male dogs, male cats and prepuberal bitches In cats, deslorelin is characterized by a longer duration of efficacy with respect to dogs [2,3] which makes it a useful alternative to surgical gonadectomy when dealing with patients at high anesthetic risk, breeding cats or when surgery becomes impractical or less advantageous such as with feral cats. The median duration of efficacy of the 4.7 mg implant in queens is 20 months [4–7] (albeit with marked individual variability (4–34 months) [4,5]. Very little is known about duration of effect of the 9.4 mg deslorelin implant in domestic queens as in the only other published report using this implant queens were spayed 18.5 months after treatment [8]. The objective of this study was to assess duration of efficacy and side effects of the 9.4 mg deslorelin implant in adult queens.

According to manufacturer's indications deslorelin implants should be placed in the interscapular area. A periumbilical placement has recently gained popularity due to the ease in locating the implant in case it has to be removed [9–11]. However, no information on efficacy and duration of action of deslorelin implants placed in periumbilical as opposed to the interscapular area is available in queens. Thus, a secondary aim of the study was to know if implant location influences treatment efficacy and/or duration.

* Corresponding author.
E-mail address: mariacarlos.pereira@phd.unipd.it (M. Pereira).

https://doi.org/10.1016/j.theriogenology.2024.10.021
Received 3 July 2024; Received in revised form 22 October 2024; Accepted 22 October 2024
Available online 22 October 2024

2. Materials and methods

The University of Padova Ethics Committee approved the study (Project n ° 64 bis/2011). The clinical trial was conducted from 2011 to 2014. Fourteen healthy adult, privately owned European queens of different weights (2.6–3.9 kg) and ages (6 months-5 years) presented for control of reproduction were recruited with owner's consent to be treated with a subcutaneous 9.4 mg deslorelin implant (Suprelorin™, Virbac, Carros, France). Only healthy queens in postpubertal state with no history of reproductive or general disease were included. A thorough clinical exam and blood collection for hematology (ADVIA 120 Hematology system, Siemens, Munich, Germany), biochemistry (hepatic and renal profile, glycemia, cholesterol and triglycerides) (BT 1500, Biotecnica, Rome, Italy) and hormonal assay was performed at the beginning of the study in order to assess suitability of each individual animal health and reproductive status. Blood samples were collected from fasted animals by jugular venepuncture, centrifuged (at 1750 g), serum harvested and kept at −20 °C until assay with chemiluminescence (Immulite, Medical System, Genova, Italy). Body weights (BW) were checked with an electronic scale (Wunder, VT 2 model, Milan, Italy). The post-pubertal state of each queen was assessed through reproductive history, a general clinical exam and a thorough reproductive check including a vaginal smear and collection of a 3.0 cc blood sample for serum progesterone (P4) assay. Relevant clinical observations (clinical exam, assessment of vaginal smears, hematology, biochemistry and serum P4 results) were performed independently by two operators. Vaginal smears were performed using a cotton swab (normal human ear cleaning cotton bud) moisturized with tap water inserted for approximately 2 cm into the queen's vagina, immediately pulled out, rolled onto a glass slide, stained with Diff-Quik (Dade Ltd, Milan, Italy) and examined at 40X and 100X. Proestrus and estrus were diagnosed based on percentage of keratinized vaginal epithelial cells (<50 % and >50 %, respectively) accompanied by heat signs, whereas anestrus was defined by presence of small amounts of non-keratinized cells. Interestrus (the phase between two estruses without ovulation) was diagnosed based on presence of a mixed population of keratinized (<50 %) and non-keratinized cells with P4 < 2.0 ng/ml. Diestrus was diagnosed whenever P4 was >2.0 ng/ml. Only queens not in estrus or diestrus were treated: queens with more than 50 % keratinized vaginal epithelial cells (estrus) or with P4 ≥ 2.0 ng/ml (diestrus) were not enrolled in the study.

On day 0 all queens were administered a 9.4 mg deslorelin implant (Suprelorin™, Virbac, Carros, France). Deslorelin implants were inserted randomly in 2 different subcutaneous localizations: interscapular (IS - in between the dorsal aspects of the shoulder blades) and periumbilical (UMB - about 1.0 cm cranial to the umbilical scar). In order to monitor efficacy and length of treatment, queens were periodically checked for general (clinical exam, BW) and reproductive health (reproductive behavioral history, vaginal smear and blood collection for hormonal assay). General and reproductive health were checked weekly during the first month, then every 2 months until 6 months and then every 6 months with the exception of blood collection for P4 assay which was done only at 0 (pre-treatment) and 60 days post-treatment (PT), and then every 6 months. At each visit owners were interviewed with questions on general and reproductive behavior. Resumption of fertility was based on observation of a >50 % keratinized vaginal smear and/or presence of oestrous behavior [12] and/or on ovulation documented by high (≥2.0 ng/ml) serum P4 concentration and/or evidence of mating. Queens were ovariectomized upon owners' request, following return to fertility and ovarian histology was carried out in order to confirm return to normal reproductive function. After fixation of the ovaries in 10 % paraformaldehyde solution (Sigma-Aldrich, Milan, Italy), 4 μm serial sections of each ovary stained with hematoxylin and eosin were examined with an optical microscope (Olympus BX40, Segrate, Italy) to evaluate ovarian parenchyma.

Statistical Analysis - Data collected was analyzed using ANOVA (GLM procedure of SIGMASTAT 2.03). Normality of the quantitative variables was tested using a Shapiro-Wilk test. Duration of treatment effect was investigated with a Two-Way ANOVA (GLM procedure, SIGMASTAT 2.03) using time post treatment (PT) (0 = day of implant, 1 = 1–30 days PT, 2 = 31–60 days PT, 3 = 61–180 days PT, 4 = 181–360 days PT, 5 = 361–600 days PT, 6 = 601–740 days PT, 7 = 741–1158 days PT) and implant localization (IS vs UMB) as independent variables, and BW, vaginal epithelial cells and P4 concentrations as dependent variables. When more than one BW, P4 concentration or keratinized cells percentage value was available for each period per cat, the latest observation of each period was used for the calculations. Whenever serum P4 was <0.1 ng/mL (minimum detection level), it was replaced as 0 ng/ml, for calculation purposes. Pearson's correlation was calculated for all parameters. Significance was set as $P < 0.05$. Differences on P4 concentrations were investigated with a one-way ANOVA, considering all the cats of the study irrespective of implant localization and using time PT as independent variable, and BW, vaginal epithelial cells and serum P4 concentrations as dependent variables. A two-sample T test was used to analyze duration of treatment between treatment groups and a paired-sample T test to compare BW before and after treatment.

3. Results

All queens were in good general health at the beginning of the study showing normal clinical, hematological and biochemical parameters. Deslorelin implants were placed in the IS and the UMB areas in 8 and 6 queens, respectively. The difference in group allocation was due to some clients objecting to UMB placement. Twelve/14 queens remained in good general health throughout the study. Queen n. 1 was admitted 18 months PT due to signs of vomiting and depression, had a severe leukopenia as the only abnormality on hematology and biochemistry, was treated with fluids and antibiotics and discharged 3 days later in good conditions. Queen n. 7 was found to have an eosinophilic granuloma on the tongue 12 months PT, was treated with monthly SC administrations of methylprednisolone acetate until the end of the study and was normal throughout the rest of the study. None of the queens showed any treatment related side effects. No local reaction at any implant injection site was observed in any treated queens.

The follow-up monitoring period ranged between 525 and 1140 days PT. Three queens were followed up for shorter periods: queens n. 14, 4 and 6 were hit by a car and died at 460-, 837- and 761- days PT, respectively. Nevertheless, these queens' data was used for the BW, serum P4 and percentage of keratinized cells analysis during the periods in which these subjects were still being followed. With regard to duration of efficacy, queen n.14 was not considered as she abandoned the study too early. Not all periodic checks could be made on time due to lack of owner's compliance.

Cat BW, serum P4 concentration, % of keratinized cells on vaginal smears and reproductive behavior during the study are shown in Figs. 1-3 and Table 1, respectively. The Shapiro-Wilk test revealed a non-normal distribution of the "variables P4 concentration" and "percentage of keratinized cells" in some of the study periods. Thus, these variables were treated non-parametrically. Average values of BW and median serum P4 and percentage of keratinized cells are shown in Table 2. Duration of efficacy of treated queens is reported in Table 3.

Vaginal smears and reproductive behavior - All owners noted a sharp decrease in reproductive behavior of treated cats already at 2 months post-implantation. During the first month PT 6/14 queens showed evidence of ovarian activity based on vaginal cytology: in queens n° 9, 12 and 13 (IS) a ≥50 % keratinized vaginal smear was observed 4, 24 and 7 days PT, while in queens n° 2, 3 and 8 (UMB) presence of ≥50 % keratinized vaginal smear was observed 10, 10 and 32 days PT, respectively (table n°1). No evidence of reproductive behavior was reported by cat owners during periods 2, 3 and 4 in both IS and UMB queens. Evidence of heat signs or vaginal cytology indicative of estrus was reported for IS and UMB queens from period 5 and period 6 onwards, respectively.

Post-treatment fertility – Breeding was first noticed for one IS queens

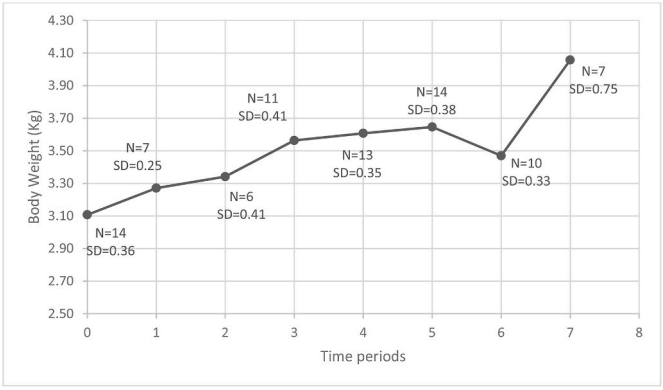

Fig. 1. Mean body weight (BW) and standard deviation (SD) in 14 adult queens treated with a subcutaneous 9.4 mg deslorelin. The study is divided into 7 time periods: 0 = day of implant, 1 = 1–30 days post treatment (PT), 2 = 31–60 days PT, 3 = 61–180 days PT, 4 = 181–360 days PT, 5 = 361–600 days PT, 6 = 601–740 days PT, 7 = 741–1158 days PT. N = number of subjects for which the body weigh was available.

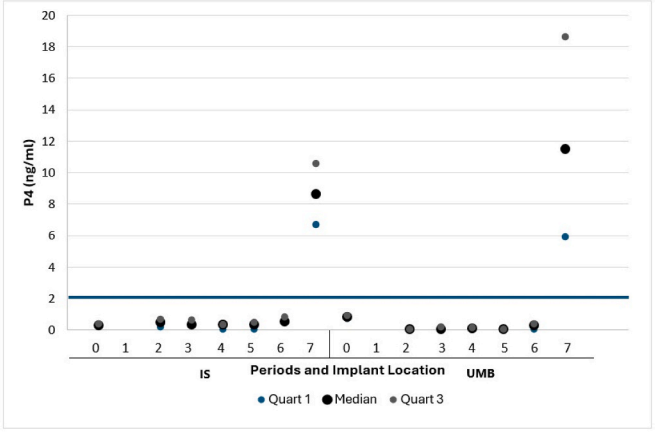

Fig. 2. Median, first and third quartiles values (Quart 1 and Quart 3, respectively) of serum progesterone (P4) in 14 adult queens treated with a subcutaneous 9.4 mg deslorelin implant in 2 different locations, interscapular (IS) and periumbilical (UMB). The study is divided into 7 time periods: 0 = day of implant, 1 = 1–30 days post treatment (PT), 2 = 31–60 days PT, 3 = 61–180 days PT, 4 = 181–360 days PT, 5 = 361–600 days PT, 6 = 601–740 days PT, 7 = 741–1158 days PT.

in period 6 and for 3 UMB queens in period 7 whereas normal pregnancy happened for both groups at period 7. Eight/13 (n. 1, 2, 3, 5, 9, 10, 11 and 14) queens resuming ovarian function were observed by their owners to mate; 6 of these (n. 1, 2, 3, 5, 9 and 10) conceived and either queened live kittens (n. 1, 5, 9 and 10) or underwent ovariohysterectomy while pregnant as per owner's request (n. 2 and 3). Queen n. 9 produced one litter of 3 at day 759 PT and when spayed at day 827 PT was again pregnant with 7 fetuses.

Body weight – Twelve/14 queens gained weight during treatment. The remaining 2 finished the study with the same weight registered before treatment. Eight queens (IS = 1, 5, 9, 10, 12 and 13; UMB = 2 and 3) expressed substantial BW increase towards the end of the study. Six of them (IS = 1, 5, 9, 10; UMB = 2 and 3) were pregnant at that moment. A significant difference was found between the weight at the time of implantation and the weight at the last appointment in each group (IS $P < 0.01$ and UMB $P = 0.023$) and in overall ($P < 0.01$).

Serum P4 - The concentration of P4 remained always around basal levels (0.0–1.1 ng/ml) during the monitoring period, began to increase after 783 days (queen n°1) and was higher during period 7 (730–1158 days PT) than at any other period ($P < 0.001$) (figure n° 2, table n° 2).

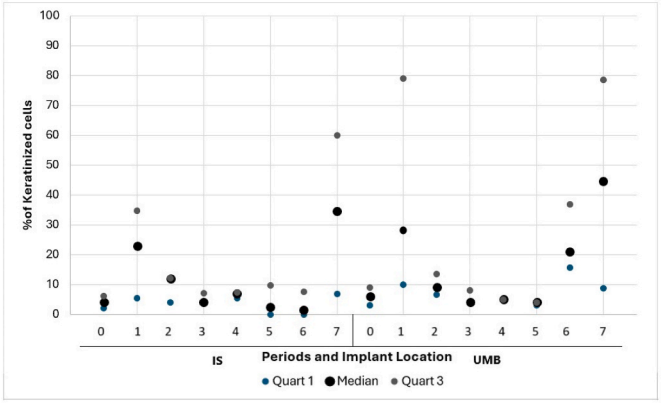

Fig. 3. Median, first and third quartiles values (Quart 1 and Quart 3, respectively) of percentage (%) of keratinized cells in 14 adult queens treated with a subcutaneous 9.4 mg deslorelin implant in 2 different locations, interscapular (IS) and periumbilical (UMB). The study is divided into 7 time periods: 0 = day of implant, 1 = 1–30 days post treatment (PT), 2 = 31–60 days PT, 3 = 61–180 days PT, 4 = 181–360 days PT, 5 = 361–600 days PT, 6 = 601–740 days PT, 7 = 741–1158 days PT.

Ovarian histology - All ovaries were normal; corpora lutea were observed on the ovaries of pregnant queens and queens who had been bred, while follicles (but no corpora lutea) were observed on the ovaries of queens who had showed signs of heat after the end of treatment efficacy but had not been mated.

Implantation site effect - No significative differences were observed for BW, percentage of keratinized cells, serum P4 and reproductive behaviour in each period between the two different implantation sites. Duration of efficacy was not significantly different ($P = 0.57$) depending on implant location: UMB queens (822.4 ± 194.4) and IS queens (768.6 ± 135.2).

4. Discussion

Deslorelin implants were well tolerated in all queens, without any evidence of erythema, dermatitis or prolonged blood loss from both injection sites. Conversely, some authors reported slight and temporary local reactions in a small proportion of queens implanted in the IS site [13] – cutaneous swelling [4], edema [8], pyodermitis [6] and an erosive lesion due to scratching [8]. Although we did not check hematology and biochemistry at the end of the study, all treated cats were in good general health conditions at the time fertility was regained based on history and clinical exam as well as on evidence of mating, conception and parturition. Adequate health status after treatment with a 4.7 mg deslorelin implant in cats has already been demonstrated by other authors either by clinical, behavioral observations or by hematology and biochemistry values within range [4,9–11,13–17]. Because of the high variability of effect of deslorelin in queens, timing of resumption of fertility is hard to predict. Estrous behavior in queens with access to outside may be difficult to observe and therefore queens may start roaming when the owner is away, which poses a health threat. Three of our queens were hit by a car towards the end of the study which may suggest that they came in heat and started roaming before the owner could realize it.

The notable increase in BW observed at the end of the study around 800-day PT (figure n. 1) is presumably due to the fact that 6/14 queens were pregnant when their BW was checked. An increase in BW in queens treated with deslorelin, verified in our study population regardless of the implantation site, should probably be expected because of the similarity of the endocrine milieu between surgically and deslorelin treated cats. However, due to some queens being pregnant and because we did not

Table 1
Progesterone (P4) concentration, estrus cycle stage, keratinized cells (%), body weight (BW) and reproductive data in 14 adult queens treated with a subcutaneous 9.4 mg deslorelin implant in 2 different locations, interscapular (IS) and periumbilical (UMB). The study is divided into 7 time periods: 0 = day of implant, 1 = 1–30 days post treatment (PT), 2 = 31–60 days PT, 3 = 61–180 days PT, 4 = 181–360 days PT, 5 = 361–600 days PT, 6 = 601–740 days PT, 7 = 741–1158 days PT.

Implant Location	Period	ID	Days PT	P4 (ng/dl)	Cycle Stage	% ker. cells	BW (Kg)	Comments
	0	1	0	0.59	interestrus	5	2.9	
		4	0	0.75	anestrus	1	3.4	
		5	0	<0.1	anestrus	8	3.9	
		9	0	0.30	interestrus	8	3.2	
		10	0	0.26	interestrus	3	2.8	
		11	0	0.26	anestrus	0	2.4	
		12	0	0.20	anestrus	6	3.1	
		13	0	0.24	anestrus	4	3.0	
		14	0	0.36	anestrus	2	2.6	
	1	9	4		estrus	88	3.3	
		4	6		anestrus	24	3.6	
		11	7		anestrus	12		
		12	7		anestrus	24		
		13	7		estrus	75		
		14	7		anestrus	7		
		1	8		anestrus	3	2.9	
		10	8		anestrus	4	3.0	
		14	14			5		
		11	24			23		
		12	24			67		
		13	24			23		
		14	30			6		
	2	5	36		anestrus	15	3.9	
		11	47			7		
		12	47			5		
		13	47			5		
		11	60	<0.1	anestrus	12	3.0	
		12	60	0.68	anestrus	4	3.5	
		13	60	0.27	anestrus	0	3.4	
		14	60	0.67	anestrus	12	2.7	
	3	4	63	0.57	anestrus	7	3.7	
		9	64	0.22		10	3.5	
		1	69	0.90	anestrus	4	3.0	
		10	77	<0.1	anestrus	3	3.3	
		5	99	<0.1	anestrus	5	4.2	
		4	125	0.71	anestrus	3	3.9	
INTERSCAPULAR		1	131	1.60	anestrus	4	3.2	
		10	148	0.34	anestrus	7	4.0	
		14	150	0.65	anestrus	0	2.7	
		9	155	0.22	diestrus	7	3.4	
		5	162	0.29	anestrus	4	4.0	
	4	1	182	0.83	anestrus	5	3.5	
		11	200	0.33	anestrus	4	3.0	
		12	200	0.35	anestrus	7	3.5	
		13	200	0.55	anestrus	4	3.6	
		4	209	0.62	anestrus	3	3.8	
		5	218	<0.1	anestrus	3	4.0	
		9	246	0.33	anestrus	8	3.3	
		5	303	<0.1	anestrus	7	4.0	
		4	307	0.28	anestrus	6	4.2	
		10	310	<0.1	anestrus	7	3.8	
		1	327	<0.1	anestrus	8	4.0	
	5	14	365	1.70	anestrus	3	3.0	
		11	410	<0.1	anestrus	0	3.2	
		9	419	0.31	anestrus	8	3.5	
		12	425	0.42	anestrus	20	3.6	
		13	425	0.80	anestrus	0	4.0	
		12	436		proestrus	32		vocalizations
		14	460					deceased
		5	484	<0.1	anestrus	15	4.2	
		4	525	0.30	anestrus	0	4.2	
		12	525	0.35	proestrus	47	4.0	vocalizations
		1	528	<0.1	anestrus	2	3.7	
		10	539		anestrus		3.7	
	6	11	603	0.38	anestrus	3	3.3	vocalizations
		13	603	1.10	anestrus	0	4.0	vocalizations
		9	698		estrus		3.4	breeding
		4	720	0.50	anestrus	21	3.8	
		10	737		anestrus	0	3.4	
	7	11	756		estrus	60	4.0	breeding
		13	756		proestrus	40	3.3	
		5	757	0.22	anestrus	21	4.4	breeding

(*continued on next page*)

Table 1 (*continued*)

Implant Location	Period	ID	Days PT	P4 (ng/dl)	Cycle Stage	% ker. cells	BW (Kg)	Comments
		9	759			5		3 kittens live born
		1	783	9.80	estrus	87	4.1	breeding
		13	783		estrus	60	3.1	breeding
		13	820		estrus	60	3.0	
		11	824		estrus	60	3.7	
		9	827		diestrus	6	5.0	neutered: 7 kittens
		4	837					deceased
		1	850		anestrus			4 kittens live born
		5	861	12.50	diestrus	5	5.0	3 kittens live born
		1	910	4.70	diestrus	9	4.5	
		10	1010		estrus	70	3.3	breeding
		10	1085					1 kitten live born
	0	**2**	0	0.61	anestrus	3	3.4	
		3	0	1.00	anestrus	6	3.3	
		6	0	<0.1	anestrus	12	3.4	
		7	0	<0.1	interestrus	9	3.1	
		8	0	<0.1	interestrus	3	3.0	
	1	**6**	6		interestrus	28	3.6	
		2	10		estrus	79	3.2	
		3	10		estrus	95	3.3	
		7	18		anestrus	3		
		8	18		anestrus	3		
		7	25		anestrus	4		
		8	25		anestrus	10		
	2	**7**	32		anestrus	6		
		8	32		estrus	74		
		7	37		anestrus	4		
		8	37		anestrus	18		
		6	40	<0.1	anestrus	9	3.6	
	3	**2**	67	0.25	anestrus	12	3.4	
		3	67	0.47	anestrus	5	3.4	
		7	99	<0.1	anestrus	3	3.8	
		8	99	<0.1	anestrus	4	3.8	
		6	103	<0.1	anestrus	1	3.8	
		2	127	0.21	anestrus	8	3.3	
		3	127	0.78	anestrus	14	3.2	
		6	166	<0.1	anestrus	4	3.9	
	4	8	210		anestrus	5	3.8	
		2	225	<0.1	anestrus	7	3.0	
PERIUMBILICAL		3	225	0.21	anestrus	2	3.1	
		6	299		anestrus	5	3.7	
		2	310	<0.1	anestrus	5	3.3	
		7	314		anestrus	9	3.7	
		8	314		anestrus	5	3.7	
	5	2	480	<0.1	anestrus	3	3.2	
		3	480	0.32	anestrus	4	3.3	
		6	488	<0.1	anestrus	23	3.9	
		7	504	<0.1	anestrus	4	3.3	
		8	504	<0.1	anestrus	3	3.8	
	6	8	609	<0.1	estrus	84		
		7	642	<0.1	anestrus	0	3.1	
		8	642		interestrus	11	3.0	
		6	655	0.41	anestrus		3.9	
		3	659	0.37	anestrus	21	3.6	
		2	660		anestrus		3.3	
		2	730	0.30	anestrus	21	3.2	
	7	6	761					deceased
		3	763		anestrus	7	3.4	
		2	801					breeding
		3	801					breeding
		2	810	11.50	diestrus	2	3.9	neutered: 3 kittens
		3	829	25.70	diestrus	11	3.8	neutered: 3 kittens
		7	840		anestrus	3	3.1	
		8	840	0.29	estrus	78	3.0	
		7	974		anestrus	2	3.2	
		7	1140		estrus	80	3.2	

have a control group no conclusions can be drawn about the increase in BW following treatment with a 9.4 mg deslorelin implant in queens.

A decrease in estrous behavior following implantation was very evident in the queens of our study. Average vaginal keratinization peaked during the first month PT, reached its minimum between 6- and 12-months PT, and started increasing again during the second year PT (Figure n. 3). Based on vaginal cytology, incidence of post implantation estrus in the queens of our study was 43 % (6/14). In the study by Toydemir et al. [8], all queens treated with a 9.4 mg deslorelin implant showed an increase in fecal estradiol compatible with an estrus-like pattern. Only 2 queens developed behavioural estrus. No post-implantation vaginal cytology was performed [8]. In two other studies using the 4.7 mg implant an incidence of 10 % [4] and 40 % [6] of estrous behavior in treated queens was also observed, but in both

Table n°2
Median and Interquartile Range (IQR) values of serum progesterone (P4) and % of keratinized cells and average and standard deviation (SD) of body weight (BW) (kg) of 14 adult queens treated with a subcutaneous 9.4 mg deslorelin implant in 2 different locations, interscapular (IS) and periumbilical (UMB). The study is divided into 7 time periods 0 = day of implant, 1 = 1–30 days post treatment (PT), 2 = 31–60 days PT, 3 = 61–180 days PT, 4 = 181–360 days PT, 5 = 361–600 days PT, 6 = 601–740 days PT, 7 = 741–1158 days PT.

	Periods	P4 (ng/ml)	+/−IQR	Ker. Cells (%)	+/−IQR	BW (Kg)	+/−SD
INTERSCAPULAR	0	0.26	0.12	4.00	4.00	3.03	0.44
	1	–	–	23.00	29.25	3.20	0.32
	2	0.47	0.47	12.00	8.00	3.29	0.48
	3	0.34	0.36	4.00	3.00	3.54	0.47
	4	0.31	0.34	7.00	1.75	3.70	0.36
	5	0.31	0.46	2.50	9.75	3.71	0.41
	6	0.50	0.36	1.50	7.50	3.58	0.30
	7	8.60	3.90	34.50	53.25	3.95	0.73
PERIUMBILICAL	0	0.81	0.20	6.00	6.00	3.24	0.18
	1	–	–	28.00	69.00	3.37	0.21
	2	0.00	0.00	9.00	7.00	3.60	0.00
	3	0.00	0.21	4.00	4.00	3.58	0.28
	4	0.11	0.11	5.00	0.00	3.47	0.33
	5	0.00	0.00	4.00	1.00	3.50	0.32
	6	0.30	0.37	21.00	21.00	3.36	0.38
	7	11.50	12.71	44.50	69.75	3.48	0.44

Table n° 3
Duration of efficacy expressed as days post treatment (PT) in 13 adult queens administered a subcutaneous 9.4 mg deslorelin implant in 2 different locations, interscapular (IS) and periumbilical (UMB). Queen n°14 was lost to follow-up early in the course of the study, therefore her data is not considered here. No significative difference of duration of efficacy between different implant location was observed.

	ID	Duration of efficacy (days)	Average ± SD	Average ± SD
INTERSCAPULAR	12	525	768.6 ±	789.3 ±
	9	698	135.2	154.9
	5	757		
	1	783		
	13	783		
	11	756		
	4	837		
	10	1010		
PERIUMBILICAL	8	609	822.4 ±	
	6	761	194.4	
	2	801		
	3	801		
	7	1140		

cases some queens were implanted in diestrus, which is known to block the flare-up effect [4,18] while queens treated in interestrus or anestrus come into heat shortly after implantation [11]. It is possible that some queens in our study experienced an estradiol surge and even ovulated without manifesting heat, as previously reported [6].

Queen n° 12 at day 436 was considered to be in proestrus due to the manifestation of vocalizations and keratinized cells of 32 %. On day 525, the queen was again classified as being in proestrus, this time with a higher percentage of keratinized cells. Contact with the owner was lost from this point onwards. Although deslorelin-induced anestrus might have been longer, the duration of deslorelin effect in this queen was conservatively judged to be 525 days. The vaginal keratinization detected on day 436 not followed by an estrus is difficult to explain. In the dog, FSH levels increase during mid- to late anestrus potentially indicating the beginning of folliculogenesis, whereas LH rises only prior to the onset of proestrus [19,20]. A similar mechanism might have occurred in cat n°12 on day 436, resulting in a limited estrogen production and consequently in vaginal epithelial cells keratinization and short-lasting signs of estrus behavior.

Serum P4 was significantly higher at the end of the study (period 7) in comparison to all other periods 0, 2, 3, 4, 5 and 6 (no blood sample was collected during period 1). Ovarian P4 production reflects ovulation taking place following mating, thereby demonstrating that the hypothalamic-pituitary-ovarian axis resumed its normal function following treatment with deslorelin. PT uterine health could not be assessed, although normal cyclicity was observed in all 11 queens completing the study, of which 8 were mated and 6 conceived (4 queened normally and 3 - including 1 that had queened before - were ovariohysterectomized during the second half of pregnancy).

Duration of efficacy of the 9.4 mg deslorelin implant was calculated extrapolating data on reproductive behavior, evidence of >50 % keratinization of vaginal epithelial cells, serum P4 concentration >2.0 ng/ml or pregnancy diagnosis. On average, duration of action of the 9.4 mg deslorelin implant in the queens of our study was 790 ± 155 days (range 525–1140 days) (Table n° 3). Had queen n°12 (follow-up lost after day 525) not been included in the statistical analysis, the average duration of effect for the IS group would have been 803 ± 100 days (more similar the UMB group: 822 ± 194 days), and for the overall population 811 + 139 days. The duration of effect is similar to the one observed in male cats treated with 9.4 deslorelin implant, in which resumption of testicular activity was reached between 750 and 850 days (average 805 days) [9]. Such duration is longer than the 4.7 mg implant whose average reported efficacy in queens is 680 days [4] although not as long as one would expect when comparing single vs double dosage. A reason for such a small difference between the two different dosages of deslorelin in queens could be individual variability (as underlined in the queens of our study by treatment durations ranging from 14 to 39 months) or resumption of ovarian function during decreasing photoperiod – as reported following removal of a deslorelin implant [21]. Unfortunately, the photoperiod at the end of the study was not assessed for our queens. Queen 7, whose duration of efficacy was the longest (1140 days), had been treated with methylprednisolone acetate from month 12 onwards until the end of the study. In mice, glucocorticoids impair ovarian and follicular development [22]. Thus, the methylprednisolone treatment of this queen may have contributed to some extent to a delay in resumption of ovarian activity after deslorelin.

5. Conclusion

Treatment with a 9.4 mg deslorelin implant in female cats in this study was effective for an average period of 790 days (range 525–1140). Implantation site did not influence duration of effect. No skin lesion or reaction were observed in any of the treated queens independently of location of administration. Upon cessation of the implant's effect ovarian function resumed in all queens completing the study (13/14), with 8/13 queens being mated and 6 of them conceiving which confirmed full reversibility of the effect of the 9.4 mg deslorelin implant

in queens. Overall, the 9.4 mg deslorelin acetate implant was a safe, efficient, reversible and long-lasting form of pharmacological sterilization in female cats. Further studies are needed to ascertain the reason behind the variability its duration of effect in queens.

CRediT authorship contribution statement

A. Baldan: Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **M. Pereira:** Writing – review & editing, Writing – original draft, Visualization, Validation, Formal analysis. **V. Pedrotti:** Methodology, Investigation, Data curation. **S. Ferro:** Writing – review & editing, Methodology. **D. Gelli:** Resources. **C. Milani:** Resources, Methodology. **Mollo A:** Resources. **C. Fontaine:** Writing – review & editing, Funding acquisition. **G.M. De Benedictis:** Methodology. **C. Stelletta:** Writing – review & editing, Resources, Formal analysis. **S. Romagnoli:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

This study was approved by the University of Padova Ethics Committee (Project n ° 64 bis/2011).

Funding

Virbac Animal Health, Carros, France provided the 9.4 mg deslorelin implants for this study.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Stefano Romagnoli reports was provided by Virbac SA. Stefano Romagnoli reports a relationship with Virbac SA that includes: funding grants and speaking and lecture fees. Co-author Stefano Romagnoli has received free 9.4 mg deslorelin implants for the study considered If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Romagnoli S, Krekeler N, de Cramer K, Kutzler M, McCarthy R, Schafer-Somi S. WSAVA Guidelines for the control of reproduction in dogs and cats. J Small Anim Pract 2024.

[2] Romagnoli S, Geretto N, Stelletta C, Milani C, Gelli D, Mollo A. Prolonged suppression of reproductive activity in male cats with a 4.7 Mg implant of deslorelin. In: 4th international symposium on non-surgical contraceptive methods of pet population control; 2010.

[3] Trigg TE, Wright PJ, Armour AF, Williamson PE, Junaidi A, Martin GB, et al. Use of a GnRH analogue implant to produce reversible long-term suppression of reproductive function in male and female domestic dogs. J Reprod Fertil Suppl 2001;57:255–61.

[4] Goericke-Pesch S, Georgiev P, Atanasov A, Albouy M, Navarro C, Wehrend A. Treatment of queens in estrus and after estrus with a GnRH-agonist implant containing 4.7 mg deslorelin; hormonal response, duration of efficacy, and reversibility. Theriogenology 2013 Mar;79(4):640–6.

[5] Munson L, Bauman JE, Asa CS, Jöchle W, Trigg TE. Efficacy of the GnRH analogue deslorelin for suppression of oestrous cycles in cats. J Reprod Fertil Suppl 2001;57:269–73.

[6] Ackermann CL, Volpato R, Destro FC, Trevisol E, Sousa NR, Guaitolini CRF, et al. Ovarian activity reversibility after the use of deslorelin acetate as a short-term contraceptive in domestic queens. Theriogenology 2012 Sep;78(4):817–22.

[7] Pisu MC, Romagnoli S. Impiego clinico di un impianto di deslorelin nel gatto. Veterinaria, Anno 26, n 1, Febbraio 2012 2012;1:9–15. 9–15. Veterinaria, Anno 26. 2012 Feb.

[8] Toydemir TSF, Kiliçarslan MR, Olgaç V. Effects of the GnRH analogue deslorelin implants on reproduction in female domestic cats. Theriogenology 2012 Feb;77(3):662–74.

[9] Romagnoli S, Baldan A, Ferro S, Righetti C, Scenna L, Gabai G, et al. Length of efficacy and effect of implant location in adult tom cats treated with a 9.4 mg deslorelin subcutaneous implant. J Feline Med Surg 2019 Jun 1;21(6):507–19.

[10] Ferré-Dolcet L, Carniello L, Ferro S, Cattai A, Romagnoli S, Mollo A. Interval between removal of a 4.7 mg deslorelin implant after a 3-, 6-, and 9-month treatment and restoration of testicular function in tomcats. Animals 2020 Sep 2;10(9):1559.

[11] Ferré-Dolcet L, Ferro S, Contiero B, Andretta F, Cattai A, Fontaine C, et al. Resumption of ovarian activity following removal of a 4.7 mg deslorelin implant in queens. Reprod Domest Anim 2022 Jan 10;57(1):3–9.

[12] Griffin B. Prolific cats : the estrous cycle. Compendium for continuing education of practicing veterinarians 2001 Dec;23(12):1049–57.

[13] Fontaine C. Long-term contraception in a small implant: a review of Suprelorin (deslorelin) studies in cats17. Journal of Feline Medicine and Surgery. SAGE Publications Ltd; 2015. p. 766–71.

[14] Celotto D, Pereira MC, Romagnoli S. Effects on general health and behaviour of a chronic treatment with a 9.4 mg deslorelin implant in female cats. University of Padova; 2023.

[15] Gontier A, Youala M, Fontaine C, Raibon E, Fournel S, Briantais P, et al. Efficacy and safety of 4.7 mg deslorelin acetate implants in suppressing oestrus cycle in prepubertal female dogs. Animals 2022 Dec 12;12(24):3504.

[16] Amaral J, Briantais P, Fontaine C, Rigaut D. Efficacy and safety of 4.7 mg deslorelin acetate implants as a neutering option in male cats: a large-scale multicentre randomised controlled study. Animals 2023 Feb 1;13(3).

[17] Goericke-Pesch S, Georgiev P, Antonov A, Albouy M, Wehrend A. Clinical efficacy of a GnRH-agonist implant containing 4.7 mg deslorelin, Suprelorin®, regarding suppression of reproductive function in tomcats. Theriogenology 2011 Mar;75(5):803–10.

[18] Romagnoli S, Ferre-Dolcet L. Reversible Control of Reproduction in Queens: mastering the use of reproductive drugs to manipulate cyclicity. J Feline Med Surg 2022 Sep 24;24(9):853–70.

[19] Kooistra HS, Okkens AC, Bevers MM, Popp-Snijders C, van Haaften B, Dieleman SJ, et al. Concurrent pulsatile secretion of luteinizing hormone and follicle-stimulating hormone during different phases of the estrous cycle and anestrus in beagle bitches. Biol Reprod 1999 Jan 1;60(1):65–71.

[20] Olson PN, Bowen RA, Behrendt MD, Olson JD, Nett TM. Concentrations of reproductive hormones in canine serum throughout late anestrus, proestrus and Estrus1. Biol Reprod 1982 Dec 1;27(5):1196–206.

[21] Ferré-Dolcet L, Ferro S, Contiero B, Andretta F, Cattai A, Fontaine C, et al. Resumption of ovarian activity following removal of a 4.7 mg deslorelin implant in queens. Reprod Domest Anim 2022 Jan 1;57(1):3–9.

[22] Wei Li, Meng Zhang, Shen Liu. Corticosterone injection impairs follicular development, ovulation and steroidogenesis capacity in mice ovary. Animals 2019 Nov 29;9(12):1047.