Research Article

The Efficiency of Melatonin Hormone Implantation in Suppressing the Estrus Cycle in Domesticated Queens (*Felis catus*)

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Abstract | The current study evaluated the efficiency of melatonin implants as a single subcutaneous dose to suppress or delay the estrus cycle in queens. Ten domesticated non-pregnant cats of known origin aged between 1-3 years were used. The cats were divided randomly into two groups. The first group (G1, n=5) was left without treatment and served as control group, and the second group (G2, n=5) was administered 18 mg melatonin implants as a single-dose subcutaneously. Before starting treatment at day zero, 3 ml of blood was collected from the jugular vein in all cats to measure estrogen and progesterone hormones. Post-treatment, the cats were monitored twice a day to determine the sexual behavior of estrus based on clinical signs, using a male detector and taking a swab from the vagina to confirm the estrus phase. Also, on days 14, 30 and 60 after treatment, reproductive hormones (estrogen and progesterone) were analyzed in the blood. The results of the current study revealed that interestrus days were significantly higher in the G2 group (111.00 ± 2.68 days) compared to the G1 group (28.00 ± 8.38 days), with a significant difference ($P < 0.05$). While there was a non-significant effect on estrus duration, with only a numerical increase ($P > 0.05$) in the estrus phase in G2 (9.20 ± 1.11 days) compared to the G1 group (7.00 ± 0.70 days). The estrogen and progesterone hormone levels were found varied in different periods, however they were remained at basal level (under 20 pg/ml and 2ng/ml respectively) in both groups. In conclusion, the melatonin implant suppresses the estrus cycle for 4 months without any adverse effects and maintains the concentration of estrogen E2 and progesterone hormones at the basal level.

Keywords | Domestic Queens, Estrus Suppression, Melatonin Implant, Hormonal Analysis, Sexual Behavior, Estrus Phase.

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INTRODUCTION

Cats are animals with highly efficient reproduction (Hassan and Saleh, 2022). The challenge of cat overpopulation is a global issue with severe consequences, including the spread of disease, and the euthanasia of millions of cats yearly (Al-Zubaidi et al., 2024). The main objective of contraceptives, fertility control in cats is to prevent reproduction by preventing the estrus cycle in females, including vocalization, rolling on the ground and a very short interestrus period, and male sexual behaviors, especially urine spraying (Johnson, 2022), owned cats and ultimately helped the management of catteries (Root Kustritz, 2018). It is imperative to understand and explore effective methods of feline reproductive control (Goericke-Pesch et al., 2014). Moreover, the current contraceptive procedures for cats have unwanted effects, some of which can be severe (Munson, 2006). Permanent reproduction control in companion cats can be accomplished through surgical

methods (e.g., ovariohysterectomy or ovariectomy) (Howe, 2006; Ibrahim and Zaid, 2017). Despite being common, the surgery may result in potential complications, including hemorrhage, peritonitis, and ovarian remnant syndrome (Karneva et al., 2017). Surgical methods are expensive and result in permanent sterilization that is unsuitable for controlling animal reproduction with future breeding value (Gimenez et al., 2009). Pharmacologic protocols, such as progestins, androgens, and gonadotropin-releasing hormone (GnRH), offer a reversible approach to controlling reproduction analogs, and immune contraception (Saxena et al., 2003). Despite their effectiveness as contraceptives, they can induce adverse effects, especially the progesterone component, that may be life-threatening (Romagnoli and Concannon, 2003; Gimenez et al., 2009). For decades, progestins have been used as an alternative to surgical spaying in female cats (Romagnoli and Ferre-Dolcet, 2022). Melatonin is another new medical contraception used in felines (Miyamoto et al., 2004). Melatonin, a neurohormone secreted by the pineal gland, is crucial in regulating cats' ovarian activity, it inhibits the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus, reducing the secretion of gonadotropins (LH and FSH) from the pituitary gland (Schäfer-Somi, 2017). This suppression of GnRH release ultimately decreases the production of sexual steroid hormones, including progesterone, and affects ovarian function. Melatonin secretion is influenced by the duration of daylight, with higher levels during periods of darkness. Administration of melatonin can disrupt the positive effects of an extended photoperiod on folliculogenesis, altering the duration of the inter-estrous interval in queens (Furthner et al., 2020).

Melatonin production from the pineal gland synchronizes the reproductive cycles of many species marked by seasonality (Hatif, 2020). By activating N-acetyltransferase, which transforms serotonin into N-acetyl serotonin, these cells function as neuroendocrine transducers of environmental changes (Al-Azawi et al., 2003). The pineal gland's melatonin production follows a circadian rhythm, with low levels produced during the day and higher concentrations during the night. However, melatonin synthesis involves the conversion of hydroxylated tryptophan to 5-hydroxytryptophan, which is then converted into serotonin. This process also controls ovarian activity in seasonal animals, such as cats (Al-Hamedawi T M et al., 2020; AL-Shammary and Al-Yasiri, 2023), with peak concentrations occurring during anestrus and interestrus (Kassim et al., 2019). Exogenous melatonin (administered orally or by injection) appears to suppress cats' estrus cycles. Melatonin may serve as the signal by which domestic female cats measure exogenous melatonin, and photoperiod melatonin may mimic the effects of decreasing photoperiod (Graham et al., 2004). Administration of 18 mg melatonin implant subcutaneously suppressed the inter-estrus, and estrus without initial estrus signs and effect lasts for 2-4 months (Fontaine, 2021). In another study, the 18 mg melatonin implant (Melovine) typically reduced ovarian activity for a longer period than the typical natural pseudopregnancy duration, particularly when it was placed during the inter-estrus phase (Schäfer-Somi, 2017). To the best of our knowledge, in Iraq, the effects of melatonin implants have not been explored in local cats. Therefore, the present study aimed to evaluate the effectiveness of melatonin implants in suppressing the estrus cycle in local domestic queens. Additionally, the study investigated the side effects of melatonin implants on female domestic queens and hormone values (estrogen and progesterone) before and after treatment.

MATERIAL AND METHODS

Ethics

Ethical approval was granted through the local animal care committee and used at the College of Veterinary Medicine within the University of Bagdad (number P.G/14 on 3/1/2024) after the end of the study.

Animals

The study was carried out at the Faculty of Veterinary Medicine, University of Baghdad's animal house facility during the cat breeding season from December 1, 2022, to June 1, 2023. The research involved ten pubertal crossbred queens, aged 1 to 3 years and weighing 2.4 to 3.2 kg, all in the interestrus phase. Additionally, three intact tomcats each 3 years old, were included as teaser males and were isolated in a metal cage within the room to stimulate estrous behavior (Vansandt, 2022). The animals were housed in a dedicated room measuring 6x5 meters and experienced a natural light photoperiod. Animals were fed premium commercial cat food and had unlimited access to water. Before the experiment began, a comprehensive evaluation of the cats' reproductive health was conducted. This involved using ultrasound to confirm the absence of pregnancy and assess the condition of the uterus. Simultaneously, vaginal swabs were collected to determine the specific phase of their estrus cycle and monitor the presence of estrus-related behavioral signs in the female cats. After meticulous verification of this data, treatments were administered during the interestrus phase according to the established experimental protocol. The entire experiment lasted for five months (1 month adaptation and 4 months estrus observation after treatment), allowing for the observation of the females' return to estrous behavior (Kutzler, 2015).

Experimental Design

The females were randomly divided into two groups: Group one (G1) without any treatment to keep as the con-

trol group. Group two (G2) received 18mg of melatonin (Melovine, Subcutaneous) as a single dose under general anesthesia and aseptic conditions. The three males were present in the same room for estrus induction and to detect the estrus when released, each one placed in a special cat cage. The cage size was 90cm in length, 60cm in width, and 60cm in height. Furthermore, they were released for one hour daily when estrus signs were observed, and the males were kept under control by preventing them from mounting females. According to Schäfer-Somi et al. (2014), the queens were monitored daily for behavioral signs of estrus. Also, vaginal cytology was assessed thrice weekly to detect the estrus cycle stage, according to Kanca et al. (2014).

Blood Collection And Hormonal Analysis

The blood collected from each cat on day zero, fourteen days, thirty days, and sixty days was approximately 2-3 ml at each time from the jugular vein or cephalic vein under aseptic condition and a general anesthesia (Al-Kalidi et al., 2017). The blood was centrifuged for 15 min at 4000 rpms (AL-Dulimy 2016) to separate the serum from the blood (Johnston et al., 2001; Axnér and Holst, 2015). The serum samples were analyzed to measure the concertations of estrogen and progesterone by an automated enzyme immunoassay system (Cobas-analyzer, Roche, Germany) according to methods of Younis and Akram, (2023).

Clinical Observations

Identification of Estrus in Queens After Treatment: A daily two-hour observation period involved systematically observing feline behavior, physiological cues, and external indicators of signal estrus, behavioral signs including lordosis, foot treading, tail positioning to expose genitalia, distinctive vocalizations, circling of male cages, and restlessness (Malandain et al., 2011). The monitoring process also considered physiological changes, including alterations in vaginal cytology and hormonal fluctuations (Al-Hamedawi et al., 2016). Detailed records of the estrus phase in each queen were kept, and the time between estrus (interestrus period) during treatment was carefully noted.

Vaginal Cytology Technique: Vaginal cytology was done to confirm the stage of the estrus cycle, according to (Kanca et al., 2014). The Diff-Quik stained slide smears were evaluated under a microscope at a magnification of 100X, 400X, and 1000X to count the intermediate, parabasal, and superficial cells through ornamentation (Reckers et al., 2022). The percentage and type of cells were used to determine the estrus cycle phase (Nawaf and Ibrahim, 2019).

Statistical Analysis

Data was statistically analyzed using Statistical Analysis System - version 9.1. The significant differences (LSD) post hoc tests and one-way and two-way ANOVA were used to assess the significance of the mean differences. Also, an independent t-test was used to compare the two means. Statistical significance was defined as $P < 0.05$ (Al-Hamedawi, 2013).

RESULTS AND DISCUSSION

Clinical Observation

Daily clinical observations were conducted to closely monitor the behavioral and physical cues of female cats, with a focus on identifying their entry into the estrus phase. These observations revealed distinct behavioral (Figure 1) and physiological changes, such as increased attraction to male cages and interactions, greater sociability with persons and other objects, unique walking patterns, and heightened vocalization during estrus; also, they were manifested when palpated by hand that raises the hind quarter and tail, which that agree with (Robinson and Noakes, 2018).

Figure 1: Behavioral sign of estrus during estrus phase in the queen

Vaginal Cytology Examination

During the anestrus phase in feline queens, vaginal smears exhibited a predominant presence of parabasal and small intermediate vaginal epithelial cells (Figure 2). These results agree with previous studies on felines (England and Friedrichs, 2014; Schäfer-Somi et al., 2014). However, during interestrus, the common cells were intermediate with few basal or parabasal and keratinized nuclear cells, this was consistent with (Solano-Gallego and Masserdotti, 2015).

In contrast, during the estrus phase in feline queens, the primary cell type observed was superficial cells with nuclear characteristics (Figure 3). This pattern is consistent with prior investigations in feline queens (England and Friedrichs, 2014). Different studies have consistently described vaginal cytology during estrus as consisting exclusively of cornified, annuclear superficial cells. The vaginal cytology in the current study exhibited >75% increase during estrus in cornified epithelium, while intermediate cells were per-

sist visible (Abid et al., 2011). Cornification may not influence 100%, as in the dog, the nucleus does not disappear altogether (Reddy et al., 2011; Johnson, 2018). These results agrees with (Kadhim et al., 2011; Kanca et al., 2014), which show that increase in estrogen concentration during estrus result in an increase in the number of layers of cells and keratinization and shedding of cells in the vaginal epithelium. Another report (Reckers et al., 2022) showed that increase in estrogen concentration might cause a 20-layered epithelium during estrus, compared to the 3-4 layers in anestrus; in addition to changes in cell size, shape, and nuclei.

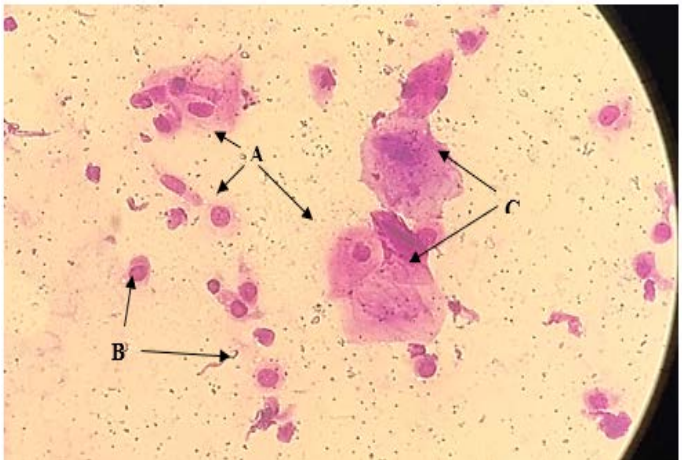

Figure 2: Vaginal cytology smear in interestrus period A. intermediate cells, B. Parabasal cells and C. Superficial cells in 400 x.

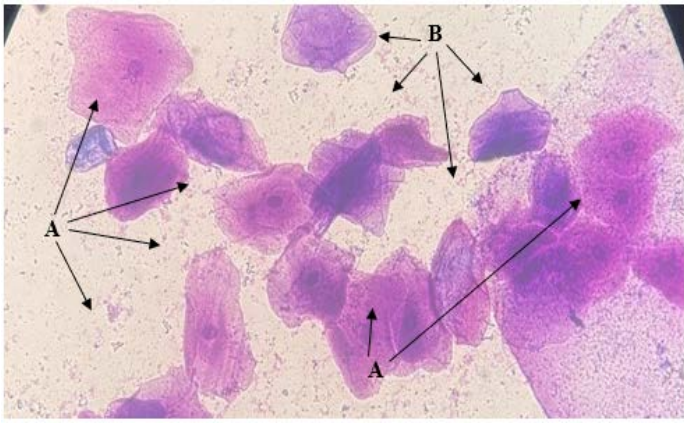

Figure 3: Vaginal cytology smear in estrus period A. nucleated Superficial cells B. unnucleated Superficial cells) in 1000x.

Animal Response And The Effect Of Treatment On The Return Of Estrous And Duration Of Estrous In Domestic Queens

All female cats in the study returned to estrus during the experiment period, which was 5 months. Both G1 and G2 cats had an estrus response of (5/5), means 100% returned to estrus during the study. These findings showed that animals in both groups did not show any significant difference ($p < 0.05$) due to the return of estrus at different periods during the experimental duration. These clinical findings of temporary hormonal estrus suppression related to the contraceptive effect of melatonin are consistent with Faya et al. (2011) who reported that melatonin administration may suppress the estrus cycle for approximately 2 to 4 months, with a range from 100% (9/9) in cats with 18 mg implants to 50% (2/4) in cats with 12 mg implants. Also, it is in agreement with (Gimenez et al., 2009) who reported that melatonin implants immediately affected estrous suppression.

Table 1: The mean ± SE effect of melatonin implant on interestrus and estrous duration in queens.

Groups	Duration of estrus (days)	Interestrous period (days)
G1	8.80±0.96 a	28.00±8.38 b
G2	9.20±1.11 a	111.00±2.68 a
P-value	0.79 NS	<0.0001

* The same letters in a column shown no significant differences (P≤0.05), and different letter indicates a significant differences (P≤0.05) between groups. G1: Control group (without treatment). G2 treatment group (implant of 18mg melatonin subcutaneously)

The present study used a melatonin implant to study its effect on suppressing the estrous cycle and duration of sexual estrous behavior. Table 1 showed that the return estrous days (interestrus duration) after melatonin implantation in G2 was 111.00 ± 2.68 days that was significantly higher ($P<0.05$) than G1 (28.00±8.38 days). That difference in the result of melatonin (G2) occurs due to the effect of melatonin treatment which acts as a temporary suppressing agent to estrus cycle in domestic queens. This result agrees with (Gimenez et al., 2009) who reported the effectiveness of a subcutaneous melatonin implant to inhibit estrus in queens (*Felis catus*). Aforementioned study verified that there were no clinically noticeable adverse effects from the subcutaneous melatonin implant and that it successfully and reversibly reduced estrus in queens for two to four months. Also, the results of the current study are consistent with the results of (Schäfer-Somi, 2017), when the 18 mg implant was used in interestrus in cats, estrus suppression was noticed without initial estrus signs, and this effect was prolonged up to 3 months. It was reported that 22/27 queens were treated this way, and estrus did not occur for an average of 103.9 days. However, the days of estrus suppression decreased in the study of (Furthner et al., 2020), who reported that 33/42 female queens had estrus inhibition following implantation of 18 mg melatonin for days (range 21-277), a mean of 86 ± 50 days. This variation showed that the treatment suppression depending on the time of implantation, females implanted during sea-

sonal anestrus, interestrus, or estrus. Our current results are also disagree with (Faya et al., 2011), who recorded the interestrus interval (63.8 ±5.4) days. The melatonin implants should be administered during the interestrous phase. However, when implanted during the anestrus stage, the duration was multiple times as long as when implanted during the estrous phase. Specifically, the duration was approximately 110 days during anestrus and 60 days during estrous (Gimenez et al., 2009).

In current study, our result regarding the duration of estrus, which recorded 8.80 ± 0.96 days and 9.20 ± 1.11 days in G1 and G2, respectively, with a non-significant difference ($p<0.05$) between groups, was in agreement with (Gulyuz et al., 2009), who during studying the effect of melatonin on the ovarian activity in Turkish Van cats concluded that the treatment was utterly reversible to ovarian activity and standard period of estrous was 7-10 days after the end of treatment.

Effects Of Melatonin Implant On The Female Reproductive System In Domestic Queens.

After using a single dose of 18 mg of melatonin implant for estrous suppression, no side effects were observed to the queens during 6 months, this result is due to the moderate dose during treatment, as well as not repeating the treatment more than once during the experiment. These results agree with (Gimenez et al., 2009), who concluded that the cats with initial estrus after melatonin application were bred during the estrus after the implantation, the cat delivered a good kitten, the period of gestation gave birth, lactation, and breeding care were ordinary. The study conducted by Johnston et al. (2001) demonstrates that the duration of behavioral estrus during ovulation was 4.71 ± 0.32 days, while in non-ovulating individuals it lasts for 6.65 ± 0.44 days. Therefore, the presence or absence of ovulation affects the length of the estrus phase, and the absence of males leads to prolonged estrous behavior. A study by (Gültiken et al., 2022) concluded that the follicular phase duration ranges from 3-16 days with an average of 7.4 days. The phase length was not altered by copulation or ovulation.

Hormonal Analysis

Effect Of Melatonin Implant On Estrogen

The study demonstrates that administering melatonin implants to domesticated queens significantly influences the concentration of estrogen hormone at various time points. The results presented in Table (2) indicate the recorded estrogen concentrations at different time intervals.

In the G1 (control group), the estrogen values were 11.56±4.00, 7.61±1.41, 10.64±2.39 and 12.78±3.40 pg/ml in different days of treatment at zero time, 14, 30, and 60 days respectively. There were no significant difference in these values at different days of study period. Also, melatonin implant group (G2) exhibited the estrogen value 5.72±0.85, 9.04±1.33, 4.76±0.32 and 4.59±0.21 pg/ml on different days of treatment viz., zero time, 14, 30, and 60 days respectively, without significant difference between days.

Table 2: The mean ± SE effect of melatonin implant on estrogen level (pg/ml) in domesticated queens.

Groups	Zero time	14 day	30 day	60 day
G1	11.56±4.00 A	7.61±1.41 aA	10.64±2.39 aA	12.78±3.40 aA
G2	5.72±0.85 A	9.04±1.33 aA	4.76±0.32 bA	4.59±0.21 bA

*Means with a different small letter in the same column are significantly different ($P<0.05$)

*Means with a different capital letter in the same row are significantly different ($P<0.05$). G1: Control group (without treatment). G2 treatment group (implant of 18mg melatonin subcutaneously)

Also, when compared between groups on day 14, the estrogen level (7.61±1.41 and 9.04±1.33) pg/ml in G1, G2 respectively, was not significantly different; however on day 30, the estrogen value (10.64±2.39, 4.76±0.32) pg/ml in G1, G2 respectively, exhibited a significant ($P<0.05$) raise in G1 than G2. Similarly, on day 60, the estrogen value (12.78±3.40, 4.59±0.21) pg/ml in G1, G2 respectively, also exhibited a significant ($P<0.05$) increase in G1 than G2. These results related to the used hormone to contraceptive lead to a decrease in GnRH hormone to act hypothalamus pituitary gonad axis to inhibit folliculogenesis which leads to decreased estrogen value, also all estrogen values remained below the normal range for estrus, which is 20 ng/ml. Johnston et al. (2001) studied the estrogen value in the estrous cycle in queens, they confirmed the mean concentration of estradiol-17B is above baseline in anestrous below (20 pg per ml) on the first day of estrus for both mated and non-mated queens, the values generally remain elevated throughout estrus and return to baseline after the onset of ovulation for ovulating queens, last 24 to 48 hours of estrus for non-ovulating queens and Estradiol- 17B concentrations, which can fluctuate dramatically, generally range between 20 to 40 pg per ml during estrus.

Also, Schmidt et al. (1983) confirm no direct relationship between the concentration or duration of circulating estradiol-17B levels and the magnitude of the LH response; however, circulating titers of this hormone were more significant than 20 pg/ml and were closely associated with sexual receptivity and the presence of visible follicle activity on the ovary.

Effect Of Melatonin Implant On Progesterone Hormone

The concentration of progesterone were recorded at different times to study the melatonin implant effect on the level of progesterone hormone in domestic queens and results were presented in Table (3). In the G1 (control) group, the progesterone values on different days after treatment were 0.90±0.15, 1.20±0.09, 1.05±0.29, and 0.94±0.09 ng/ml at zero time, 14, 30, and 60 days respectively. Also, in G2 group, the progesterone values were 0.65±0.18, 1.61±0.09, 1.27±0.14, and 1.67±0.31 ng/ml at zero, 14, 30, and 60 days respectively. In control group, no difference (P>0.05) was observed in progesterone concentration between various days, however, in G2 group, as compared to zero day, 14,30,60 day exhibited a raise (P<0.05) in progesterone level.

Table 3: The mean ± SE effect of melatonin implant on progesterone hormone level (ng/ml).

Groups	Zero time	14 day	30 day	60 day
G1	0.90±0.15 A	1.20±0.09 bA	1.05±0.29 bA	0.94±0.09 bA
G2	0.65±0.18 B	1.61±0.09 aA	1.27±0.14 bA	1.67±0.31 aA

*Means with a different small letter in the same column are significantly different (P<0.05)

*Means with a different capital letter in the same row are significantly different (P<0.05). G1: Control group (without treatment). G2 treatment group (implant of 18mg melatonin subcutaneously)

When compared between groups, on day 14 the progesterone values (1.20±0.09, 1.61±0.09) in G1 and G2 respectively, exhibited a significant raise (P<0.05) in G2 as compared to G1 group. Similarly, on day 60, the level of progesterone in G2 (1.67±0.31) was higher (P<0.05) as compared to G1 (0.94±0.09) group. The progesterone concentration typically remained basal (2 ng/ml) in all groups and days, probably because the cat without ovulation and formation corpus luteum is responsible for the excretion of progesterone hormone. This result is in agreement with (Swanson et al., 1997), who concluded that the concentrations of serum progesterone naturally remained basal (2 ng/ml) in anovulatory queens but raised above baseline in ovulatory and anovulatory females, mean progesterone increased from a normal of 1.2 ± 0.4 ng/ml to 9.6 ±1.7 ng/ml after ovulation. Also, in agreement with (Brown, 2011), who confirmed that plasma progesterone concentrations are basal during anestrus, interestrus, proestrus, and estrus before ovulation. This observation is also in line with Ström Holst and Frössling (2009), who concluded that peripheral concentrations of progesterone remain at basal levels until near the time of ovulation. Progesterone levels rise coincident with the estimated time of ovulation, increase slowly throughout the remainder of estrus, and remain elevated during the ensuing luteal phase or pregnancy. Unlike the dog, the cat does not exhibit a preovulatory rise in progesterone.

CONCLUSION

The melatonin implants (18mg of melatonin) used subcutaneously as a single dose were a suitable contraceptive in domestic queens without side effects. Also, it suppressed ovaries activity by inhibiting estrogen and progesterone hormone up to 4 months after treatment.

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NOVELTY STATEMENT

This study introduces a novel approach to feline reproductive control by evaluating the effectiveness of melatonin implants (18mg, Melovine) for suppressing the estrus cycle of domestic queens. The research demonstrates the successful suppression of estrus for 4 months without observed side effects. Furthermore, our results reveal a significant impact on estrogen and progesterone hormone levels and also showed melatonin implants as a safe and reversible contraceptive method for felines, by minimizing potential complications associated with traditional surgical and pharmacological interventions.

CONFLICT OF INTERESTS

The author declared that there is no conflict of interest.

AUTHOR CONTRIBUTION

All authors made equal contribution.

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