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Suppression of estrus in cats with melatonin implants

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Abstract

The objective of this study was to assess the efficacy of a subcutaneous melatonin implant to suppress estrus in queens (felis catus). The hypothesis was that this implant would temporarily and reversibly suppress estrus in queens without producing any clinically detectable side effects. Fourteen adult queens were maintained in cages under artificial illumination (14 h light:10 h dark) for 45 d and then randomly assigned to one of two treatments. At interestrus, queens received a single subcutaneous melatonin implant (18 mg; Melovine [CEVA Sante Animal, Libourne, France]; MEL: n = 9), or a single subcutaneous placebo implant without melatonin (0 mg; PLA; n = 5). At the next estrus, all queens received a second MEL (n = 9) or PLA (n = 5) implant. Blood samples were taken when queens displayed estrous signs and during interestrus to measure estradiol (E_2) and progesterone (P_4), respectively, by radioimmunoassay. There were no significant differences in duration of the interestrus interval in PLA cats, regardless of whether the implants were placed during interestrus or estrus (6.0 ± 9.7 d vs. 6.0 ± 9.7 d, respectively; least square means [LSM] ± SEM). However, when MEL implants were placed during interestrus, the duration of interestrus was approximately twice as long as that occurring when MEL implants were placed during estrus (113.3 ± 6.1 d vs. 61.1 ± 6.8 d, respectively; $P < 0.01$). Serum E_2 and P_4 concentrations were similar in queens with PLA and MEL implants and in queens that received implants in estrus and interestrus. In conclusion, a subcutaneous MEL implant effectively and reversibly suppressed estrus in queens for approximately 2 to 4 mo with no clinically detectable side effects.

Keywords: Contraception; Domestic cat; Melatonin implants; Reversible; Slow-release implants

1. Introduction

Overpopulation of feral cats, owned cats, and dogs in certain areas around the world is a troubling issue for many sectors of society [1]. Furthermore, although the current contraceptive protocols for cats have undesirable effects, some of which are very serious [2], there are few investigations regarding control of reproduction in cats. Therefore, there is a need to develop effective, reversible, and safe contraceptives for felids.

Permanent control of reproduction in companion animals can be achieved using surgical methods (e.g., ovariectomy or ovariohysterectomy) [3–5]. Although routine, these surgeries may result in complications, including hemorrhage [3], ovarian remnant syndrome

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[6,7], stump pyometra, fistulous draining tracts, and accidental urethral ligation [3]. Surgical methods are also expensive when performed on a large scale (e.g., to control feral cat populations). This is particularly a problem in developing countries with limited economic resources and no programs to control overpopulation with owned and feral cats. Furthermore, unwanted dogs and cats may be reservoirs or vectors of transmissible human or animal diseases. Lastly, surgical methods result in a permanent sterilization that is not suitable for controlling reproduction in animals with future breeding value [3–5].

Reversible control of reproduction can be achieved using pharmacologic methods such as progestins [8–10], androgens [4,5,10], gonadotropin-releasing hormone (GnRH) analogues [2], and immunocontraception [11–13]. Steroids such as progestins and androgens are currently available for contraception. In spite of their efficacy as contraceptives, they can induce side effects that may be life-threatening [4,5,8,9]: potential side effects include (i) cystic endometrial hyperplasia-pyometra complex [14]; (ii) mammary fibroadenomatosis [15,16]; (iii) mammary neoplasia [17]; and (iv) hyperglycemia-glucosuria syndrome due to insulin resistance [18–20]. Although safe contraception can be achieved for 14 mo with GnRH analogues, some undesirable effects, including estrus induction shortly after implant placement and variable duration of estrus suppression, have been described [2]. Immunocontraception is still under investigation [11–13].

Goats and sheep are short-day breeders; sexual activity occurs during autumn and winter [21]. In these species, subcutaneous controlled-release melatonin implants advance the reproductive season [22–25]. In contrast, the administration of melatonin in long-day breeders such as hamsters, horses, and cats had variable results. Although the administration of melatonin infusions inhibited reproductive activity in hamsters [26–28], results in melatonin-treated horses were contradictory [29–33]. In cats, 5 mg melatonin given intravenously every other day suppressed ovarian activity in animals maintained under continuous (24 h) light [34]. Perhaps melatonin is the signal by which the female domestic cat measures photoperiod, and exogenous melatonin may mimic the effect of decreasing photoperiod [34]. More recently, Graham et al. gave queens 30 mg melatonin orally for 35 d [35]. This treatment effectively inhibited ovarian activity for the first 25 d, without any apparent side effects. Although oral administration of melatonin to cats is impractical in clinical practice, subcutaneous melatonin implants, which maintain constant serum melatonin concentrations [36], could be useful to control feline reproduction.

The objective of the current study was to assess the efficacy of a subcutaneous melatonin implant to reversibly suppress estrus in queens. The hypothesis was that a subcutaneous melatonin implant would temporarily and reversibly suppress the estrous cycle in queens (felis catus) without producing any clinically detectable side effects.

2. Materials and methods

2.1. Experimental design

Fourteen adult mixed breed queens, aged between 12 and 14 mo and weighing between 2 and 4 kg, were used in a randomized design (Fig. 1). In addition, a 3-yr-old intact tomcat was included in the study as a teaser male and for breeding.

The queens were housed alone or in pairs in stainless steel cages and were fed commercial cat food (Fit 32; Royal Canin, Buenos Aires, Argentina) and water ad libitum. The tom was housed separately and fed the same diet. All queens were maintained in a controlled environment (room dimensions, 3.5×4.6 m) with artificial incandescent illumination (14 h of daily bright light from five 100-watt lights, approximately 50 cm from the cats [37]. After 45 d of acclimation, queens were assigned to one of two treatments.

Animal care, housing, and experimentation complied with the International Guiding Principles for Biomedical Research Involving Animals (38]. This study was approved by the Graduate School Committee of the Faculty of Veterinary Sciences at University of La Plata.

At interestrus, queens assigned to one treatment received a single subcutaneous melatonin implant (18 mg; Melovine [CEVA Sante Animal, Libourne, France]; MEL: n = 9, first period). At the same stage of the estrous cycle, queens assigned to the other treatment received a single subcutaneous placebo implant without melatonin (0 mg; PLA: n = 5; first period). On a daily basis, queens were observed to detect behavioral estrus and receptivity to the male, and vaginal cytologies were obtained. A new implant was inserted during the next estrus in all queens (MEL, n = 9; PLA, n = 5; second period).

During the next behavioral and cytologic estrus after the second implant was inserted, each queen was placed with the tom. First mating was documented, and pregnancies were confirmed by an ultrasonographic examination done 25 d after the first mating, with a 5.0/7.5 sector transducer (Tringa; Pie Medical, Maastritch, Holland). One queen from each treatment group was not mated (due to limited space to accommodate offspring).

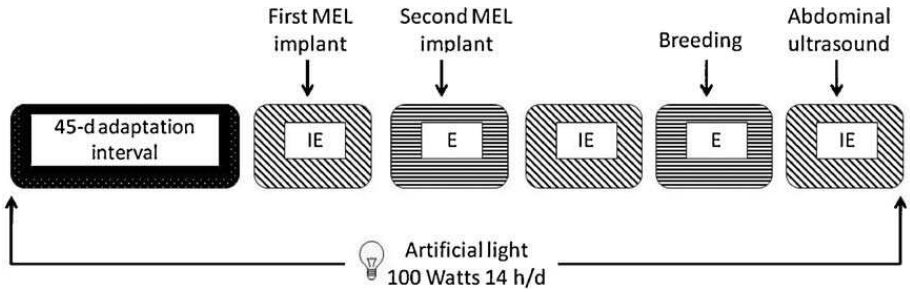

Fig. 1. Experimental design for queens treated with melatonin or placebo implants. Timelines are represented as follows: solid color, 45-d adaptation interval; diagonal bars, IE, interestrus; horizontal bars E, estrus. All queens (n = 14) were maintained in a controlled environment under artificial incandescent illumination (14 h of daily bright light). At IE, queens received a single subcutaneous melatonin implant (18 mg; Melovine [CEVA Sante Animal]; MEL: n = 9) or a single subcutaneous placebo implant without melatonin (0 mg; PLA: n = 5). At the next E, the queens received a second single subcutaneous MEL implant or a second single subcutaneous PLA implant. At the following E, queens were placed with the tom, and 25 d after breeding, abdominal ultrasound was performed.

2.2. Implant insertion

All animals were sedated with 0.05 mg/kg acepromazine (Acedan; Holliday Scott SA, Buenos Aires, Argentina), given subcutaneously. The interscapular space was clipped and aseptically prepared. Lidocaine (Lidocaína 2%; Over SA, Santa Fe, Argentina) was administered subcutaneously (1 mL), and a 1.5-cm incision was made. The implant was inserted in the subcutaneous tissue 1 cm distal from the insertion point. The incision was closed with a single nylon suture, which was removed 10 d later. After the implant was inserted, the site was inspected daily for 3 d for signs of inflammation. A physical exam was performed once weekly, and abnormal findings were recorded.

2.3. Vaginal cytologies

Vaginal cytologies were obtained daily to confirm behavioral estrus. Swabs 3 mm in diameter and 6 cm long were used for sample collection. Swabs were moistened with sterile saline water, introduced into the vagina approximately 1.5 cm, and quickly and gently rotated against the floor and lateral walls of the vagina. Smears were air-dried and stained with methylene blue. Stained slides were examined at 100× and 400× magnification to enumerate parabasal, intermediate, and superficial cells. Stage of the estrous cycle was determined according to the percentage and type of cells present [39].

2.4. Measurement of serum estrogen and progesterone concentrations

Once a month, blood samples were taken to measure serum concentrations of progesterone (P_4) to detect presence of corpus luteum and pseudopregnancy. Thirty-six hours after the start of estrus, blood samples were taken to measure serum estradiol (E_2) concentrations to confirm estrus.

All samples were centrifuged and stored at–20 °C until E_2 and P_4 were measured by a solid-phase radioimmunoassay (RIA) using I^{125} (Coat-A-Count, Estradiol; Coat-A-Count, Progesterone; Diagnostic Product Corporation, Los Angeles, CA, USA). The intra-assay CVs for high-pool and low-pool P_4 (5 and 1 ng/mL) were 3.4% and 6.1%, respectively, whereas for high-pool and low-pool E_2 (30 and 8 pg/mL), they were 5.0% and 2.9%.

2.5. Exclusion criteria

Queens that developed postsurgical tissue reactions, signs of disease, or elevated P_4 indicative of presence of a corpus luteum (CL) during the study were eliminated from the trial and excluded from the statistical analysis.

2.6. Statistical analyses

Comparisons between treatments (MEL vs. PLA) and stage of cycle when implants were placed (interestrus vs. estrus), were analyzed by least square means (LSM) ANOVA with the GLM procedure of SAS [40]. The mathematical model included the main effects of treatment, stage of cycle when the implants were inserted, the interaction between treatment and stage of cycle, and the residual error. The dependent variables analyzed were interestrus interval, E_2 concentration, and P_4 concentration. Data are represented as LSM ± SEM. Significance was defined as $P < 0.05$.

The statistical model is

$$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + E_{(ijk)}$$

Table 1
Least square means (±SEM) of the interestrus interval and serum concentrations of E_2 and P_4 in cats given melatonin or a placebo.

Treatment	Stage of cycle	Interestrus interval (d)	E_2 (pg/mL)	P_4 (ng/mL)
Melatonin (n = 9)	Interestrus	113.3 ± 6.2^a	20.71 ± 5.1	0.37 ± 0.32
	Estrus	61.1 ± 6.9^b	24.75 ± 6.1	0.88 ± 0.36
Placebo (n = 5)	Interestrus	6.0 ± 9.7	40.27 ± 8.05	0.47 ± 0.51
	Estrus	6.0 ± 9.7	13.70 ± 9.29	0.25 ± 0.51

[a,b]Within a column and treatment, values without a common superscript differed ($P \leq 0.05$).

where Y_{ij} is the response variable in the ith treatment with the jth stage of cycle; μ is the overall mean; α_i is the effect of the ith treatment; β_k is the effects of the jth stage of cycle; $(\alpha\beta)_{ij}$ is the interaction of the ith treatment with the jth stage of cycle, and $E_{(ijk)}$ is the residual error term.

3. Results

In the second period, one queen from the PLA group had increased serum P_4 concentrations (14 ng/mL) and was excluded from the study.

There were no significant differences in interestrus interval between queens that had a PLA implant inserted in interestrus or estrus (Table 1). However, queens that had a MEL implant inserted in interestrus had an interestrus interval approximately twice as long as that in those who had the implant inserted during estrus (treatment by stage of estrous cycle interaction, $P < 0.01$; Table 1).

Serum E_2 concentrations during estrus did not differ between treatment groups ($P = 0.57$; Table 1) or between stage of cycle when treatment was administered ($P = 0.14$). Serum P_4 concentrations during the interestrus interval did not differ between treatment groups ($P < 0.54$) or between stage of cycle when treatment was administered ($P < 0.73$).

Within 3 to 9 d after the implant insertion during interestrus, 33% (3 of 9) of MEL queens had superficial cells present in their vaginal cytologies and estrus behavior for 2 d. Within 9 to 11 d after implant insertion during estrus, 78% (7 of 9) of MEL queens had superficial cells present on vaginal cytologies and estrous behavior for 2 to 3 d. Queens that received PLA implants during interestrus and estrus did not have superficial cells immediately after implant placement. However, PLA queens had superficial cells in vaginal cytologies, estrous behavior, and receptiveness to the male at regular intervals for 6 to 10 d.

At the end of the experiment, the queen from the PLA group that was excluded was not exposed to the tom, resulting in a final group of four queens. One queen from the MEL group was not mated due to limited space to accommodate the offspring, resulting in a final group of eight queens. After mating, all (4 of 4) PLA queens and 75% (6 of 8) of MEL queens became pregnant and had an apparently normal pregnancy.

It was noteworthy that none of the queens had any clinically detectable side effects during treatment.

4. Discussion

The duration of interestrus and mean serum P_4 and E_2 concentrations during PLA treatment were similar to those previously reported [4,5]. Neither the interestrus interval nor steroid hormone concentrations were modified by treatment with PLA implants. Furthermore, queens in the PLA group cycled at regular intervals when exposed to 14 h of continuous light. This confirmed previous findings of Leyva and colleagues [34], who reported that folliculogenesis was stimulated in queens exposed to continuous light but inhibited in queens exposed to an 8-h light regimen.

In contrast, duration of the interstrus interval was extended 2 to 4 mo when queens were treated with MEL implants, supporting our initial hypothesis that MEL could temporarily and reversibly suppress estrous cyclicity in queens. To some extent, these results were consistent with Griffin and colleagues who administered MEL implants to cats and thereby suppressed estrus for 75 d [41]. In the present study, low serum P_4 concentrations throughout the prolonged interestrus interval clearly indicated that spontaneous ovulation and pseudopregnancy did not occur. Only one queen from the PLA group had serum P_4 concentrations high enough to indicate the presence of a functional CL and pseudopregnancy; this queen was removed from the study.

The reproductive suppressive effects of the MEL implants were no longer present at the time of the next estrus, based on serum E_2 concentrations and pregnancy rate. Thus, treatment with a MEL implant could offer a method to control the estrous cycle in domestic queens while preserving future reproductive potential.

Length of the interestrus interval in the MEL group was strongly influenced by stage of estrous cycle when

implants were placed. This interaction could be explained by high E_2 concentrations when implants were inserted or by photorefractoriness (42]. In rats, high serum E_2 concentrations decrease expression of MEL ovarian receptors via downregulation (43]. The existence of ovarian MEL receptors has been reported in rats (43,44(and humans (45–47(but has not been studied in queens. If queens had ovarian MEL receptors, high E_2 concentrations might have downregulated MEL ovarian receptors and thus explained the shorter interestrus interval in queens treated with MEL implants during estrus. Photorefractoriness has been reported in rams (48–50(, sheep (51,52(, seasonal rodents (53(, mustelids (54(, and silver foxes (55]. This phenomenon occurs in animals that are maintained on a long and constant photoperiod (>14 h light) and undergo spontaneous reversion to the opposite photoperiod (42]. In the current study, perhaps prolonged elevated MEL concentrations (due to repeated treatments) produced photorefractoriness in treated queens. This may explain the shorter interestrus interval in queens when they were treated for the second time during estrus. However, because no reports of ovarian MEL receptor or photorefractoriness in queens are available, further studies are needed to confirm this line of reasoning.

In cats, intravenous administration of 5 mg MEL every other day, starting on the second day of follicle growth, suppressed E_2 synthesis [34]. Graham and colleagues [35] were able to suppress occurrence of estrus in queens with oral administration of MEL for 30 d. In their study, 30 mg/d MEL given orally to queens 3 h before lights-off effectively and reversibly suppressed estrus. More recently, Griffin and colleagues administered five MEL implants (12 mg each) to four cats, temporarily inhibiting estrus in three of four cats [41]. In the current study, an 18 mg MEL implant inserted during the interestrus interval reversibly suppressed estrus for 4 mo. However, when the implant was inserted during estrus, it reversibly suppressed estrus for only 2 mo. Although serum MEL concentrations were not measured, based on estrus suppression and P_4 and E_2 serum concentrations, the implant was apparently able to deliver enough MEL to maintain folliculogenesis, and estrus signs were suppressed. Further studies are under way to study serum MEL concentrations in queens with one or two implants inserted over a 4-mo period to confirm the clinical findings reported here.

The MEL implants used in the current study were reported to maintain plasma MEL concentrations in ewes to near physiologic nighttime values (300 to 1000 pmol/L) over a period of 10 wk [56]. Those implants are routinely used for a minimum of 40 d and up to 70 d during spring or early summer to advance seasonal breeding in several breeds of sheep [56]. Further studies are needed in queens to confirm that implants are capable of delivering near physiologic nighttime MEL concentrations over the period of 16 wk that estrus was suppressed.

Three to 11 d after insertion of MEL implants, some queens had behavior and vaginal cytologies, consistent with estrus, which lasted for 2 d. During this short interval, queens did not display receptiveness to the male. Unfortunately, blood samples were not taken to measure E_2 concentrations to confirm estrus. If estrus had been confirmed in these queens, these results could be related with those reported by Graham et al. and Griffin et al. [35,41]. Graham and colleagues reported that at least 30 d of oral MEL treatment was necessary to suppress follicular activity in all queens [35]. Similarly, Griffin and colleagues administered subcutaneous MEL implants to cats and reported a mean interval from implantation to estrus suppression of 20 d (range, 17 to 26 d) [41]. Based on the current study, we inferred that at least 11 d of subcutaneous MEL treatment is necessary to suppress follicular activity in the domestic cat. However, further studies are needed to confirm this assertion.

In this study, none of the queens had any clinically apparent side effects. In contrast, Griffin and colleagues reported uterine pathology as a side effect in queens given subcutaneous MEL implants [41]. Because these authors performed ovarian and uterine histopathology only after treatment and only in treated animals [41], they could not confirm that the pathology was due to MEL treatment. Although uterine and ovarian histopathology were not done in the current study, based on the high pregnancy rate obtained at the first breeding after treatment, we inferred that this treatment did not cause substantial uterine alterations.

In conclusion, a subcutaneous MEL implant effectively, reversibly, and safely suppressed estrus in queens for 2 to 4 mo. Additional studies are needed toward suppressing estrus in queens for the entire breeding season.

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