Animal Reproduction Science 195 (2018) 302–308

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

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

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ARTICLE INFO

Keywords:
GnRH agonist
Deslorelin
Contraception
Semen
Testicular volume
Testosterone

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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https://doi.org/10.1016/j.anireprosci.2018.06.008
Received 2 February 2018; Received in revised form 7 June 2018; Accepted 15 June 2018
Available online 18 June 2018
0378-4320/

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,

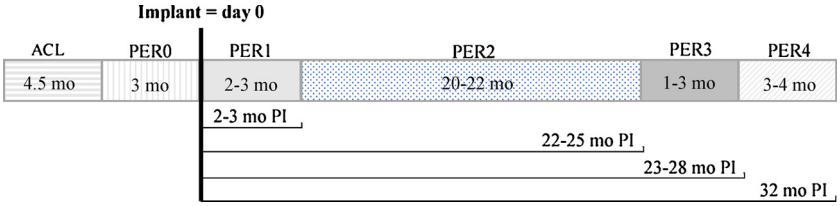

Fig. 1. Experimental design and semen collection of animals. Time lines are represented as follow: horizontal bar, acclimatization period (ACL); PER0, vertical bar, pretreatment period; PER1, solid light grey bar, post-implant stimulating period; PER2, dotted bar, post-implant sperm suppression period; 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.