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Reestablishment of sperm quality after long-term deslorelin suppression in tomcats

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ABSTRACT

The aim of the study was to determine the time after treatment with a 4.7 mg deslorelin implant until Tomcat spermatogenesis activity was restored, and seminal parameters reached pre-implant values. Tomcats (n = 6) were randomly assigned to one of two treatments. Three cats (n = 3) received a deslorelin implant (4.7 mG; Suprelorin®, Virbac, France) in the interscapular subcutaneous region whereas three (n = 3) received no implant and served as control group. Semen samples were collected by electroejaculation every 4 wk from 3 mo before treatment (pretreatment samples) until reestablishment of pre-treatment sperm quality, 32 mo post-implant insertion (PI). Each semen sample was assessed for motility, velocity, concentration, total sperm count, viability, acrosome integrity, plasma membrane integrity and sperm morphology. After semen collection, testicular volume and presence/absence of penile spines were recorded. Additionally, blood samples were taken to measure testosterone concentration. An increase in sperm concentration and total sperm count was present 1 mo PI despite of an abrupt decrease in serum testosterone concentrations after 2–4 weeks. This initial stimulatory effect was followed by a decrease in seminal parameters, reduction of testicular volume and disappearance of penile spines 2 mo PI. A single Suprelorin® 4.7 mg implant suppressed sperm production for 22–25 months. No clinically side effect was observed during the study period. All toms returned to their initial seminal quality 23–28 months after treatment. Therefore, we conclude that Suprelorin® 4.7 mg is a safe option for reversible reproduction control during long periods in tomcats.

1. Introduction

GnRH agonist implants have been used as efficient alternative to surgical castration in dogs and cats (Goericke-Pesch et al., 2011; Novotny et al., 2012; Romagnoli et al., 2012; Lucas, 2014; Novotny et al., 2015). It has been reported that in toms, a single 4.7 mg deslorelin implant was effective in reducing libido, mating behavior and urine marking (Goericke-Pesch et al., 2011; Pisu and Romagnoli, 2012). It was also reported that the effect of deslorelin implants on sexual behavior was highly variable. Pisu and

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Romagnoli (2012), reported that the mean duration to return to normal libido was 15 ± 3 mo, while Goericke-Pesch et al. (2014), observed that normal libido was reached 22–25 mo after treatment. Goericke-Pesch et al. (2011), showed a decreased in testicular volume of approximately 25% 4 weeks after treatment, about 60% at week 12, and 73.5% on week 36 after treatment compared to pre-treatment values. Serum testosterone concentration slightly increased 2 d after treatment, then mean testosterone concentration was significantly reduced to basal levels (< 0.1 ng/mL) on day 20 and remained basal until 252 d after treatment. In another study, the same authors observed that serum testosterone remained basal for 15–25 mo after treatment, and testosterone levels rapidly increased reaching values > 0.5 ng/mL 2–3 weeks later (Goericke-Pesch et al., 2014). Novotny et al. described a significantly decrease in sperm concentration 4 mo PI (Novotny et al., 2012). Likewise, Romagnoli et al. (2017) described total absence of sperm 62–72 days after treatment in 4/7 cats with the use of 9.4 mg deslorelin implants. Some studies in cats have shown the effect of GnRH implant on libido, sperm production and serum testosterone concentration. However, the effect on sperm quality (plasma membrane integrity, sperm morphology, viability and acrosome integrity) during treatment and time until reestablishment of pre-treatment sperm parameters has not been studied. The aim of the study was to determine the time after treatment with a 4.7 mg deslorelin implant until Tomcat spermatogenesis activity was restored, and seminal parameters reached pre-implant values.

2. Materials and method

2.1. Experimental design

Six sexually mature tomcats aged between 2 and 4 years and weighting 4.3 ± 0.5 Kg, were included in this experiment. All males were housed alone and were fed with commercial cat food (pH control; Vital can, Buenos Aires, Argentina) and water *ad-libitum*. Tomcats were maintained in a controlled environment (room dimensions, 3.5 x 4.6 m) with artificial incandescent illumination giving 150–300 lux at floor level. Light schedule was stablished with alternated 2-month photoperiod cycles to maintain semen quality (Nuñez Favre et al., 2012). Tomcats were kept in the environment during 4.5 mo (45 d acclimatization + 1.5 spermatogenesis cycle & maturation) before the beginning of the study to become familiar with the new environment, handling and lighting schedule. After acclimatization pre-treatment samples were collected and then toms were randomly assigned to one of two treatments. Three cats (n = 3) received a deslorelin implant (4.7 mg; Suprelorin®, Virbac, France; TRT) whereas three (n = 3) received no implant and served as untreated control group (CON). Samples were collected until semen reached and maintained pre-treatment values for at least 3–4 mo in Suprelorin® implant cats (Fig. 1).

A physical examination was performed once a week, and clinical adverse effects and abnormal findings were recorded. In addition, behavioral, food and water intake and fecal changes were recorded daily. Animal care, housing, and experimentation complied with the International Guiding Principles for Biomedical Research Involving Animals (CIOMS, 2012). This study was approved by the Graduate School and the Animal Care and Use Committees of Laboratory Animals of the School of Veterinary Sciences at University of La Plata (#39.1.13B).

Implant insertion

Implants in all toms were placed subcutaneously cranial to the interscapular region under sedation. To place the implant, a disposable syringe coupled with the preloaded implanted provided by Virbac® was used. After the implant insertion, the site was inspected daily for 3 d for signs of inflammation. A physical exam was performed once weekly, and abnormal findings were recorded.

Semen samples collection and evaluation

Semen collection was performed by electroejaculation. Toms were anaesthetized with a combination of xylazine (0.5 mg/kg im; Kensol®, Köning SA, Argentina) and ketamine (20 mg/kg im; Ketamina 50®, Holliday-Scott SA, Argentina). Each tom received a total of 80 stimuli divided in three sets (30, 30 and 20) with 2–3 min of rest between sets. The first set consisted of 10 stimuli at 2 V, 10 at 3 V and 10 at 4 V. The second set consisted of 10 stimuli at 3 V, 10 at 4 V and 10 at 5 V. The third set consisted of 10 stimuli at 4 V and 10 at 5 V (Howard et al., 1990). Semen sample was collected into a 1.5 mL pre-warmed plastic tube and immediately assessed.

Samples were collected every 4 wk from 3 mo before treatment (pre-treatment samples) until 32 mo post-implant insertion (PI). Each ejaculate was assessed for motility (MOT; % motile), velocity (VEL; 0–5), volume (VOL; μL), sperm concentration (SC; $x10^6$/mL), total sperm count (TSC; $x10^6$), viability (VIA; % alive; eosin–nigrosin stain), acrosome integrity (AI; % intact; FITC-PSA), plasma membrane integrity (PMI; % intact; CFDA-PI) and sperm morphology (SM; % normal; Tinción 15®, Biopur, Rosario, Santa Fe,

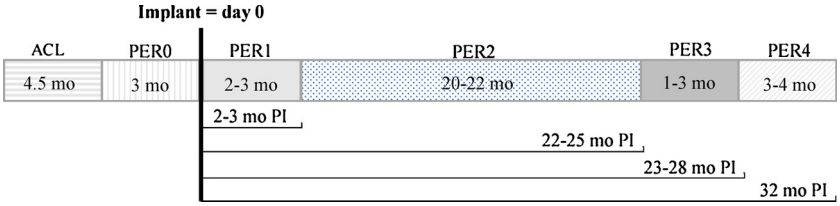

Fig. 1. Experimental design and semen collection of animals. Time lines are represented as follow: horizontal bar, acclimatization period (ACL); PER0, vertical bar, pretreatment period; PER1, solid light grey bar, post-implant stimulating period; PER2, dotted bar, post-implant sperm suppression period; PER3, solid dark grey bar, onset of sperm production period; PER4, diagonally bar, reestablishment of sperm production. Semen was collected every 4 wk since 3 mo before treatment (pretreatment samples), immediately before implant insertion (day 0), and every 4 wk until 32 mo post-implant insertion (PI). Duration of each period is indicated inside bars.

Argentina).

Before electroejaculation visual examination of the penis was performed to evaluate penile spines and after that, measurement of testicular length (a), width (b) and depth (c) (mm) was recorded to calculate testicular volume using the equation of a modified sphere (volume [mm^3] = 4/3 * π * (½a * ½b * ½c); Goericke-Pesch et al., 2011). In addition, blood samples were taken every two weeks to measure serum testosterone concentration from PER0 until the end of the study. Blood samples were taken in the morning, 4 h after the light was turned on. All samples were centrifuged, and serum stored at −20 °C until testosterone concentration was measured by electrochemiluminescence immunoassay (Testosterone II, Elecsys and Cobas e analyzers, Roche®, Mannheim, Germany; detection limits were 0.025–15.0 ng/mL, 0.085–52.0 nmol/L).

2.2. Statistical analysis

Data were divided into 5 periods (PER, Fig. 1); PER0 = pretreatment + implant day samples (from 3 mo before treatment, until immediately before implant insertion [day 0]); PER1 = PI stimulating period (from implant insertion until 2–3mo PI); PER2 = PI sperm suppression (from the end of PER1 until 22–25 mo PI), PER3 = PI onset of sperm production (from the end of PER2 until 23–28 mo PI) and PER4 = PI sperm reestablishment to pre-implant values (from the end of PER3 until 32 mo PI); periods duration varies within cats.

Six lineal regression models with time repeated measures were fitted to assess the effect of treatment on VOL, SM, testicular volume and serum testosterone concentration. Six generalized lineal regression models with Poisson distribution and time repeated measures were fitted to assess the effect of treatment on MOT, VEL, SC, TSC, VIA, AI and PMI. Treatment, time and time by treatment interaction were added to models as fixed effects and compound symmetry was defined for correlation structure of repeated measure within same individual through time period. Mean differences between treatments were estimated in each time period (Proc Glimmix, SAS 9.4). Significance was defined as P value less than 0.05.

3. Results

No local reaction in the implant area neither other clinical side effect was observed at any time during the study period.

Complete seminal evaluation is shown in Table 1. Seminal evaluation at PER0 showed no statistical differences between groups. During PER1 treated cats rapidly increased SC and TSC showing the implant stimulatory effect but, at the same time, normal SM decreased (Fig. 2). After this initial stimulatory effect, semen quality began to fall until sterility occurred 3–4 mo PI (begging of PER2). During PER2 it was not possible to measure most of seminal samples because of very low seminal VOL and reduced TSC in TRT cats compared to CON group (VOL, 26.2 ± 5.5 vs 90.2 ± 6.2 µL, $p < 0.0001$; TSC: 0.02 ± 0.04 vs. 8.88 ± 2.55 × 10^6, $p < 0.01$; Fig. 2). One cat still had ejaculates with sperm 6–7 mo PI, but it was considered sterile since spermatozoa were without motility (0%) and only 3% had normal sperm morphology. First ejaculate with sperm cells after complete downregulation was achieved 22, 23 and 25 mo PI in treated cats. This event marks the beginning of PER 3 (PER2 vs PER3; VOL, 26.2 ± 5.5 vs 41.3 ± 9.0 µL, $p < 0.15$; and TSC, 0.02 ± 0.04 vs. 0.63 ± 0.33 × 10^6, $p < 0.05$). Pre-treatment values were achieved 1–3 mo later, marking the beginning of PER4 (TRT vs CON, TSC 6.11 ± 2.70 vs. 15.11 ± 9.35 × 10^6, $p > 0.25$). Seminal evaluation of control cats showed no significant differences throughout the study.

Penile spines decreased in treated cats 8 wk PI. One of the TRT cats showed almost complete atrophy during the study period while the others showed total absence of penile spines from 20 wk PI until the end of PER2. Reappearance of penis spines was observed during PER3. Fig. 3 illustrates the appearance of penis spines during the study.

Table 2 shows testicular volume during the study period in both groups. Testicular volume at PER0 showed no statistical differences between groups. In TRT group, during PER1 testicular volume decreased 23% and in PER2 49% relative to initial values (1431 ± 174 vs 1857 ± 165 mm^3, $p < 0.01$; 957 ± 107 vs 1857 ± 165 mm^3, $p < 0.0001$, respectively). During PER3, testicular volume increased and reached pre-treatment values at PER4. No differences were observed in testicular volume between PER4 vs PER0 (1882 ± 181 vs 1857 ± 165 mm^3, $p > 0.75$). The control group showed no differences in testicular volume throughout the study. No differences were observed in testicular volume between TRT vs CON in PER0 and PER4 (1857 ± 165 vs. 1525 ± 179, $p > 0.49$; 1882 ± 181 vs. 1706 ± 95 mm^3, $p > 0.79$, respectively).

Serum testosterone concentrations decreased to basal levels 2wk PI (in two cats) and 4wk PI (in one cat). Testosterone concentration remained basal for 21 ± 2 mo PI, approximately 6–8 wk before the onset of sperm production (PER0, 2.45 ± 0.66; PER1, 0.08 ± 0.62; PER2, 0.46 ± 0.58; PER3, 4.41 ± 1.07; PER4, 3.82 ± 0.76 ng/mL). No differences were observed in testosterone concentration between PER4 vs PER0 (3.82 ± 0.76 vs. 2.45 ± 0.66 ng/mL, $p > 0.12$). Differences were observed in testosterone concentration between CON and TRT tomcats (PER1, 3.26 ± 0.72 vs 0.08 ± 0.62, $p < 0.01$; PER2, 3.61 ± 0.60 vs 0.46 ± 0.58 ng/mL, $p < 0.01$; Fig. 4).

4. Discussion

Our results agree with previous studies which demonstrate that 4.7 mg Suprelorin® implants suppress hypothalamic-pituitary-gonadal axis in tomcats (Goericke-Pesch et al., 2011; Novotny et al., 2012; Pisu and Romagnoli, 2012; Romagnoli et al., 2017). However, to our knowledge this is the first study to report reestablishment of sperm production after complete azoospermia due to the presence of a deslorelin implant in male cats.

In our study, testosterone concentration decreased to basal levels 2–4 wk after implant insertion. Similar findings were observed

Table 1
Semen evaluation parameters of control (CON) and deslorelin treated (TRT) tomcats during the 32 mo study period.

	PER0		PER1		PER2		PER3		PER4	
Semen parameter	CON	TRT	CON	TRT	CON	TRT	CON	TRT	CON	TRT
Motility	91.8 ± 16.0	70.8 ± 12.1	57.1 ± 14.2	59.1 ± 10.9	69.8 ± 11.6^{A}	0.04 ± 0.06^{B}	85.8 ± 9.0^{A}	31.2 ± 5.3^{B}	80.7 ± 13.4	65.6 ± 8.4
Velocity	5.0 ± 0.9	4.2 ± 0.5	3.81 ± 0.8	4.3 ± 0.6	4.3 ± 0.3^{A}	0.1 ± 0.1^{B}	4.6 ± 0.7	3.2 ± 0.53	4.4 ± 0.6	4.0 ± 0.6
Volume (μL)	94.7 ± 13.7	85.4 ± 7.5	92.2 ± 10.5^{A}	51.4 ± 8.1^{B}	90.2 ± 6.2^{A}	26.2 ± 5.5^{B}	89.0 ± 10.5^{A}	41.3 ± 9.0^{B}	99.8 ± 8.5^{A}	53.3 ± 9.2^{B}
Sperm concentration (10^6/mL)	91.7 ± 67.0	59.7 ± 38.4	82.0 ± 61.8	480.6 ± 238.0	105.5 ± 45.0^{A}	0.4 ± 0.5^{B}	89.8 ± 50.0^{A}	13.6 ± 9.2^{B}	134.0 ± 97.1	97.9 ± 64.9
Total sperm count (10^6)	8.6 ± 4.6	4.6 ± 2.0	8.8 ± 5.8	22.1 ± 6.9	8.8 ± 2.5^{A}	0.0 ± 0.0^{B}	8.6 ± 4.2^{A}	0.6 ± 0.3^{B}	15.1 ± 9.3	6.1 ± 2.7
Viability (%)	69.4 ± 7.1	57.0 ± 3.9	60.7 ± 5.4	64.3 ± 4.3	66.2 ± 3.1		73.3 ± 5.4	52.7 ± 8.5	70.8 ± 4.3	63.9 ± 5.0
Acrosome integrity (%)	77.5 ± 7.8	57.1 ± 8.3	71.5 ± 9.7	52.3 ± 8.7	72.6 ± 4.4		62.9 ± 11.1	57.1 ± 8.5	64.1 ± 6.2	65.3 ± 7.6
Plasma membrane integrity (%)	82.0 ± 4.8	78.6 ± 3.9	79.3 ± 10.2	73.0 ± 11.7	71.2 ± 2.8		71.6 ± 8.3		64.7 ± 11.3	70.0 ± 3.6
Sperm morphology (%)	66.7 ± 9.4	57.1 ± 6.2	64.1 ± 6.2^{A}	27.2 ± 5.5^{B}	52.1 ± 4.5		69.3 ± 6.2	58.8 ± 12.8	66.6 ± 5.5	59.8 ± 6.0

Tomcat semen evaluations performed in control (CON) and treated with 4.7 mg deslorelin implant, Suprelorin® (TRT); since 3 mo before and the implant day (PER0), post-implant stimulating period (PER1), post-implant sperm suppression (PER2), post-implant onset of sperm production (PER3) and reestablishment to pre-implant sperm values (PER4). Data are presented as LSM ± SEM. Different superscripts letters in same row are different at $p < 0.05$.

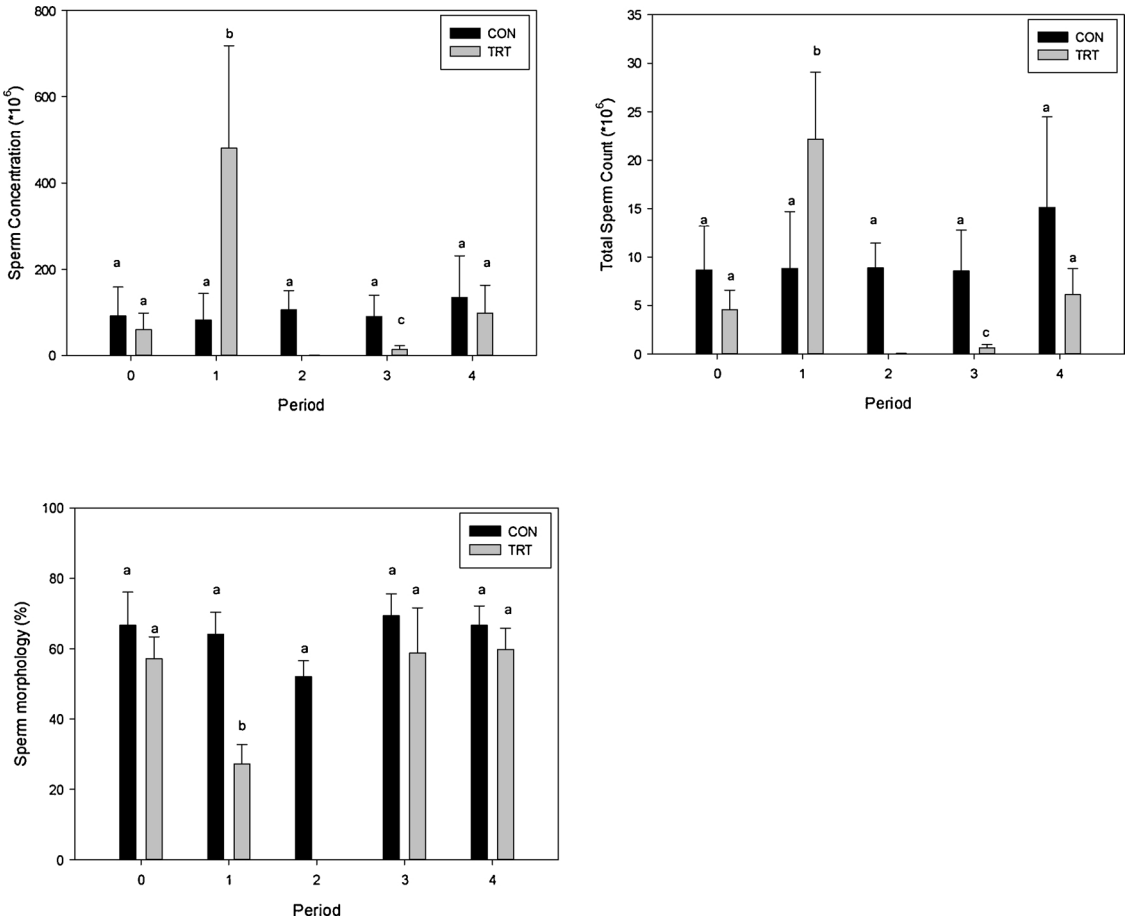

Fig. 2. Least square means ± SEM of sperm concentration, total sperm count and sperm morphology in cats' semen from control (CON) and deslorelin implant treatment (TRT) groups during the study (CON vs TRT; different letters represent differences in sperm parameter between treatment at different periods, $p < 0.05$). Period 0, 3 mo before and implant day sample; Period 1, post-implant stimulating period; Period 2, post-implant sperm suppression; Period 3, post-implant onset of sperm production; PER4, reestablishment of sperm production.

in previous studies in male cats and dogs (Junaidi et al., 2009; Goericke-Pesch et al., 2011; Pisu and Romagnoli, 2012; Goericke-Pesch et al., 2013; Lucas, 2014). Novotny et al. (2012) have reported in tomcats, a considerable increase in the total number of spermatozoa one month after treatment. This could be due to the initial hyperstimulation of the hypothalamic-pituitary axis and consequent stimulation of spermatogenesis. Similar findings were recently reported with the use of 9.4 mg deslorelin implants. The study showed an improvement of motility and sperm concentration 20 days after implant insertion (Romagnoli et al., 2017). In male dogs, Romagnoli et al. (2012), reported an increase in total sperm count, motility and seminal volume 23–32 days after treatment with 4.7 mg deslorelin implant. This stimulatory effect was followed by a decrease in seminal parameters. In our work, due to the initial stimulatory effect, an increased in sperm concentration and total sperm count was observed in PER1. This initial stimulatory effect was followed by a gradual and continuous decreased in seminal parameters. Junaidi et al. (2009), reported in male dogs that the decrease in seminal parameters was accompanied by a progressive increase in sperm abnormalities. Romagnoli et al. (2017) reported an increased in sperm abnormalities from day 33 PI in one cat and since day 111 PI in another cat using 9.4 mg deslorelin implants. Similarly, in our work we found an increase in sperm abnormalities 2–3 mo PI (Table 1, PER0 vs. PER1; Fig. 2).

In dogs, a progressive decrease in semen volume was observed 28–35 days PI (Junaidi et al., 2009) and 23–32 days PI (Romagnoli et al., 2012) using 4.7 mg deslorelin implants. Junaidi et al (2009) reported that ejaculates could no longer be obtained from 35 to 42 days PI, while Romagnoli et al (2012) reported a volume of 0 cc. in ejaculates 60–74 days PI. In our work, the decrease in seminal parameters was paralleled with a 64% decrease in seminal volume in PER2. Even though seminal collections were attempted, it was impossible to continue with seminal examinations due to the low volume. The decrease in seminal volume could be due to a shrinkage of the prostate gland caused by deslorelin as reported in dogs (Junaidi et al., 2009).

A fall of median total sperm count from 16 to 0.001×10^6 in tomcats can occur 4 months after insertion of a 4.7 mg deslorelin implant (Novotny et al., 2012) and recently, Romagnoli et al. (2017) have reported total absence of sperm 62–72 days PI in most of the cats treated with 9.4 mg deslorelin implants (4/7 cats). However, sterility was achieved as early as 40 days PI in one cat and 97–111 days PI in two cats. Our findings agree with those above since concentration and total sperm count decreased approximately

Fig. 3. Appearance of penis spines during de study period. a) implant day picture, penis spines are well developed; b) 5 mo post implant insertion, c) total absence 7 mo post implant insertion, d) end of study period (32 mo post implant insertion) penis spines are redeveloped.

Table 2
Testicular volume (mm^3) of control (CON) and deslorelin treated (TRT) tomcats during the 32 mo study period.

Period	CON	TRT
0	1525 ± 179^{A}	1857 ± 165^{A}
1	1512 ± 172^{A}	1431 ± 174^{B}
2	1675 ± 67^{A}	957 ± 107^{C}
3	1895 ± 121^{A}	1690 ± 191^{A}
4	1706 ± 95^{A}	1882 ± 181^{A}

Period 0, 3 mo before and implant day sample; Period 1, post−implant stimulating period; Period 2, post−implant sperm suppression; Period 3, post−implant onset of sperm production; Period 4, reestablishment to pre−implant sperm values. Data are presented as LSM ± SEM. Different superscripts indicate differences between Periods of the same column at $p < 0.05$.

99% in PER2 compared with initial values (Table 1, PER0 vs PER2). In agreement with previous findings, in our study during PER2 one cat had ejaculates with 0% of motility (Ackermann et al., 2012). These findings agree with other authors since, atrophy of seminiferous tubules with elongated spermatids were observed in histological observations of tomcats testicles 5 months after deslorelin implant insertion (Goericke-Pesch et al., 2013; Novotny et al., 2015).

Testicular volume has been reported to decrease by about 60% on week 12 post implant insertion and by > 70% on week 36 post implant insertion (Goericke-Pesch et al., 2011). In the same way, we observed a decrease of about 50% during PER2, even though the period was much longer than that of previous studies (20–22 mo).

Several authors agree with the high variability in the duration of the deslorelin implant effect. Pisu and Romagnoli (2012), established the duration of implant effect by recording testosterone concentration. These authors observed a decrease to basal levels 20–30 days after implant insertion remaining basal for 15 ± 3 months. Goericke-Pesch et al. (2014), reported full downregulation of testicular function for about 15–25 months. Our findings agree with those authors since serum testosterone concentrations decreased to basal levels 2–4 wk PI, and onset of sperm production and first semen evaluation after complete downregulation was achieved 22–25 months PI. Subsequently sperm parameters improved, reaching pre-implant values 23–28 mo PI, sperm production and quality was maintained until the end of the study (32 mo PI).

To our knowledge this is the first report which documents the effect of a deslorelin implant on sperm quality and the

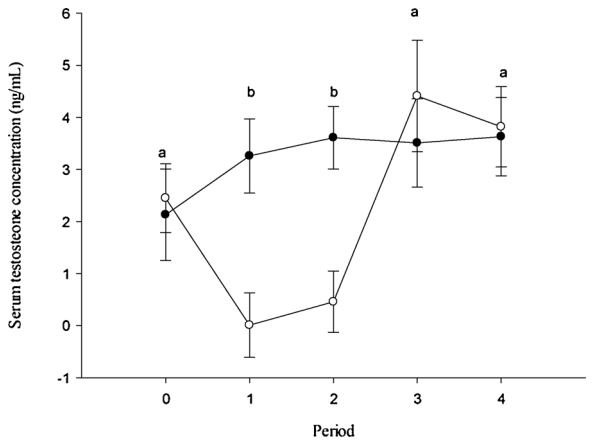

Fig. 4. Least square means ± SEM of serum testosterone concentration in tomcats from control (black circle) and treated with deslorelin implant (white circle) groups during the study period. Differences between treatment are represented by different letters, $p < 0.05$. Period 0, 3 mo before and implant day sample; Period 1, post-implant stimulating period; Period 2, post-implant sperm suppression; Period 3, post-implant onset of sperm production; Period 4, re-establishment of sperm production.

reestablishment of normal sperm parameters and spermatogenesis in tomcats. Therefore, these findings show that deslorelin is a reversibly and safe option for reproduction control during long periods in tomcats, allowing the return of semen quality to pre-treatment values.

5. Conclusion

The insertion of a single Suprelorin® 4.7 mg implant in tomcats appears to suppress hypothalamic-pituitary-gonadal axis for 22–25 mo with no clinically side effect observed during the study period. All toms returned to their initial seminal parameters 23–28 mo after treatment and maintained seminal quality until the end of study period (32 mo PI). We conclude that Suprelorin® 4.7 mg is a reversibly and safe option for reproduction control during long periods in tomcats.

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