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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