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Effect of melatonin implants on spermatogenesis in the domestic cat (*Felis silvestris catus*)

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ABSTRACT

The aim of this study was to assess the efficacy of subcutaneous melatonin implants to temporarily and reversibly suppress spermatogenesis in male cats. Tomcats (n = 8) were housed in a conditioned room with alternating long and short 2-month photoperiod cycles to maintain sperm production and quality. Animals were randomly assigned to one of the two treatments. Four animals received a subcutaneous melatonin implant (MEL, 18 mg; Syntex, Argentina), whereas the other four received a subcutaneous placebo implant (PLA, 0 mg; Syntex). Semen samples were collected by electroejaculation every 14 days for 252 days. Sperm parameters were evaluated in all ejaculates, and data were analyzed by ANOVA. Melatonin-implanted cats significantly decreased their sperm quality in all the parameters studied compared with the control group (MEL vs. PLA; least squares means ± SEM; motility, 71.3 ± 3.4 vs. 82.1 ± 3.6; velocity, 3.4 ± 0.1 vs. 4.6 ± 0.1; total sperm count, 2.6 ± 2.2 vs. 19.4 ± 3.3; acrosome integrity, 48.7 ± 5.6 vs. 62.8 ± 5.6; plasma membrane integrity, 52.2 ± 4.7 vs. 72.9 ± 5.5; normal sperm morphology, 45.8 ± 3.3 vs. 63.7 ± 3.4; $P < 0.05$). Conversely, volume and serum testosterone concentrations were similar in both groups (volume, 0.15 ± 0.02; serum testosterone concentrations, 1.1 ± 0.1; CV 18.9%; $P > 0.05$). At 91 ± 7 days after implant insertion, sperm motility decreased 38.5%, velocity 26.5%, total sperm count 82%, acrosome integrity 22%, plasma membrane integrity 30%, and normal sperm morphology decreased 32% of preimplant values. This effect was present until 120 ± 15 days after implant insertion. After that, seminal parameters started to increase and reached preimplant values at about 140 ± 7 days after implant insertion. Nevertheless, treated animals conserved the capacity to produce semen during the treatment period. In conclusion, a single subcutaneous melatonin implant effectively and reversibly reduced sperm production and quality in male domestic cats for approximately 120 ± 15 days without clinically detectable adverse effects.

1. Introduction

Feral cat overpopulation is an important problem in many countries. Diseases and parasites affecting feral cats lead to public health risks. A variety of options are available for feline population control. The permanent control of reproduction in cats can be achieved using surgical methods. Surgical methods (e.g., orchiectomy or vasectomy; ovariectomy or ovariohysterectomy) are expensive when performed on a large scale (e.g., to control feral cat populations) [1,2]. This is particularly a problem in developing countries with limited economic resources and no programs to control overpopulation with owned and feral cats. In addition, surgical methods result in a permanent sterilization that is not

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suitable for controlling reproduction in animals with future breeding value [2,3]. For these animals, nonsurgical methods of contraception are the best option [1]. Although nonsurgical contraception in queens has attracted the interest of research in the last decade, there are few studies concerning this topic in toms [1]. Bisdiamines are amebicidal drugs that specifically arrest spermatogenic activity in various domestic and wild animals [4]. Bisdiamina (WIN 18,446, Fertilisin, SAF Bulk Chemicals, USA) mixed in food daily for 76 days has the potential to arrest spermatogenesis during the treatment period, and normal spermatogenesis was restored by Day 152 after treatment [4]. In the last decade, GnRH has been one of the targets for immunocontraception. Levy et al. [5] showed that a vaccine made with synthetic GnRH coupled with a foreign protein (KLH) and combined with mycobacterial adjuvant was effective to reduce testosterone concentration and to induce testicular atrophy 3 months after vaccine administration. However, more studies are necessary to determine the duration of immunity and true rate of efficacy of GnRH immunocontraception in cats. More recently, the use of a single dose of GnRH antagonist (330 μg/kg acycline) was efficient to impair spermiogenesis, spermatocytogenesis, and sperm motility for 14 days [6]. In addition, the use of a GnRH agonist implant (4.7 mg deslorelin; Suprelorin, Virbac, France) during 252 days proved to be effective to reduce testis size, libido, mating behavior, and urine marking. Castrated-related effects were observed approximately 112 days after implant insertion. Additionally, no adverse effects were observed after treatment [7]. Furthermore, none of these studies have included the effect of seasonality or light refractoriness as a study factor in the experimental designs.

Queens are seasonal breeder. Estrous cyclicity is present during long photoperiod and is associated with low serum melatonin concentrations [8,9]. Tomcats produce semen throughout the year with moderate annual variations, improving their semen quality during female breeding season [10–12]. Oral and subcutaneous melatonin administration effectively and reversibly suppressed estrous cycles in queens kept under long photoperiod conditions without adverse effects [13,14]. However, daily oral or every other day, subcutaneous administration of melatonin is impractical in clinical practice. Conversely, subcutaneous melatonin implants proved to be effective to reversibly suppress estrus in queens approximately for 2 to 4 months with no clinically detectable adverse effects [15]. In the same way, melatonin implants could be used in male cats. Therefore, the objective of this study was to assess the efficacy of a subcutaneous melatonin implant to reversibly suppress spermatogenesis in tomcats. The hypothesis was that a subcutaneous melatonin implant would temporarily and reversibly suppress spermatogenesis in tomcats without producing any clinically detectable adverse effects.

2. Materials and methods

2.1. Experimental design

2.1.1. Animals

Eight adult toms, mixed short hair breeds, aged between 3 and 5 years, and weighing 4.5 and 5 kg, were used. All males were housed alone in stainless steel cages and were fed with commercial cat food (Fit 32; Royal Canin, Buenos Aires, Argentina) and water *ad libitum*. Animals were maintained in a controlled environment with artificial lighting [15,16]. All males were fertile, as they had fathered litters before the start of this experiment. Animal care, housing, and experimentation complied with the International Guiding Principles for Biomedical Research Involving Animals [17]. The Graduate School and the Laboratory Animal Care and Use Committees of the Faculty of Veterinary Sciences at National University of La Plata approved this study.

2.1.2. Light regimen

Tomcats were maintained in a controlled environment (room dimensions, 3.5 × 4.6 m) with artificial incandescent illumination using 100-W lights at approximately 50 cm from the cats [15,16]. To maintain sperm production and quality and to avoid refractoriness to long photoperiod during the study period, long photoperiod cycles (LPCs, 56 days) were alternated with short photoperiod cycles (SPCs, 28 days) as previously described by Nuñez Favre et al. [18]. Briefly, animals were maintained on long photoperiod (12 hours light–12 hours dark, 7 am to 7 pm) during 140 days (acclimation + two spermatogenesis cycles). After that, LPC and SPC were alternated using LPC for 56 days, and then light was decreased from 12 to 8 hours at a rate of 8 min/day over 28 days. Animals stayed 28 days with SPC (8 hours light–16 hours dark, 7 am to 3 pm), and, subsequently, the lighting increased at the same rate but at inverse rate as the decline (8 min/day, 28 days). This light regimen was repeated during the whole study period (252 days; Fig. 1).

2.1.3. Semen collection and evaluation

Semen collection was performed by electroejaculation. Toms were anesthetized 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). As previously described by Howard et al. [19], all cats received 80 stimuli divided in three series (30, 30, and 20) with 2 to 3 minutes rest between sets. Briefly, 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. Semen sample was collected into a 1.5-mL prewarmed plastic tube and immediately assessed. After 140 days (acclimation + two spermatogenesis cycles), semen was collected from all males every other week during 84 days to select experimental animals (Fig. 1). During this period, seven semen samples of each cat were collected. Only toms with greater than 70% motility, greater than 4 velocity, greater than 12×10^6 sperm concentration, greater than 50% acrosome integrity, greater than 70% viability, greater than 60% plasma membrane integrity, and greater than 50% normal sperm morphology were included in the experiment. Immediately after animal selection, toms were randomly assigned to one of two treatment groups. Animals assigned to the treatment group received a subcutaneous melatonin implant (18 mg; Syntex, Argentina; MEL, n = 4), whereas animals assigned to the placebo group received a subcutaneous placebo implant without melatonin (0 mg; Syntex, PLA, n = 4). After implant insertion (Day 0), the

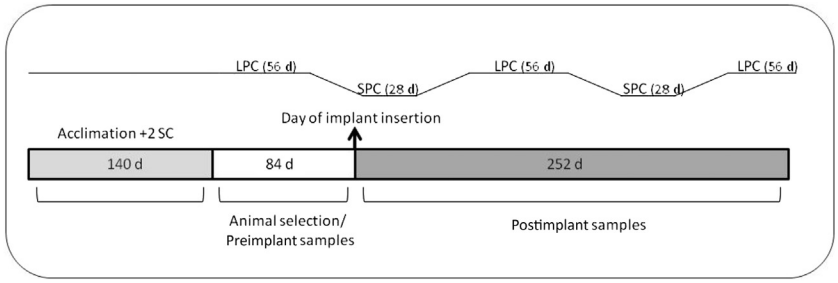

Fig. 1. Experimental details regarding design, light regimen, and semen collection of study animals. LPCs, long photoperiod cycles; SPCs, short photoperiod cycles; SCs, spermatogenesis cycles.

semen samples were collected from all males every other week during 252 days (Fig. 1). Each ejaculate from each tom was individually assessed for motility (% motile), velocity (0–5), volume (mL), sperm concentration ($\times 10^6$/mL), total sperm count ($\times 10^6$), viability (% alive; eosin–nigrosin stain), acrosome integrity (% intact; FITC-PSA), plasma membrane integrity (% intact; CFDA-PI), and sperm morphology (SM, % normal; Tincion 15, Biopur, Argentina). In the morning (4 hours after the light was turned on), after electroejaculation, blood samples were taken to measure serum testosterone concentrations. All samples were centrifuged, and serum was stored at −20 °C until testosterone was measured by a solid-phase RIA using I^{125} (Coat-A-Count, Total Testosterone; Coat-A-Count; Diagnostic Product Corporation, Los Angeles, CA, USA). A physical examination was performed once a week, and adverse effects and abnormal findings were recorded. In addition, weight and rectal temperature were recorded once a week and behavioral and fecal changes were recorded on a daily basis.

2.1.4. Statistical analysis

Serum testosterone and sperm production were analyzed by the Mixed procedure of SAS [20] as repeated measures. The model included fixed effect of treatment (MEL vs. PLA), the random effect of tom and the fixed effect of time (sampling day), and the interaction of treatment by time. Time-point comparisons between treatments were made with the PDIFF and/or SLICE options of the Mixed procedure. Data are presented as least squares means ± SEM. Significance was defined as P value less than 0.05.

3. Results

Sperm parameters were lower in MEL compared with PLA samples (MEL vs. PLA; motility, 76.2 ± 1.6 vs. 86.3 ± 1.8; velocity, 3.9 ± 0.1 vs. 4.7 ± 0.1; total sperm count, 4.1 ± 1.5 vs. 25.8 ± 1.9; acrosome integrity, 52.3 ± 2.5 vs. 61.0 ± 2.6; plasma membrane integrity, 61.3 ± 2.8 vs. 76.4 ± 3.0; and normal sperm morphology, 54.3 ± 3.0 vs. 70.1 ± 3.2; $P < 0.05$). Similarly, MEL samples tended to have lower viability than PLA samples (62.4 ± 2.6 vs.70.9 ± 2.8; $P = 0.06$). However, volume and serum testosterone concentrations were similar in both groups (0.15 ± 0.02; 1.1 ± 0.1; CV 18.97%; $P > 0.05$).

In addition, motility, velocity, total sperm count, and normal sperm morphology changed during sampling dates (day; $P < 0.05$; Fig. 2). Furthermore, at 91 ± 7 days after implant insertion, sperm motility decreased 38.5%, velocity 26.5%, total sperm count 82%, acrosome integrity 22%, plasma membrane integrity 30%, and sperm morphology decreased 32% of preimplant values. This effect was present until 120 ± 15 days after implant insertion. After that, seminal parameters started to increase and reached preimplant values at about140 ± 7 days after implant insertion. It is noteworthy to point out that none of the toms had any clinically detectable adverse effects during treatment.

4. Discussion

In contrast with our hypothesis, a subcutaneous melatonin implant did not completely suppress spermatogenesis. The implant, however, temporarily and reversibly reduced spermatogenesis in tomcats without producing any clinically detectable adverse effects.

Long time ago, the queen was described as a seasonal breeder; however, photoperiod variations in sperm production in tomcats have been reported recently [10–12]. Therefore, male and female cats could be classified as long-day breeders with sexual activity ceasing under decreasing photoperiod and high melatonin serum concentration and resuming with increasing photoperiod and low melatonin serum concentration. The effect of a melatonin implant to suppress ovarian activity in queens was shown [15]. However, the effect of melatonin to suppress spermatogenesis in the tomcat has not been studied yet. This is the first study to show that melatonin is effective to reduce sperm production in toms without clinically adverse effect.

Gimenez et al. [15] reported that a single 18-mg subcutaneous MEL implant was effective, reversible, and safe to suppress estrus in queens for 2 to 4 months. Our results show that the same melatonin implant was effective to reduce sperm parameters for 120 ± 15 days in tomcats. During this period, MEL-treated animals had sperm parameters similar to those reported by Tsutsui et al. [21] during nonbreeding season and to those reported by Nuñez Favre et al. [18] in cats during the photo-refractory period.

Seasonality on sperm morphology was previously reported by Axner and Linde Forsberg [22]. These authors reported the highest percentage of normal spermatozoa in toms during the female reproductive season when serum melatonin concentrations decrease [22]. In the same way,

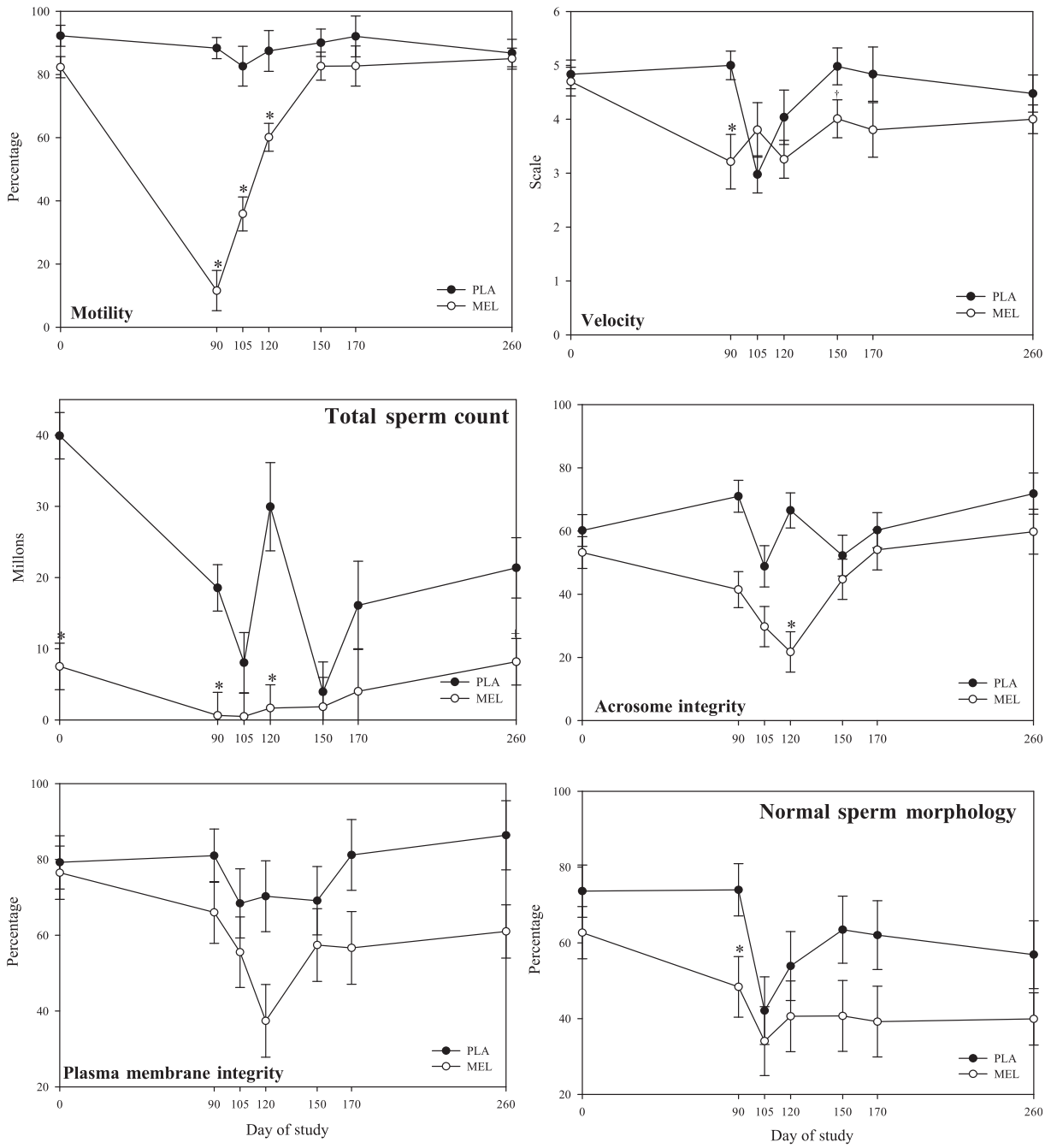

Fig. 2. Least square means ± SEM of motility, velocity, total sperm count, acrosome integrity, plasma membrane integrity, and normal sperm morphology in tomcats semen from placebo (PLA) and melatonin (MEL) groups during the study (MEL vs. PLA; differences in sperm parameter between treatment at different sampling dates are represented by superscript symbol *$P < 0.05$; $^{+}$observations with superscript differ at $P < 0.07$. Day 0: implant insertion (MEL and PLA).

the percentage of morphologically normal spermatozoa in the present study was higher in PLA-treated animals compared with MEL-treated animals.

Some researchers have reported poor semen quality in toms during the female nonbreeding season in agreement with short photoperiod and high melatonin serum concentration. Blottner and Jewgenow [10] found a significantly lower percentage of motile and morphological intact spermatozoa after castration of free-roaming cats during the winter days from December to March. Additionally, in a previous study we reported that free-roaming toms castrated during decreasing daylight showed lower number of morphologically normal sperm and sperm plasma membrane integrity and tended to have lower motility and total sperm count compared with toms castrated during increasing daylight [11]. These findings are

in agreement with the present study in which MEL-treated animals had significantly lower values than PLA-treated animals.

Some authors have demonstrated seasonality in serum testosterone concentrations with higher concentrations during breeding season and lower concentrations during nonbreeding season [21]. Similar results were found in a German feral cat population, in which testicular testosterone concentration showed higher concentrations during spring and significantly lower concentrations during fall [10]. However, other authors have found hardly any differences in serum testosterone concentration during breeding and nonbreeding season [23]. In addition, a previous study in Argentina, including short-hair feral cats in natural photoperiod, showed a clear seasonal variation in sperm quality with moderate variation in serum testosterone concentration [11]. Similarly, in the present study, serum testosterone concentrations showed no differences comparing MEL- and PLA-treated animals. In agreement with a previous study by Blottner and Jewgenow [10], in the present study we found very high variation in serum testosterone concentrations between animals. Furthermore, differences in serum testosterone concentrations in the same animal at different time points during sampling were found. This observation could be explained by individual fluctuations in diurnal peripheral blood testosterone levels as other authors previously reported [23,24].

During the treatment period, spermatogenic activity of melatonin-treated cats was similar to the result obtained by Tsutsui [21] in cats in nonbreeding season with higher serum melatonin concentration. In this way, treatment with melatonin implants seems to offer an alternative to safely reduce spermatogenesis in tomcats for at least 4 months. However, a fertility trial needs to be performed to determine if queen became pregnant when matted with melatonin-implanted tomcats with low sperm production.

In queen, exogenous melatonin was used as contraceptive to mimic its physiologic effect on reproductive activity. Tomcats show better epididymal sperm quality under natural long photoperiod [11]. However, when tomcats were maintained under long photoperiod (12 hours/light/day) for several months, the sperm production decreased. Recently, photo-refractoriness was described in tomcats [18]. Refractoriness and reduced sperm production and sperm quality induced by a prolonged long photoperiod can be restored after placing tomcats to a short photoperiod [18]. So melatonin implant could mimic short photoperiod and reduce sperm production. In PLA animals, light regimen could produce fluctuations on serum melatonin concentration, and this could allow maintain good semen quality. This fact was previously described in silver fox, bucks, and rams. Besides, there are some findings that suggest that this is the case for cats too [25–28].

4.1. Conclusion

In conclusion, a subcutaneous MEL implant effectively, reversibly, and safely reduced spermatogenesis in male cats for 120 ± 15 days. Seminal parameters were restored 261 ± 22 days after implant insertion with no adverse effects.

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