



# Semen quality and interval to sterility in tom cats treated with a 9.4 mg deslorelin implant

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## Abstract

**Objectives** Gonadotropin-releasing hormone (GnRH) agonists like deslorelin are being increasingly used in tom cats for their efficacy in controlling reproductive behaviour and fertility. Deslorelin implants have been widely available in Europe since 2008. Little, if anything, is known about the interval between treatment and onset of sterility, as well as semen quality, after treatment in tom cats. The purpose of this study was to investigate semen quality and interval treatment sterility in tom cats treated with a 9.4 mg deslorelin implant.

**Methods** Fifteen healthy adult tom cats were treated with a 9.4 mg deslorelin implant (Suprelorin 12). On each cat, semen collection and a GnRH stimulation test (intramuscular administration of 50 µg gonadorelin [Fertagyl], followed by blood sampling 1 h later, to assay serum testosterone) were performed on the first consultation and then repeated every 15 days until complete sterility was achieved. Semen collection was performed by introducing a 14 cm, open-end feline catheter (Argyle) 9 cm into the distal urethra 10 mins after sedation by intramuscular injection of 100 µg/kg medetomidine (Domitor).

**Results** Semen collection was not successful in all cats at each attempt. In the first month after treatment, the semen of only four cats could be evaluated, while the semen of eight cats could be evaluated during the second and third months of the study. Semen quality (ejaculate volume, progressive motility and morphological abnormalities) improved slightly during the first 19–25 days in 2/4 cats, and in 1/4 cats motility was still very high (80%) 25 days post-treatment (PT), but we have no data of fertility prior to treatment in this cat. The last cat never produced spermatozoa. Subsequently, semen quality gradually worsened in all cats from 30 days onwards. At 70 days PT, one cat was still potentially fertile. After 72 days all cats were sterile.

**Conclusions and relevance** Semen quality increased slightly in treated cats during the first month after treatment, and then gradually decreased over the following months. Complete sterility was reached within 40–72 days following implantation.

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## Introduction

Non-surgical control of cat reproduction is becoming increasingly important, with attention progressively shifting from the use of steroids to drugs that actually block steroid production, such as gonadotrophin-releasing hormone (GnRH) agonists. Deslorelin is a GnRH agonist that has become widely available in Europe and Australia for use in male dogs.<sup>1–4</sup> Clinical research has shown that extra-label use of deslorelin in male cats is as safe as and effective for a longer time than in male dogs.<sup>5</sup> This is causing an increased interest in the use of deslorelin from cat breeders and owners who may want to temporarily stop their tom cats from roaming and

impregnating queens in the cattery. Once a cat has been implanted with deslorelin, one of the first questions a cat breeder will ask is how long it takes for a treated cat to

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become sterile. The objective of this paper is to report on semen quality and interval from treatment to sterility in cats treated with a 9.4 mg deslorelin implant.

## Materials and methods

This study was approved by the University of Padova Ethics Committee (Project 64 bis/2011). Fifteen adult, privately owned tom cats of various breeds (13 Europeans, one Birman and one Turkish Angora), age (5 months–5 years) and weight (3.0–5.8 kg) were used with owners' consent (Table 1). Health conditions were assessed at the beginning of the study through clinical and reproductive examinations, including semen collection, which was performed before and every 2 weeks for the first 3 months following treatment with a 9.4 mg deslorelin implant (Suprelorin 12; Virbac). At each visit a GnRH stimulation test was also performed in each cat through intramuscular (IM) administration of 50 µg gonadorelin (Fertagyl; Intervet), followed by blood sampling 1 h later to assay testosterone. The sequence of events of the protocol of this study is summarised in Table 2. Serum testosterone was assayed regularly during the study but these data will not be shown in this paper. Semen collections were performed every 2 weeks during the first 2 months until complete sterility was observed.<sup>6</sup> Sterility was diagnosed based on total number of spermatozoa  $\leq 0.5 \times 10^6$ , sperm motility  $< 20\%$  and normal morphology  $< 20\%$ .

Semen collection was performed by introducing a 14 cm, open-end feline catheter (Argyle; Covidien) 9 cm into the distal urethra 10 mins after injecting cats intramuscularly with 100 µg/kg medetomidine (Domitor; Pfizer). Once semen was collected, cats were immediately awakened with atipamezole (Antisedan; Pfizer). Semen was transferred from the catheter into a 1.0 cc Eppendorf vial, its volume estimated using a Gilson 20 µl micropipette, then the sample was diluted with phosphate-buffered saline to obtain a volume of 100 µl. Progressive motility was estimated under light microscopy at 10×. Then semen was diluted 1:40 with saline to obtain a volume of 100 µl, in order to count sperm in a Bürker chamber. Sperm morphology was assessed on a Diff-Quik (Dade Spa)-stained semen smear under light microscopy at 60×. Morphology was evaluated by observing 100 spermatozoa and considering the following characteristics: detached heads, head abnormalities, abnormalities of the neck, abnormalities of the tail and the presence of cytoplasmic droplets. Data collected and analysed using the GLM procedure of SIGMASTAT 2.03 software, with one-way ANOVA (repeated measures). For statistical purposes, the study period was divided into five intervals: time 0 = day 0, time 1 = days 19–25, time 2 = days 30–41, time 3 = days 42–70 and time 4 = days 72–111.

**Table 1** Identification and signalment for 15 cats treated with a 9.4 mg deslorelin implant (Suprelorin 12; Virbac)

| Cat | Breed          | Age (months) |
|-----|----------------|--------------|
| 1   | European       | 5            |
| 2   | European       | 48           |
| 3   | European       | 8            |
| 4   | European       | 8            |
| 5   | European       | 12           |
| 6   | European       | 52           |
| 7   | European       | 52           |
| 8   | European       | 9            |
| 9   | European       | 9            |
| 10  | Turkish Angora | 8            |
| 11  | European       | 24           |
| 12  | Birman         | 24           |
| 13  | European       | 9            |
| 14  | European       | 7            |
| 15  | European       | 6            |

**Table 2** Sequence of events on any given day in which a semen collection was performed in each of the 15 cats receiving the 9.4 mg deslorelin implant

| Time (mins) | Event                                   |
|-------------|---|
| 0           | Clinical examination                    |
| 20          | First (basal) blood sampling            |
| 25          | GnRH administration                     |
| 70          | Sedation with medetomidine              |
| 80          | Semen collection                        |
| 85          | Second (poststimulation) blood sampling |
| 90          | Administration of atipamezole           |

GnRH = gonadotropin-releasing hormone

## Results

Results of the timing of collections, motility, morphology and concentration of semen samples of the 15 cats of the study, as well as average volume ( $\pm$  SD), motility and morphology, are reported in Tables 3 and 4, respectively. Semen collection with medetomidine was simple and relatively easy to perform, although in 2/35 collections the catheter could not be inserted through the urethral meatus and/or advanced through the urethra, possibly due to a urethral spasm. Strong contractions of leg muscles were observed in three cats (cats 2, 7 and 13). This was observed in another study using a dose of 50 µg/kg of medetomidine, but the contractions disappeared using a higher dose (130 µg/kg).<sup>7</sup> An incomplete sedation may have caused the strong muscle contractions noted in this study. One episode of salivation was observed in cat 2, which was successfully treated with subcutaneous administration of an antiemetic drug (maropitant citrate, 1 mg/kg [Cerenia; Pfizer]).

Semen collection could be performed in most cases, but it could not be achieved five times/cat (as originally

**Table 3** Days post-treatment (PT), presence (P) or absence (A) of spermatozoa in the sample; volume of ejaculate; and sperm quality parameters in 15 cats,\* treated with a 9.4 mg deslorelin implant (Suprelorin 12, Virbac)

| Time period | Cat | Days PT | Sperm | Semen volume | Motility (%) | Normal morphology (%) | Abnormal morphology (%) | Head abnormality | Neck abnormality | Tail abnormality | Cytoplasmic droplet | Detached head | Concentration (spermatozoa/ml) |                      |
|-------------|-----|---------|-------|--------------|--------------|-----------------------|-------------------------|------------------|------------------|------------------|---------------------|---------------|--------------------------------|----------------------|
| 0           | 1   | 0       | P     |              | 40           | 60                    | 40                      | 1                | 2                | 2                | 19                  | 15            | 42 × 10 <sup>6</sup>           |                      |
|             | 3   | 0       | P     |              | 50           | 50                    | 50                      | 1                | 6                | 5                | 9                   | 34            |                                |                      |
|             | 4   | 0       | P     | 2            | 50           | 70                    | 30                      | 0                | 5                | 8                | 1                   | 12            |                                |                      |
|             | 6   | 0       | P     | 10           | 60           | 60                    | 40                      | 1                | 4                | 11               | 10                  | 10            | 20 × 10 <sup>7</sup>           |                      |
|             | 8   | 0       | P     | 20           | 40           | 60                    | 40                      | 2                | 6                | 10               | 21                  | 6             | 34 × 10 <sup>7</sup>           |                      |
|             | 9   | 0       | P     | 10           | 50           | 60                    | 40                      |                  |                  |                  |                     |               | 0                              |                      |
|             | 10  | 0       | A     | 2.5          |              |                       |                         |                  |                  |                  |                     |               |                                |                      |
|             | 11  | 0       | P     | 5            | 50           | 60                    | 40                      | 1                | 3                | 7                | 3                   | 29            | 0                              |                      |
|             | 12  | 0       | A     | 10           |              |                       |                         |                  |                  |                  |                     |               |                                |                      |
|             | 13  | 0       | A     | 1            |              |                       |                         |                  |                  |                  |                     |               | 0                              |                      |
|             | 14  | 0       | A     | 15           |              |                       |                         |                  |                  |                  |                     |               | 0                              |                      |
|             | 15  | 0       | P     |              |              |                       |                         |                  |                  |                  |                     |               | 0                              |                      |
|             | 1   | 7       | 25    | P            | 5            | 80                    | 70                      | 30               | 0                | 5                | 15                  | 1             | 8                              | 46 × 10 <sup>7</sup> |
|             |     | 8       | 20    | P            | 20           | 80                    | 60                      | 40               | 0                | 4                | 20                  | 0             | 16                             | 52 × 10 <sup>7</sup> |
|             |     | 9       | 20    | P            | 20           | 20                    | 60                      | 40               | 1                | 5                | 8                   | 2             | 27                             | 32 × 10 <sup>7</sup> |
| 10          |     | 19      | A     |              |              |                       |                         |                  |                  |                  |                     |               | 0                              |                      |
| 3           |     | 40      | P     | 1            | 20           | 50                    | 50                      | 1                | 9                | 10               | 0                   | 29            |                                |                      |
| 4           |     | 40      | P     | 5            | 50           | 30                    | 70                      | 0                | 8                | 37               | 4                   | 18            |                                |                      |
| 5           |     | 40      | P     | 1            | 20           | 40                    | 60                      | 0                | 4                | 37               | 4                   | 18            |                                |                      |
| 6           |     | 40      | P     | 5            | 0            | 70                    | 30                      | 1                | 9                | 8                | 0                   | 7             | 70 × 10 <sup>7</sup>           |                      |
| 8           |     | 33      | P     | 2            | 20           | 80                    | 20                      | 0                | 6                | 10               | 0                   | 8             |                                |                      |
| 9           |     | 33      | P     | 2            | 20           | 30                    | 70                      | 0                | 5                | 41               | 0                   | 20            |                                |                      |
| 10          |     | 35      | A     | 15           |              |                       |                         |                  |                  |                  |                     |               | 0                              |                      |
| 11          |     | 41      | P     | 5            | 40           | 60                    | 40                      | 2                | 5                | 28               | 0                   | 10            |                                |                      |
| 2           |     | 65      | A     |              |              |                       |                         |                  |                  |                  |                     |               | 0                              |                      |
| 4           |     | 65      | A     |              |              |                       |                         |                  |                  |                  |                     |               | 0                              |                      |
| 3           |     | 7       | 70    | P            | 10           | 80                    | 90                      | 10               | 2                | 4                | 2                   | 5             | 0                              |                      |
|             | 8   | 48      | P     | 1            | 0            | 30                    | 70                      | 0                | 8                | 22               | 0                   | 40            |                                |                      |
|             | 8   | 62      | A     | 2            |              |                       |                         |                  |                  |                  |                     |               | 0                              |                      |
|             | 9   | 48      | P     | 1            | 0            | 30                    | 70                      | 0                | 0                | 22               | 0                   | 51            |                                |                      |
|             | 9   | 62      | A     | 2.5          |              |                       |                         |                  |                  |                  |                     |               | 0                              |                      |
|             | 11  | 64      | A     | 10           |              |                       |                         |                  |                  |                  |                     |               | 0                              |                      |
|             | 1   | 72      | A     |              |              |                       |                         |                  |                  |                  |                     |               | 0                              |                      |
|             | 4   | 6       | 111   | P            | 2            | 0                     | 60                      | 40               | 0                | 7                | 18                  | 4             | 13                             |                      |
|             |     | 7       | 97    | P            | 10           | 0                     | 30                      | 70               | 2                | 7                | 16                  | 5             | 45                             |                      |

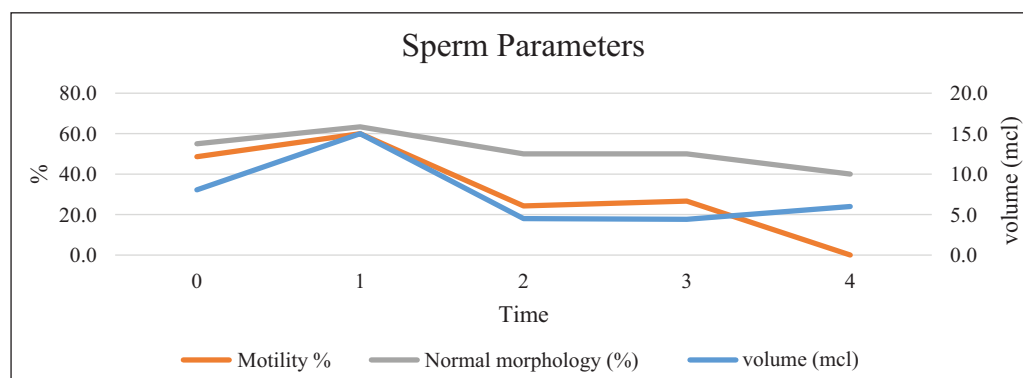
\*PT periods were divided as follows: time 0 = day 0 (treatment); time 1 = PT days 19–25; time 2 = PT days 30–41; time 3 = PT days 42–70; time 4 = PT days 72–111. Semen was collected 10 mins after an intramuscular injection of 100 µg/kg medetomidine (Domitor; Pfizer)

**Table 4** Time changes in semen quality in 15 cats treated with a 9.4 mg deslorelin implant (Suprelorin 12; Virbac)

| Time period* | Motility (%) | Normal morphology (%) | Abnormal morphology (%) | Head abnormality | Neck abnormality | Tail abnormality | Cytoplasmic droplet | Detached heads |
|--------------|--------------|-----------------------|-------------------------|------------------|------------------|------------------|---------------------|----------------|
| 0            | 48.6 ± 6.9   | 60.0 ± 6.3            | 40.0 ± 6.3              | 1.0 ± 0.6        | 4.3 ± 1.6        | 7.2 ± 3.3        | 10.5 ± 8.1          | 17.7 ± 11.2    |
| 1            | 60.0 ± 34.6  | 63.3 ± 5.8            | 36.7 ± 5.8              | 0.3 ± 0.6        | 4.7 ± 0.6        | 14.3 ± 6.0       | 1.0 ± 1.0           | 17.0 ± 9.5     |
| 2            | 24.3 ± 16.2  | 51.4 ± 19.5           | 48.6 ± 19.5             | 0.6 ± 0.8        | 6.6 ± 2.1        | 24.4 ± 14.7      | 1.1 ± 2.0           | 15.7 ± 7.9     |
| 3            | 26.7 ± 46.2  | 50.0 ± 34.6           | 50.0 ± 34.6             | 0.7 ± 1.2        | 4.0 ± 4.0        | 15.3 ± 11.5      | 1.7 ± 2.9           | 30.3 ± 26.8    |
| 4            | 0.0          | 45.0 ± 21.2           | 55.0 ± 21.2             | 1.0 ± 1.4        | 7.0 ± 0          | 17.0 ± 1.4       | 4.5 ± 0.7           | 29.0 ± 22.6    |

Data are mean ± SD

\*Post-treatment (PT) periods were divided as follows: time 0 = day 0 (treatment); time 1 = PT days 19–25; time 2 = PT days 30–41; time 3 = PT days 42–70; time 4 = PT days 72–111



**Figure 1** Average percentage sperm motility, percentage normal morphology and volume ( $\mu\text{l}$ ) of semen samples collected from 12/15 cats before and up to 4 months following treatment with a 9.4 mg deslorelin implant (Suprelorin 12; Virbac). Average values during the monitoring period. Numbers on the horizontal axis refer to the division of experimental time into four periods: time 0 = day 0; time 1 = days 19–25; time 2 = days 30–41; time 3 = days 42–70; time 4 = 72–111

planned) in all 15 cats (Table 3). Owing to the side effects of medetomidine and/or lack of owner compliance, only 1–2 semen collections could be performed in eight cats; 3–5 semen collections could be performed in 7/15 cats (cats 4, 6, 7, 8, 9, 10 and 11); therefore, the following discussion, as well as conclusions, of this study are, in general, based on the results from these seven cats.

Sperm production was monitored for 3 months and in two cats up to 4 months after the initial consultation (prior to implantation) (Figure 1). Within each time interval the number of ejaculates collected was 12/15 cats on day 0 (pretreatment), 4/15 cats on days 19–25, 8/15 cats on days 30–41, 6/15 cats on days 48–70 and 3/15 on days 72–111 (see Table 3).

In the seven cats from which semen was collected regularly (cats 4, 6, 7, 8, 9, 10 and 11 were collected from 3–5 times) throughout the study, the observed changes in sperm characteristics are discussed below.

#### Improvement of semen quality

In three cats (cats 7, 8 and 9), semen was collected as early as 20 days after implantation: semen quality in these cats was very high (cat 7 had a motility of 80%, but

semen could not be collected prior to treatment) or higher than prior to implantation (cats 8 and 9). In cat 8, sperm motility doubled at day 20 compared with the pretreatment value observed at the beginning of treatment, although the percentage of morphological abnormalities was unchanged (60% normal spermatozoa). In this cat, sperm concentration more than doubled during the first 20 days from  $20 \times 10^7/\text{ml}$  to  $52 \times 10^7/\text{ml}$ . In cat 9, sperm motility was decreased at 20 days (from 50% to 20%), despite a reduction of all percentages of morphological abnormalities. Sperm concentration in this cat remained fairly constant during the first 20 days.

#### Worsening of semen quality

After the initial improvement in seminal quality during the first month, semen parameters (motility and normal morphology) progressively worsened from 30 days PT onwards (time periods 2, 3 and 4; see Table 3 and Figure 1). Semen could still be considered potentially fertile (motility  $>20\%$ , normal morphology  $>20\%$ ) on post-treatment days 33, 33, 40, 41 and 70 in cats 8, 9, 4, 11 and 7, respectively (cats 3 and 5, from which semen could be collected less than three times, were still

potentially fertile at 40 days post-implantation [see Table 3]). This was observed despite a relevant reduction in the size of penile spikes and serum testosterone in all five cats (data not shown). Each one of the abovementioned five cats was diagnosed as sterile (motility <20%, normal morphology <20%) at the subsequent semen collection. Cat 6 was already sterile at 40 days post-treatment, and cat 10 never produced sperm before treatment and throughout the study – its semen was always azoospermic, although it regained fertility after the end of the study (data not shown); therefore, semen data from this cat were not included in the overall mean.

Semen was present but spermatozoa were dead in 4/7 cats (cats 6, 8, 9 and 7) on days 40, 48, 48 and 97, respectively. A high incidence of morphological abnormalities (70%) was observed in 3/4 cats. In one of these four cats (cat 6), morphological abnormalities at day 40 were only 30%, but at the subsequent check, at day 111, they increased to 40%.

Total absence of sperm in previously potentially fertile animals was observed in 4/7 cats (cats 4, 8, 9 and 12) on post-treatment days 62–72. In 2/7 cats (cats 6 and 7), dead spermatozoa were still present at 97–111 days PT. The penile spikes were regressing in most cats during the second month PT and disappeared in all cats during the third month PT.

#### Side effects of medetomidine

Semen collection with medetomidine was simple and relatively easy to perform, although several limits were observed. Lack of owner compliance and side effects of medetomidine made it difficult to regularly visit all cats.

Only one semen collection could be performed in 6/15 cats, owing to the cat being lost to follow-up ( $n = 2$ ) and because of the side effects of medetomidine (strong contractions of leg muscles while attempting catheterisation), and/or lack of owner compliance ( $n = 4$ ). Owing to lack of owner compliance, only two semen collections could be performed in 2/15 cats. On the subset of the 7/15 cats with at least three semen collections, a total of 25 semen collections were performed: the 10 missing collections were owing to technical problems relative to inserting the urinary catheter (in one case the catheter could not be inserted through the external urethral orifice; in another case the catheter was inserted but could not be progressed more than 4 cm), strong contractions of leg muscles or lack of owner compliance. Occasionally, the amount of semen recovered was not sufficient to perform the entire semen evaluation: in these cases, semen count was not performed, and therefore only motility and morphology were evaluated.

Statistical analysis showed a significant decrease of motility ( $P < 0.05$ ) between time periods 0 and 4. Instead, with regard to spermatozoan morphology, no statistically significant difference was found. However, a

decreasing trend of normal sperm morphology was observed ( $P = 0.065$ ).

## Discussion

This is the first study to document semen quality in cats treated with a subcutaneous implant of 9.4 mg deslorelin. Prior to treatment onset, semen quality in the cats was comparable with what has been observed by other authors using the medetomidine method for cat semen collection.<sup>7</sup> Sperm concentration was higher in this study than in other studies in which electroejaculation was used.<sup>8</sup> This could be owing to the fact that electroejaculation allows collection of a relevant amount of fluid from the accessory glands, while alpha ( $\alpha$ )-agonists simply displace seminal fluids from the deferens into the urethra.<sup>7,8</sup>

During the first 30 days PT, semen quality showed an increase in 2/4 cats (cats 8 and 9, from which semen was collected during the first month PT). Cat 8 had an increase in both motility (from 40% to 80%) and concentration (from  $20 \times 10^7/\text{ml}$  to  $52 \times 10^7/\text{ml}$ ), while cat 9 had a decrease in the percentage of morphological abnormalities (from 50% to 30%). Semen quality was still very good at day 25 in cat 7 (80% motility, 70% normal morphology,  $46 \times 10^7$  sperm/ml), although, unfortunately, for this cat no semen data are available for day 0 (treatment). In mammals, spermatogenesis is promoted by testosterone.<sup>9</sup> The initial increase in semen quality observed in some of the cats in our study mirrors our observations in dogs implanted with deslorelin,<sup>3</sup> and may have been caused by the stimulating effect of deslorelin on testosterone production by the seminiferous tubules.

During the second month PT, average motility appeared to be decreased (from  $48.6 \pm 6.9\%$  on day 0 [treatment] to  $24.3 \pm 16.2\%$  at days 30–41), and morphological abnormalities tended to increase from  $40 \pm 6.3\%$  (prior to treatment) to  $48.6 \pm 19.5\%$  during the second month PT, and to  $50 \pm 34.6\%$  during the third month PT. As a result, during the second month PT semen quality started to decrease and 3/7 cats were sterile (spermatozoa were still present but all dead) at 40–48 days PT (cats 6, 8 and 9), while 3/7 cats were diagnosed as fertile at 40–70 days PT (cats 4, 7 and 11) and 1/7 cats (cat 10) were azoospermic on three different occasions between day 0 (treatment) and day 35 PT; this cat was probably nearing puberty at the time of treatment (it had already had adult-size penile spikes) and the treatment may have shut down its initial sperm production altogether. This cat subsequently resumed fertility at the end of the study (data not shown). Cats 6 and 7, which had been diagnosed as fertile at 40 and 70 PT days, respectively, still had spermatozoa present, albeit all dead, at 111 and 97 PT days, respectively. The wide range in which treated cats achieved sterility (40–48 to 97–111 days PT) shows the variability of individual response of cats to deslorelin, which has also been demonstrated following the use of the 4.7 mg implant.<sup>5,10–12</sup>

Deslorelin is quite effective in causing sterility in treated tom cats, although the quality of semen obtained from cat 7 at 70 days PT (80% motility and 90% normal morphology) shows that such an effect may, on occasion, be reached well into the third month PT. Despite occasional failures, in general semen collection with medetomidine was easy, fairly reliable and allowed us to assess semen quality over time in all cats in the study. This method of sampling, however, requires access and familiarity with sedative drugs and their antidotes because of the possible occurrence of side effects due to the high dose of medetomidine administered. As non-selective  $\alpha$ -agonists such as medetomidine may induce vomiting, semen collection was not performed when the cat's owner had not properly withheld food for 12 h before sedation.

## Conclusions

The subcutaneous implant of 9.4 mg deslorelin caused an improvement in semen quality in two cats during the first month PT, while complete sterility was reached in all cats between 40 and 70 days PT. This is similar to what has been reported in dogs.<sup>3</sup> Therefore, with regard to cats, it is also important to inform owners that a male cat implanted with 9.4 mg deslorelin may remain fertile up to 70 days PT.

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

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