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Effects of Melatonin on the Onset of Ovarian Activity in Turkish Van Cats

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Abstract: With the present study, we aimed to investigate the effects of melatonin on the initiation of breeding season in the female cats. For this purpose, 12 female cats and 2 tom-cats (male cats) (vasectomized) at age of 2-6 years old were used. The female cats were randomly divided into control (group 1, n = 6) and melatonin-treated groups (group 2, n = 6). Sham (placebo) and melatonin implants (containing 18 mg of melatonin) were placed subcutaneously into the females during the late anoestrous in groups 1 and 2, respectively. Sexual behaviors, vaginal smears and serum progesterone levels for the animals were recorded. The effect of the daylight (varying from 9.5-12.5 h, December through April) on the cyclic activity were also assessed during the study period. The animals were sexually silent during the anoestrus period. Upon the commencement of breeding season, while the female cats in group 1 showed estrus signs, none but one of the female cats in group 2 possessed the indications of the estrus. Moreover, the findings of cytology were not statistically different in both groups during the study period ($p \geq 0.05$). Although, the progesterone levels in breeding season were higher in group 1 ($p \leq 0.05$) than in un-breeding season, no such difference was present in group 2. The progesterone levels in breeding season were markedly elevated ($p \leq 0.01$) in group 1 with comparison to group 2. In the latter group, melatonin effectively postponed cyclic activity of the females. The present results suggest that the administration of exogenous melatonin might prolong un-breeding season, presumably via the suppression of hypothalamic centers. Besides, we found that exposure to daylight of 10.5 or more hours per day were also, sufficient for the onset of cyclic activity in the female cats.

Key words: Cat, melatonin, estrus, vaginal cytology, progesterone, season

INTRODUCTION

Over the last decades, although the effects of photoperiod and melatonin on the regulation of reproductive events in various animal species have been widely studied, few studies are available concerning the reproductive functions of domestic felids (Arendt, 1998; Bittman *et al*., 1983; Peltier *et al*., 1998). In seasonal breeders, like the felids, annual transitions between the breeding and non-breeding seasons constitute a natural process of gonadal activation and deactivation (Arendt *et al*., 1993; Bittman *et al*., 1983). Melatonin appears to exert its effect on the reproduction through the hypothalami-hypophysial-gonadal axis (Reiter, 1980). Female cats are seasonal breeders when, they are exposed to natural photoperiod. The ovarian activity of the female cat is suppressed with decreasing light period and it resumes with increasing light period (Leyva *et al*., 1989b). The exposure of the female cats to the prolonged dark period of 16 h light/8 h dark cycle is shown to suppress the ovarian activity through increasing the melatonin synthesis (Leyva *et al*., 1989a). Furthermore, exogenous melatonin suppresses the ovarian activity in domestic cat even under 24 h light cycle (Leyva *et al*., 1984).

According to earlier observations on cats, the breeding season commences in January and February and continues, until the autumn, under the daylight conditions in the Eastern Turkey (latitude 38.03 °N) (Gulyuz *et al*., 1994).

Since, few studies are existing regarding the reproductive functions of domestic felids (Arendt, 1998; Bittman *et al*., 1983; Peltier *et al*., 1998), we planned to determine the influence of exogenous melatonin, as a subcutaneous implant, on the estrus cycle of the domestic cats, on the ovarian dynamics during the breeding and non-breeding seasons and on the prolongation of the anoestrus period in transition period (from anoestrus to estrus).

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MATERIALS AND METHODS

This study was conducted during the non-breeding season when, all the females were in anoestrus, continued during transition period and ceased when the third estrus were observed in most of them between December 17th, 2003 and April 04th, 2004. Twelve adult female and 2 vasectomized male cats, aged 2-6 years, were randomly selected from the cats that were housed in Van Cats Research Center (38°N), Van, Turkey. The cats were fed daily with a commercial cat food (Chicken Cat Food, IAMS Co., USA) and with drinking water provided *ad libitum*.

The females, previously exposed to 16 h dark cycle for 3 weeks, were randomly separated into control and melatonin groups. On December 17th, 2003, all the female cats received subcutaneous implants into the scruff of their neck. The hormone implants contained 18 mg of melatonin (Melovine®, Sanofi Sante Animals, UK) while, sham implants had no active substance. Subsequently, all the cats were simultaneously exposed to natural photoperiod conditions. The weekly mean day length varied during the study as follows: on December 21st, 9 h 36 min, on January 28th, 10 h 30 min, on February 25th, 11 h 35 min and on March 17th, 12 h 28 min.

The study was ceased on April 4th when, the light cycle was 13 h and the implants were removed from the animals in both groups.

The ovarian activity of the animals was assessed through the observation of sexual behaviors (Ishida *et al*., 2001; Shille *et al*., 1979), analysis of the serum progesterone levels (Dieleman and Schoenmakers, 1979) and the examination of the vaginal cytology (Concannon and Digregorio, 1986; Shille *et al*., 1979) with some minor modifications. All the females in both groups were subjected to thorough estrus observation with a teaser male for 90 min every day in separated rooms. Sexual behaviors of the males and females were observed and the data obtained were recorded. The blood samples were collected weekly using cephalic vein (1-2 mL cat^{-1}). The serum samples were obtained by centrifugation at 1000 rpm for 5 min and stored at -20°C, until the analyses. Progesterone levels were measured by Radioimmunoassay (RIA) (Dieleman and Schoenmakers, 1979). In order to avoid induction of ovulation, the samples of vaginal cytology were collected weekly. The vaginal cells were collected and stained with Toluidine blue. The cell types were determined by vaginal smears to reveal the progression of follicular development (Johnston *et al*., 1996).

Statistical analyses of the data on vaginal smears and hormonal analysis were performed by the Paired Samples t-test, using the SPSS for Windows Release 7.5.1 Standard Version, 1989-1996 and the data obtained were expressed as percentages.

RESULTS

The effects of melatonin on the responses to photoperiod and the male receptivity of females at both anoestrus and the transition period (initiation of the normal breeding season) are summarized in Table 1.

During the anoestrus season, the female cats in both groups neither exhibited any sexual behavior nor mated. When, the length of daylight reached 10.5 h, the estrous behaviors were observed in all the females in control group (6/6), but only one female displayed estrus signs 16 days after the beginning of breeding season in melatonin group, as confirmed with a decreased progesterone level and increased vaginal intermediate and superficial cells in that period.

Melatonin was found to be especially, effective to prolong the non-breeding season in the majority, except one, of the females in melatonin group. Upon the commencement of breeding season, all the female cats in the control group showed estrus and mated, which was verified by the presence of decreased progesterone levels and vaginal smear findings. In addition, during the study, one female in melatonin group also exhibited estrus behaviors and mated.

The females in control group exhibited their first estrus, with some individual differences, during the new season mainly because of increasing daylight, reaching or exceeding 10.5 h day^{-1} in this location (38°N). Surprisingly, we also noticed that there was a tendency of synchronization of non-estrous females by the presence of the individuals with estrous towards the end of the present study.

The effects of melatonin implants on the vaginal cytology of the females in both estrus and anoestrus periods are summarized in Table 2. The relative percentage of vaginal cell types of females in control and melatonin groups was not statistically different during the breeding and non-breeding seasons. In general, distributions of the vaginal exfoliative cell rates in melatonin group were similar in both the breeding and non-breeding seasons. However, the parabasal and intermediate cells were encountered more in non-breeding season than in

Table 1: The effects melatonin implant on the appearance of sexual behaviors (%) in female cats

Groups	Anoestrus period (n = 6)	Oestrus period (n = 6)
Control	0	100
Melatonin	0	16.66

Table 2: Distribution of vaginal exfoliative cells in control and melatonin groups

Groups	Cell types	Anoestrus no: 42 (%)	Oestrus no: 70 (%)
Control	Basal	7.69±0.74	5.49±0.49
	Para basal	18.47±1.31	12.90±0.76
	Intermediate	52.58±1.38	45.11±0.98
	Superficial	21.81±1.26	36.50±1.65
Melatonin	Basal	9.06±0.84	6.61±0.71
	Para basal	21.28±1.38	16.40±1.37
	Intermediate	46.25±1.45	48.16±0.72
	Superficial	23.14±1.63	28.83±2.08

Table 3: Progesterone concentrations in control and melatonin groups

Groups	Anoestrus season	Oestrus season
Control	0.32±0.12^{a}	18.78±3.25^{b**}
Melatonin	0.36±0.12	2.73±0.69*

^{a,b}: Statistical differences ($p \leq 0.05$) within the groups. *,**: Statistical differences ($p \leq 0.01$) between the groups

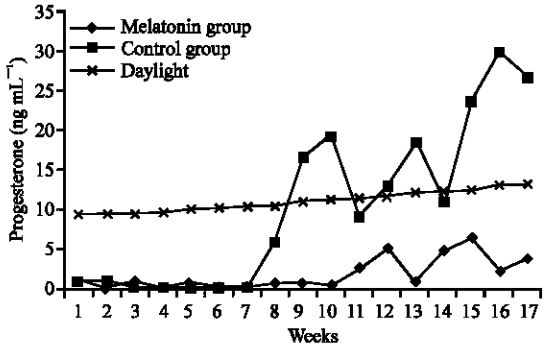

Fig. 1: The weekly plasma progesterone levels in control and melatonin groups during light cycle

breeding season. Additionally, the superficial cells were more common in breeding season than in non-breeding season.

The weekly serum progesterone concentrations in control and melatonin groups are shown in Fig. 1. The mean progesterone level of the females in control group was statistically higher ($p \leq 0.05$) in breeding season than in non-breeding season. However, there was no such difference ($p > 0.05$) between the non-breeding and breeding seasons in melatonin group. Furthermore, the mean progesterone level in control group was statistically higher ($p \leq 0.01$) than in melatonin group during the breeding season (Table 3).

We also, noted that 3-5 days after the onset of the estrus the serum progesterone concentrations increased gradually and exceeded 1 ng mL^{-1} during the first 2 days and continued elevating over the 10 ng mL^{-1} between the 3rd-5th days. The progesterone level ranged from 8-40 ng mL^{-1} during the diestrum period.

DISCUSSION

Cats are long-day breeders and seasonally polyestrous; however, the season can be quite long. In the Northern hemisphere, the breeding season begins in January as the days begin to lengthen and often continues until September. The non-breeding season starts in September as the days begin to shorten and often continues, until February. In the present study, the females of groups 1 and 2 exposed to natural light of daily 9-10 h, did not show any signs of sexual behavior during the non-breeding season, as confirmed by the vaginal smears and hormonal analyses. The progesterone concentrations (<1 ng mL^{-1}) were found to be similar in both groups during the non-breeding season. Hence, the present findings indicate that the sexual silence of the females exposed to longer dark cycle is their physiological response, regulated by the increased secretion of melatonin.

Several studies exist concerning the role of melatonin in the regulation of breeding in animals. Exposure to dark for 16 h is shown to increase the endogenous melatonin secretion, leading to the cessation of ovarian activity under the natural conditions (Leyva *et al*., 1989a). The endogenous melatonin exerts its main effect at the Hypothalamic-Pituitary-Gonadal Axis (also HPGA). In the present study, the exogenous melatonin treatment was found to be as effective as the endogenous melatonin and prolonged the non-breeding season (anoestrous period) in 83% of the female cats (5/6). In addition, the melatonin implants markedly lowered ($p \leq 0.05$) the progesterone concentration with regard to the controls. The working mechanism of the melatonin can be explained briefly as follows: The melatonin implants caused melatonin concentrations to remain high, thereby suppressing the GnRH secretion through exerting a negative feedback effect on the hypothalamus (Graham *et al*., 1998; Leyva *et al*., 1984, 1989a). Even though, melatonin implants ceased estrus sings or any sexual behaviors in most of the female cats (83%), one female cat still showed estrus sings. Indeed, the progesterone concentration in this female cat was higher than the rest of the females in the melatonin group. This result may indicate that even though, melatonin can markedly postpone the breeding season via suppressing the ovarian function, it still cannot completely block ovarian activity and prolong the non-breeding season. By contrast, as the length of daylight increased (10.5 h or more), the female cats in control group (n = 6) showed estrus behaviors and mated. As the daylight cycle increases, the synthesis of melatonin is suppressed and then the inhibitory effect of melatonin on the hypothalamus disappears. This leads a higher gonadotropin release by the pituitary gland that triggers the follicular development in the ovaries. Thus, the cyclic activities of females in the control group were in parallel with the features of typical breeding season. In the present study, we also noticed that the cyclic activities did not initiate in the control group unless the dark cycle

was about 13.5 h or less. This pattern is similar to a previous study (Leyva *et al*., 1989a), reporting that the exposure to dark cycle for 16 h leads to an increased melatonin secretion, thus, blocking the sexual cycle of female cats. A possible explanation for the difference would be that the dark cycle of 13.5 h or less might be minimal threshold level for the cats. In fact, a comparable finding also indicates that a minimum light cycle of 10 h is enough to initiate the estrus cycles in cats (Shille and Sojka, 1995).

Besides, the relative percentages of vaginal exfoliative cell types within and between the groups were alike. Surprisingly, the examination of these cells during the non-breeding season in both groups depicted a mixture of various cells types. During the anoestrus period, the dispersion of superficial cells was observed to be around 20% in both groups. The presence of the similar results during the breeding and non-breeding seasons in the control group seems to reflect the estrus and diestrus phases of the estrous cycle. Additionally, the occurrence of 60-65% of superficial cells in vaginal cytology does not generate a firm base for the identification of the estrous cycle in the female cats. Nevertheless, the changes in the distribution and appearance of the vaginal cells with regard to the phases of the estrus cycle are useful for the recognition of the sexual activities and behaviors (Shille *et al*., 1979; Shille and Sojka, 1995). The intermediate cells were commonly present in vaginal smears during both the breeding and the non-breeding seasons; therefore, this type of exfoliative cells may not be a reliable tool for predicting the onset of estrus and the phases of sexual cycle in the cat. Nevertheless, the frequencies of the vaginal cells in melatonin group were similar in both the breeding and the non-breeding seasons, indicating that most of the females in this group did not show estrus cycle and sexual activity during the course of the observations.

Moreover, the occurrence of the intermediate and parabasal cells in the non-breeding seasons was encountered more frequently than in the breeding season in both groups. Actually, these cells are observed commonly in prepuberty, proestrus, metestrus and anestrus in feline vaginal smears (Gulyuz *et al*., 1994).

CONCLUSION

Overall, the present results indicated that the exogenous melatonin treatment may prolong anoestrus, presumably by the suppression of hypothalamic centers. In addition we further showed that the exposure to light cycle of 10.5 or more hours was likely to initiate the reproductive activities in female cats even though, some individual differences may occur. More detailed investigations on the effects of melatonin levels at cellular and molecular levels remained to be done to elucidate mechanism of melatonin on the regulation of reproductive functions in the cats.

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