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Comparative Assessment of Melatonin Implants and Progesterone Hormone Effect on the Testicular Changes in Tomcats

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Abstract

The purpose of study to evaluate the effectiveness of melatonin implants and progesterone hormone as non-surgical methods for controlling reproductive activity in adult male cats. Twenty-four healthy toms, aged 3–4 years, were equally divided into three groups: a control group, a melatonin-treated group (18mg S.C.) and progesterone-treated group (megestrol acetate 2.5 mg every 10 days for two months). All cats were housed in comparable environmental conditions, and behavior observations as well as serum testosterone concentrations were taken 20, 40 and 60 days after treatment.

melatonin implants and progesterone hormone both led to testicular regression ($p < 0.05$) of treated tom cats comparative to control. Testicular length and width, scrotal circumference, seminiferous tubule diameter, interstitial tissue thickness, number of interstitial cells, and height of germinal epithelium all showed a gradual decrease over the course of 60 days. Regression was modest with melatonin (MLT) and stronger and earlier with progesterone. By day 60, testicular length and width decreased to approximately 17 mm (MLT) and 14 mm (in P4-treated cats), while scrotal circumference was reduced to 2.44 cm (MLT) and 1.52 cm (P4-treated). Histologically, melatonin led to a gradual vacuolation and desquamation of germ cells, whereas progesterone induced marked seminiferous atrophy, change of spermatogenic cells and expansion of the interstitium. There were no local or systemic adverse reactions. Results indicate that both have an inhibitory effect on testicular structure and reproductive activity, the suppression being reversible and progesterone having more potent inhibition.

Keywords: Melatonin implants, Progesterone, Tomcats, Testes.

I. Introduction

Surgical castration is the most common form of tomcat reproductive control; however, reversible and non-invasive methods are now in demand. particularly for Persian cat's breeders generating interest in hormonal treatments. Pharmacological approaches using products like Gonadotrophin-Releasing Hormone agonists, and melatonin implants provide for temporary suppression of testosterone and spermatogenesis that is reversible without permanent infertility (**Ferré-Dolcet & Romagnoli, 2023; Kutzler, 2015; Al- Shammary S.M. and Al-Yasiri E. A. 2023)** Testosterone level is affected by age (**Al-Shammary et al., 2013; Saaed and Zaid 2019**), species (**ZAID, 2017**), season (**Hussain et al., 2017**). Cats are seasonal breeders that undergo natural reproductive suppression during short photoperiods, when melatonin serum levels rise and the hypothalamic pituitary gonadal axis is down-regulated **(Luño et al., 2023; Rahawy et al., 2017)**. A testicular morphometry evaluation testis length, width and scrotal circumference allow using more objective criteria of gonadal function and the reversibility of the suppression **(Madrigal-Valverde et al., 2025; Al-Shammary S.M 2015**). Histologically, these changes are also evident and constitute modifications at the germinal epithelium level (height of the seminiferous tubules and number of interstitial cells), indicating the extent of hormonal suppression (**Diagone et al., 2012**).

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Thus, pharmacological ablation by the combination melatonin and progesterone appears as a promising and reversible alternative to classic orchiectomy in tomcats. This study was to compare their relative effectiveness in suppression of reproductive behavior, hormonal concentration as well as a safer, less invasive alternative for control over its reproduction in domestic cats.

II. MATERIALS AND METHODS

Ethical Approval

All procedure that done in our study conformist ethical permission was obtained from the local committee of animal care and use at college of veterinary medicine, Baghdad university. (No: P-G/266/, 20/10/2025).

Experimental Animals

The study was conducted with 24 healthy sexually mature male cross-mix domestic cats of 3–4 years old and weighing 3.5–5 kg. All animals were kept under the same environmental and feeding conditions at the Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Baghdad, between mid-January to mid-March 2025; all animals were reported clinically healthy prior to their enrollment.

Experimental Design

Twenty-four tom cats were allocated randomly to receive melatonin (18 mg S.C.), progesterone (megestrol acetate 2.5 mg every 10 days for 2 months) and untreated control groups of eight animals each. Biometric (testicular measurements) and histological (structural morphometric analysis) studies in two tomcats from each group (18 tomcat for the histological examination) were conducted on days 20, 40 and 60 to evaluate the changes in the testis morphology and structure induced by the hormones.

Scrotal assessment

All toms were sedated with intramuscular injection of a cocktail of Ketamine hydrochloride (10%, Alfasan, Holland; 15 mg/mL BW) and Xylazine (2 % Alfasan, Holland; 1.1 mg/kg BW) in combination with Atropine sulfate (Bio-Kana, Hsinchu, Vietnam; 0.05 mg/kg BW) (**Al-Bdeery et al., 2015**). All the animals were restrained in a head-down position and testicular length was measured with digital vernier caliper (**Kumar et al., 2023**).

Histological examination

The two testes remove from each group(18 tomcat for the histological examination) and the sample taken without the epididymis and fixed in 10% neutral formalin for 48 hrs. with the solution changed every 24 hours to make sure of adequate preservation (**Sampedro et al., 2021**). Paraffin embedding was carried out according to the routine histological procedures including fixation, dehydration, clearing, impregnation, embedding, sectioning, staining and mounting. Sections of the tissue were stained by hematoxylin and eosin (**Isaac et al., 2023**).

Statistical analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). Two-way, three-way ANOVA with interaction and least significant differences (LSD) post hoc test was performed to assess significant differences among means. $P < 0.05$ is considered statistically significant.

III.RESULTS & DISCUSSION

Testicular length and width

Significant ($p < 0.05$) bilateral decrease in the testis dimensions – length and right/left width were induced by both implants of melatonin and progesterone compared with controls. In the control testes remained constant in size over the 60-day period of experimentation indicating normal gonadal activity. By contrast, melatonin-treated cats had a symmetrical and progressive decline of right and left testicular sizes there was no significant difference between sides. At day 60 testicular length was reduced to approximately 17 mm, right and left width were respectively mean values of 14.9 mm and 14.3 mm indicating a moderate and fully reversible gonadal regression.

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The bilateral reduction was earlier and more pronounced with progesterone treatment. Great atrophy occurred as early as day 20, and the length and width of testis reduced gradually to approximately 14.5 mm in length and 12~12.5mm in width for both sides on day 60. The symmetrical reduction in both the right and left testes argues against local, and rather for systemic endocrine suppression.

These results indicate that both hormones inhibit the hypothalamic-pituitary-gonadal (HPG) axis, resulting in testicular degeneration based on decreased gonadotropin and testosterone levels. The effects of melatonin were associated with a slow and photoperiod-like inhibition, whereas those of progesterone (acting directly on GnRH production and Leydig steroidogenesis) resulted in the quicker and stronger regression observed above (**Stocco, 2001; Ferré-Dolcet & Romagnoli, 2023**). The bilaterally symmetrical regression corresponds to earlier described cases for felids and other seasonally breeding species, in which hormonal suppression leads to semetrical testicular atrophy with reduced spermatogenic activity (**Amelkina et al. 2022; Naidenko et al., 2022**).

Table (3.1) Effect of progesterone or melatonin implants on the Right length testes of tom cat (Means ± SE).

Groups	20 days	40 days	60 days
Control	A20.30±0.89ab	A21.03±0.80a	A20.78±1.03a
Melatonin (18mg)	A19.02±1.05abc	A19.25±0.99abc	A17.40±0.88bc
Progesterone (2.5mg)	A17.15±0.91cb	A16.47±1.12cb	A14.88±1.19b
LSD		3.04	

Table (3.1) Effect of progesterone or melatonin implants on the left length testes of tom cat (Means ± SE).

Groups	20 days	40 days	60 days
Control	A20.11±0.91a	A20.93±0.87a	A20.63±1.06a
Melatonin (18mg)	A18.71±1.10ab	A19.08±1.08ab	A17.05±0.91bc
Progesterone (2.5mg)	A16.84±1.00bc	A16.22±1.10bc	A14.48±1.11c
LSD	3.04		

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)

Table (3.2). Effect of progesterone or melatonin implants on the right testicular width of tom cat (Means ± SE).

Groups	20 days	40 days	60 days
Control	A16.58 ± 0.65^{a}	A16.72 ± 0.55^{a}	A16.88 ± 0.71^{a}
Melatonin (18 mg)	A14.41 ± 0.38^{bc}	A15.28 ± 0.64^{ab}	A14.93 ± 0.73^{ab}
Progesterone (2.5 mg)	A14.29 ± 0.71^{bc}	A13.93 ± 0.83^{bc}	A12.68 ± 1.11^{c}
LSD	2.06		

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)

Table (3.3). Effect of progesterone or melatonin implants on the left testicular width of tom cat (Means ± SE).

Groups	20 days	40 days	60 days
Control	A16.19 ± 0.68^{ab}	A16.53 ± 0.50^{a}	A16.63 ± 0.71^{a}
Melatonin (18 mg)	A14.21 ± 0.42^{bc}	A15.08 ± 0.64^{abc}	A14.33 ± 0.77^{bc}
Progesterone (2.5 mg)	A14.21 ± 0.71^{bc}	A13.78 ± 0.83^{cb}	A12.25 ± 1.20^{b}
LSD	2.06		

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)

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Scrotal circumference (S.C.)

The data in Table (3.4) reveal a progressive reduction in scrotal circumference (S.C.) following hormonal treatment compared with the control group. In control tom cats, S.C. remained relatively stable throughout the experiment, with mean values of 3.00 ± 0.07 cm at 20 days, 3.33 ± 0.13 cm at 40 days, and 3.25 ± 0.23 cm at 60 days. Melatonin-treated cats showed a gradual decline, recording 2.96 ± 0.14 cm at 20 days, 2.82 ± 0.15 cm at 40 days, and 2.44 ± 0.28 cm at 60 days. The progesterone group exhibited the most significant reduction, with values decreasing from 2.54 ± 0.17 cm to 1.94 ± 0.17 cm and 1.52 ± 0.14 cm across the same intervals. With an LSD value of 0.50, the results indicate that both treatments significantly reduced scrotal circumference, with progesterone exerting the strongest effect.

Table (3.4). Effect of progesterone or melatonin implants on the scrotal circumference (S.C.) of tom cat (Means ± SE).

Groups/SC (cm)	20 days	40 days	60 days
Control	A3.00 ± 0.07^{a}	A3.33 ± 0.13^{a}	A3.25 ± 0.23^{c}
Melatonin (18 mg)	A2.96 ± 0.14^{a}	AB2.82 ± 0.15^{b}	B2.44 ± 0.28^{b}
Progesterone (2.5 mg)	A2.54 ± 0.17^{a}	B1.94 ± 0.17^{c}	B1.52 ± 0.14^{c}
LSD	**0.50**		

These findings are consistent with previous reports showing that exogenous hormones suppress LH/FSH secretion and testicular activity, leading to scrotal contraction (**Goericke-Pesch et al., 2013; Amelkina et al., 2022**). Comparable reductions were noted in seasonal species where melatonin shortens the breeding period and reduces testicular and scrotal size (**Rosa et al., 2012**). Progesterone's effect corresponds to its direct suppression of Leydig-cell function and androgen output (**Stocco, 2001**).

Seminiferous tubule diameter

Table (3.5) demonstrates the effect of progesterone and melatonin implants on seminiferous tubule diameter in tom cats. The seminiferous tubule diameter in the control group showed an almost constant measurement throughout the experimental interval, with readings of 69.90 ± 1.88 µm at 20, 40, and 60 days, showing no significant difference between recording periods. The tubule diameter in melatonin-treated cats was generally larger than other groups at 20 days, recording 99.26 ± 2.51 µm and progressively decreased to 81.51 ± 3.82 µm at 40 days and 48.65 ± 4.18 µm at 60 days. The statistical significance of the difference among these periods was achieved Progesterone-treated cats exhibited the most dilated difference among all days of the study, recording mean values of 66.01 ± 1.29 µm at 20 days, comparing with 48.28 ± 1.59 µm at 40 days and 32.26 ± 3.23 µm at 60 days. the difference among the recording days was statistically significant and also when compared to control and melatonin groups, this reflects the strong anti-gonadal activity of the progesterone hormone. Consequently, both hormonal implants revealed a time-course atrophy of seminiferous tubules, one of which excites an earlier and severe degenerative alteration compared to the other.

Table (3.5). Effect of progesterone and melatonin implants on seminiferous tubule diameter (Mean ± SE) in tom cats at different time intervals.

Groups	20 days	40 days	60 days
Control	A69.90±1.88b	A69.90±1.88ab	A69.90±1.88a
Melatonin	A99.26±2.51a	B81.51±3.82a	C48.65±4.18ab
Progesterone	A66.01±1.29b	AB48.28±1.59b	B32.26±3.23b
LSD	0.24		

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)

The current results demonstrated a significant decrease in seminiferous tubule diameter of the tom cat with the administration of progesterone and melatonin implants where the reduction effect was most pronounced with progesterone treatment. Throughout the experimental period, in control groups, there were no changes in the size of the tubules diameter it was 69.90 µm. No change in diameter in the observation group can be seen as the normal condition of the testicular state without hormonal effects on it. Results of melatonin implant demonstrated an initial high at 20 days where it 99.26 µm, then there was a gradual significant decrease, with a diameter of 81.51 µm after 40 days and of 48.65

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µm due to 20 days which indicated that melatonin has a time-dependent effecting inhibiting in the testes. the most affected groups were combined with progesterone where it dramatically decreased before and with continued time. on 20 days there was 66.01 µm and on the last day of the study, it was 32.26µm evaluated histomorphologically due to the inhibition of the hypothalamic-pituitary-gonadal axis by hormonal implants in terms of seminiferous epithelial degeneration and tubular apathy. the progressive decreases an in-tubule size elicited by melatonin here is consistent with its physiological role in seasonal modulation of reproduction (**Houda et al., 2021**). it is warranted that this effect has been described for other species where long-term melatonin administration decreases the level of gonadotropins, inducing a complete reversible depression of the testes (**Rosa et al., 2012**). The visualized serious effect of progesterone can also be explained by its strong negative feedback effect on the hypothalamic GnRH and the anterior tropic hormones LH and FSH, as well as its direct inhibitory effect on Leydig and Sertoli cells, which leads to the depletion of germ cells, the atrophy of the interstitium, and the apathy of the tubules (**Stocco, 2001**). Similar results induced by progestogens have been reported in dogs, ferrets, and rodents (**Goericke-Pesch et al., 2013**). However, as evidenced by some researchers our observation, the effect of melatonin is species-specific or depends on the dosage and can be reversible in behavior of the physiological conditions (**Ferré-Dolcet & Romagnoli, 2023**). Thus, the study showed a gradual time-dependent decrease in tubules diameter of the tom cat with both progesterone and melatonin treatments, at the same time, directly taking a more pronounced time atrophy.

Number of interstitial cells

The effect of progesterone and melatonin implants on the number of interstitial cells in tom cats is presented in Table (3.7). In control animals, interstitial cell counts remained stable across all time points (4.60 ± 0.40) with no significant changes ($p > 0.05$). Melatonin treatment caused a progressive reduction in interstitial cell numbers compared with controls. Counts declined from 3.20 ± 0.37 at 20 days to 2.20 ± 0.37 at 40 days, reaching the lowest value of 1.40 ± 0.40 at 60 days ($p < 0.05$ vs. control). Progesterone induced a more pronounced suppression, with interstitial cell numbers significantly lower than controls as early as 20 days (1.60 ± 0.50). Values remained depressed at 40 days (1.80 ± 0.37) and decreased further to 1.00 ± 0.31 by day 60.

Table (3.7) Effect of progesterone or melatonin implants on the Numbers of interstitial cells of tom cat (Means ± SE).

Groups	20 days	40 days	60 days
Control	A4.60±0.40a	A4.60±0.40a	A4.60±0.40a
Melatonin	A3.20±0.37b	AB2.20±0.37b	B1.40±0.40b
Progesterone	A1.60±0.50c	A1.80±0.37b	A1.00±0.31b
LSD	**1.14**		

In this study, both progesterone and melatonin implants significantly reduced the number of interstitial cells in tom cats, with progesterone exerting the earliest and most pronounced effect. Control cats maintained stable interstitial cell counts across the experimental period, consistent with normal Leydig cell maintenance under physiological androgenic regulation. Melatonin-treated cats showed a progressive decline, with reductions evident by 20 days and reaching the lowest levels at 60 days, reflecting its delayed but cumulative suppression of gonadotropins and testosterone production. Progesterone-treated cats exhibited a more rapid and sustained suppression, with significant reductions already present at 20 days and further declines over time, confirming its potent inhibitory effect on GnRH, LH, and FSH secretion and its direct impact on Leydig cell survival. These findings are in line with previous studies reporting that hormonal contraceptives, particularly progestogens, cause Leydig cell depletion and interstitial regression in felids and canids (**Andrews, 2023**). Similar decreases in interstitial cell numbers have been described in seasonal breeders during testicular regression induced by melatonin, where Leydig cells undergo apoptosis and shrinkage due to reduced trophic stimulation (**Monageng et al., 2023**). The stronger effect of progesterone observed here is biologically plausible given its dual mechanism of action: suppression of pituitary gonadotropins and direct inhibition of Leydig cell steroidogenesis via downregulation of steroidogenic enzymes (**Oduwole et al., 2021**). Previous studies in rodents and dogs exposed to long-

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term progestogens also demonstrated marked Leydig cell depletion (**Kutzler, 2015**), supporting our findings. Overall, the progressive reduction in interstitial cell numbers highlights the central role of these cells as sensitive markers of endocrine suppression, with progesterone exerting a more immediate and profound effect than melatonin in tom cats.

Histological findings

Histological findings (control group, 20 days)

Figure (3.4) shows a histological section of the testis from a control tom cat at 20 days. The seminiferous tubules appear normal in both shape and size, lined with well-differentiated germinal epithelial cells (g). The arrangement of the spermatogenic layers is intact, and the lumen contains developing germ cells. The interstitial tissue appears normal, with clearly visible interstitial cells (arrows) distributed between the tubules. These findings indicate preserved testicular structure and function in the control group.

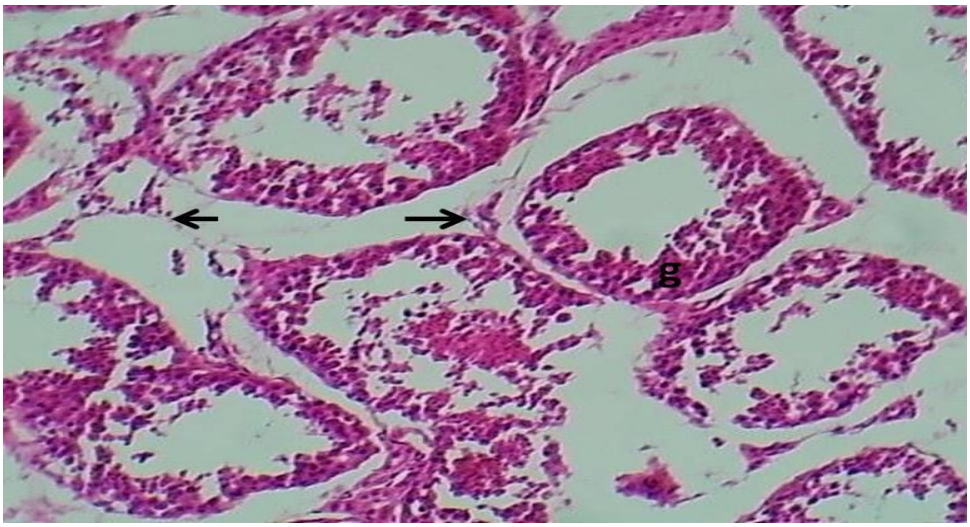

Figure (3.4): section of testis (control group/20 days) shows normal shape and size of seminiferous tubules that comprised of will differentiate germinal cells (g) and normal interstitum (Arrows0 .H&E.100x)

The histological findings of the control group at 20 days revealed normal seminiferous tubules lined with well-differentiated germinal epithelial cells and intact spermatogenic layers, with normal interstitial tissue containing clearly visible Leydig cells, confirming preserved testicular architecture and spermatogenesis. These results are consistent with the observations of (**Johnson, 2022**), who reported preserved testicular structure in untreated tom cats, and with (**Lapuente et al., 2020**), who noted that structurally intact seminiferous tubules in healthy cats support normal spermatogenesis. Similarly, (**Heinrich & DeFalco., 2020**) confirmed that in the absence of hormonal manipulation, the testicular histology remains unchanged, with organized germinal epithelium and active interstitial cells. In contrast, (**Rosa et al., 2012**) suggested that seasonal variations in melatonin secretion could induce minor alterations in spermatogenic activity even in control animals, which differs from our findings of consistent normal morphology; this variation may be attributed to environmental and photoperiodic differences between studies.

Histological findings (melatonin group, 20 days)

Figure (3.5) shows a histological section of the testis from a melatonin-treated tom cat at 20 days. The seminiferous tubules largely maintained their normal shape and size, with preserved germinal epithelium. However, early degenerative changes were evident, particularly in the form of vacuolated alterations within the germinal cells (red arrows). Some germ cells appeared slightly disorganized within the tubules (black arrow), indicating the onset of mild structural disruption compared with controls. Despite these changes, spermatogenic layers were still recognizable at this stage.

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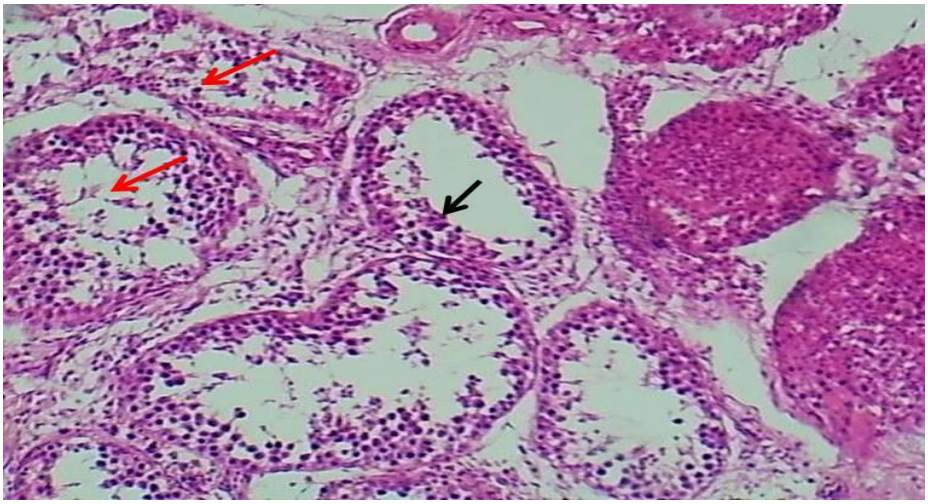

Figure (3.5): Section of testis (Group-Melatonin/20 days) shows normal shape and size of seminiferous tubules that revealed marked little vaculated changes within germinal cells (arrows).H&E.100x

The histological examination of the melatonin-treated group at 20 days demonstrated seminiferous tubules that largely retained their normal shape and size with preserved germinal epithelium; however, early degenerative changes were noted in the form of vacuolated germinal cells and slight disorganization of some spermatogenic layers, suggesting the onset of mild disruption compared with controls. These findings align with (**Heidarizadi et al., 2022**), who highlighted that melatonin can influence testicular activity by altering germ cell organization, and with (**ViviD & Bentley, 2018**), who emphasized the role of melatonin in modulating reproductive seasonality and its potential to affect spermatogenesis. Similarly, (**Ferré-Dolcet & Romagnoli, 2023**) reported that hormonal interventions in tom cats may lead to early histological changes before significant spermatogenic impairment occurs. Conversely, (**Essawy et al., 2023**) indicated that melatonin treatment alone, especially at early stages, may not always produce evident degenerative alterations, suggesting that the mild vacuolation observed in this study could be dose- or duration-dependent (**Isaac et al., 2023**).

3.6.3. Histological findings (progesterone group, 20 days)

Figure (3.6) shows a histological section of the testis from a progesterone-treated tom cat at 20 days. The seminiferous tubules are reduced in size and display irregular shapes compared with the control group. Severe degenerative changes are evident within the germinal epithelium, including the presence of large vacuoles (macro-vacuolation) within germinal cells (black arrows). The number of spermatogonia is markedly decreased (red arrows), indicating impaired spermatogenic activity. In addition, interstitial edema is observed, accompanied by thinning of the testicular interstitium (blue arrows). These findings reflect the early onset of structural damage in the testis following progesterone treatment.

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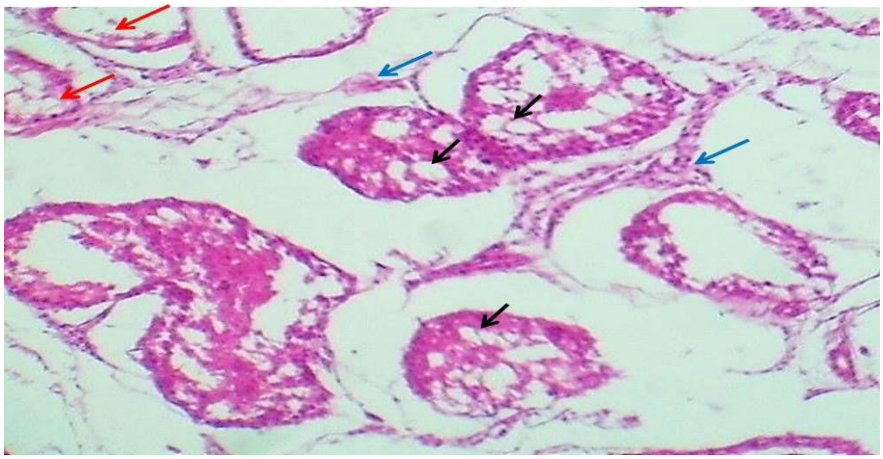

Figure (3.6): Section of testis (Group-progesterone/20 days) shows reduce numbers and sized with marked irregular shape of seminiferous tubules that revealed marked severe vacular changes (Macrovacules) within germinal cells (Black arrow), marked little spermatogonium cells (Red arrows), interstitial edema with thinning of testicular interstitum (Blue arrows).H&E.100x

The histological findings of the progesterone-treated group at 20 days revealed pronounced testicular alterations, including reduced size and irregular shape of seminiferous tubules, severe vacuolation within germinal epithelial cells (macro-vacuolation), a marked reduction in spermatogonia, and interstitial edema with thinning of the testicular interstitium, all of which indicate early impairment of spermatogenic function. These results are in agreement with (**Ijaz et al. 2019**), who reported that chemical and hormonal fertility control in male cats leads to degenerative histological changes, including reduced spermatogonia and vacuolated germ cells. Similarly, (**Goericke-Pesch et al., 2013**) emphasized that exogenous hormonal manipulation can disrupt the organization of spermatogenic layers and compromise seminiferous tubule integrity. (**Sikka & Wang, 2008**) also noted that endocrine interference significantly alters reproductive physiology, often leading to disrupted germ cell development. In contrast, (**Webster et al., 2015**) indicated that the severity of histological damage following progesterone treatment may vary with dose and exposure duration, and in some cases, early administration did not completely abolish spermatogenesis. These differences suggest that while our findings clearly demonstrate early and severe testicular degeneration, variations across studies may be explained by differences in treatment protocols, hormone dosage, and animal sensitivity.

Histological findings (melatonin group, 40 days)

Figure (3.7) shows a histological section of the testis from a melatonin-treated tom cat at 40 days. The seminiferous tubules appear slightly reduced in size compared with the control group, with noticeable degenerative changes. Moderate vacuolated alterations are present within the germinal epithelium (black arrows), while desquamated germinal cells are seen within the lumen (n), indicating disruption of normal spermatogenesis. The interstitial tissue shows reduced numbers of interstitial cells (blue arrow), suggesting early impairment of Leydig cell function.

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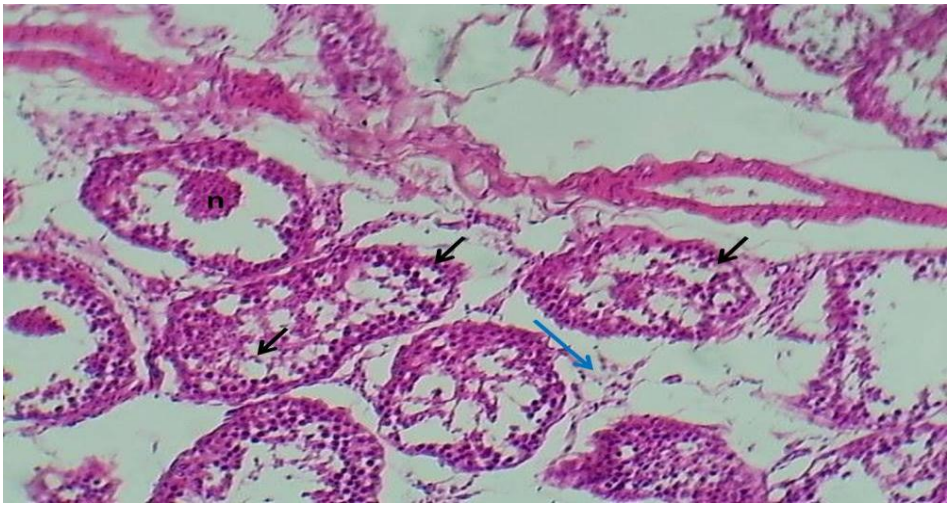

Figure (3.7): Section of testis (Group-Melatonin/40 days) shows slightly normal size of seminiferous tubules that revealed moderate vaculated changes within germinal cells (Black arrows), desquamated cells (n) & reduce numbers of interstitial cells (Blue arrows) .H&E.100x

The histological findings of the melatonin-treated group at 40 days demonstrated a slight reduction in seminiferous tubule size compared with the control group, with moderate vacuolated changes in the germinal epithelium, the presence of desquamated germinal cells in the lumen, and reduced numbers of interstitial cells, indicating progressive disruption of spermatogenesis and early impairment of Leydig cell activity. These results are consistent with (**Soleimani Mehranjani et al., 2022**), who noted that prolonged melatonin exposure can induce degenerative testicular changes, including disorganization of spermatogenic layers and reduction of interstitial cell populations. Similarly, (**Prochowska & Niżański., 2022**) reported that extended hormonal intervention in tom cats results in vacuolation and desquamation within seminiferous tubules, confirming our observations. (**Sikka & Wang, 2008**) also highlighted that melatonin has strong regulatory effects on reproductive seasonality, which may accelerate degenerative changes under experimental conditions. In contrast, (**Kutzler, 2015**) indicated that melatonin administration alone does not universally lead to significant histological damage and that observed effects may depend on treatment duration, dosage, and environmental conditions. These variations suggest that while our study supports melatonin's degenerative impact on testicular histology over time, discrepancies across studies could be attributed to differences in experimental design and biological responsiveness.

Histological findings (progesterone group, 40 days)

Figure (3.8) shows a histological section of the testis from a progesterone-treated tom cat at 40 days. The seminiferous tubules exhibit marked structural damage, with evident severe atrophy and thinning of the germinal epithelium. Spermatogonia are markedly deteriorated and reduced in number (black arrows), indicating profound suppression of spermatogenesis. The interstitial tissue contains only a few interstitial cells (red arrow), while mild fibroplastic changes are observed in the surrounding stroma (blue arrow). These findings highlight the progressive degenerative impact of progesterone treatment on testicular tissue by 40 days.

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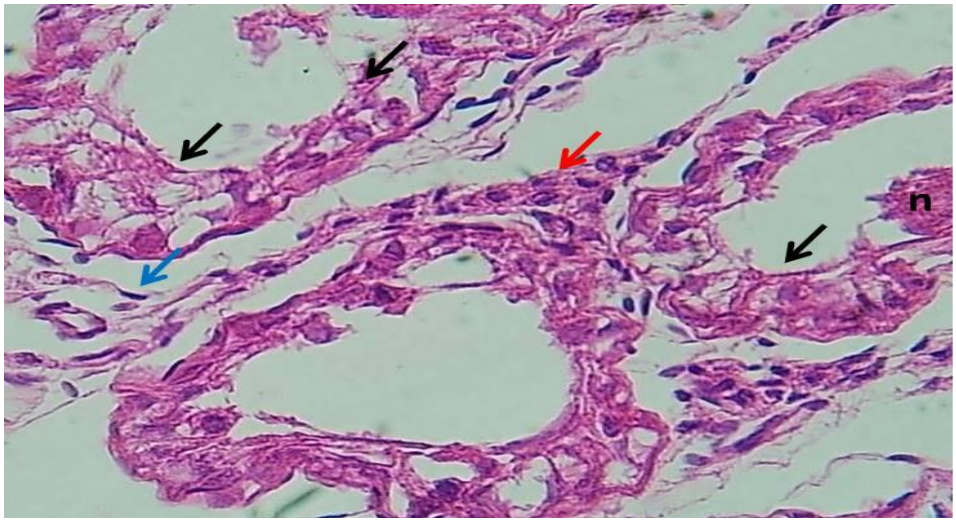

Figure (3.8): section of testis (group-progesterone/ 40 days) shows seminiferous tubules revealed severe atrophy of seminiferous tubules associated with deterioration of spermatogium cells (Black arrows), few numbers of interstitial cells (Red arrows), & mild fibroplasia (Blue arrows) .H&E.100x

The histological findings of the progesterone-treated group at 40 days showed pronounced testicular degeneration, characterized by severe atrophy and thinning of the germinal epithelium, marked deterioration and reduction of spermatogonia, a sparse population of interstitial cells, and mild fibroplastic changes within the stroma, indicating progressive suppression of spermatogenesis and compromised testicular function. These results are in agreement with (**Fagundes et al., 2014**), who reported that chemical and hormonal fertility control induces advanced degenerative changes in male cat testes, including loss of spermatogenic cells and stromal alterations. (**Li et al., 2024**) also demonstrated that prolonged hormonal manipulation leads to severe testicular atrophy and disruption of the seminiferous epithelium. Likewise, (**Sikka & Wang, 2008**) highlighted that endocrine interference can significantly impair germ cell development through disrupted hormonal regulation. In contrast, (**Sharma & Agarwal, 2018**) suggested that the degree of degeneration varies depending on dosage and treatment duration, with some cases showing only partial suppression of spermatogenesis rather than the extensive damage observed here. These discrepancies emphasize that while our findings strongly support progesterone's detrimental effects on testicular histology, differences in treatment protocols and animal variability may influence the severity of changes reported across studies.

Histological findings (melatonin group, 60 days)

Figure (3.9) shows a histological section of the testis from a melatonin-treated tom cat at 60 days. The seminiferous tubules appear reduced in size with a narrowed lumen. Marked degenerative changes are observed, characterized by the presence of numerous desquamated germinal cells sloughed into the lumen (arrows). The germinal epithelium is markedly disrupted, reflecting advanced impairment of spermatogenesis compared with earlier stages.

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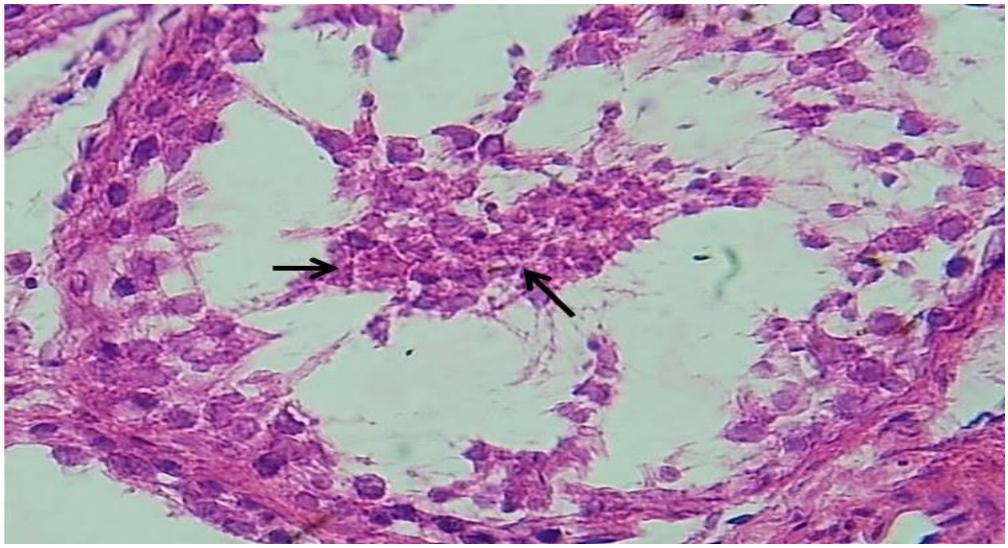

Figure (3.9): Section of testis (Group-Melatonin/60 days) small size seminiferous tubules marked luminal desquamated cells (Arrows) .H&E.400x

The histological findings of the melatonin-treated group at 60 days revealed advanced testicular degeneration, with reduced seminiferous tubule size, narrowed lumina, and a high number of desquamated germinal cells sloughed into the lumen, accompanied by a markedly disrupted germinal epithelium, indicating severe impairment of spermatogenesis compared with earlier stages. These results agree with (**Dehdari Ebrahimi et al., 2021**), who reported that prolonged melatonin exposure leads to progressive testicular degeneration, including germinal epithelium disruption and luminal desquamation. Similarly, (**Warren, 2014; Diagone et al., 2012**) confirmed that sustained hormonal intervention in tom cats causes advanced degenerative changes, often culminating in near-complete spermatogenic arrest. (**Yu, 2018**) also noted that long-term alterations in melatonin signaling can strongly impair reproductive function by disturbing germ cell development and hormonal balance. On the other hand, (**Li et al., 2024**) suggested that melatonin's effects can sometimes be reversible and not always result in extensive permanent histological damage, which contrasts with the pronounced degeneration observed in this study. These differences indicate that while our findings confirm melatonin's strong inhibitory effects on testicular histology with longer exposure, variability in dosage, treatment duration, and animal sensitivity may explain the discrepancies reported across studies.

3.4.8. Histological findings (progesterone group, 60 days)

Figure (3.10) shows a histological section of the testis from a progesterone-treated tom cat at 60 days. The seminiferous tubules display severe atrophy with marked reduction in spermatogonia. Necrotic changes within spermatogonia are evident (black arrows), indicating extensive cellular damage. The interstitial tissue is markedly expanded (asterisk), suggesting fibrosis or edema in response to tubular degeneration. These findings demonstrate advanced degenerative changes and near-complete suppression of spermatogenesis following prolonged progesterone treatment.

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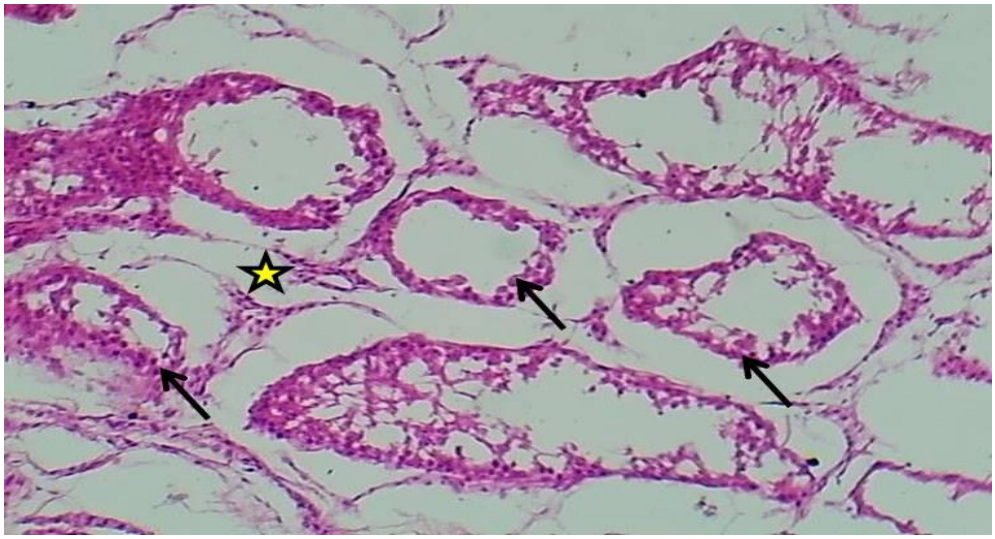

Figure (3.10): section of testis (group-progesterone/ 60 days) shows seminiferous tubules revealed severe atrophy with marked little of spermatogonium cells associated that associated with necrosis of spermatogium cells (Arrows) increased area of interstitial tissue (asterisk),.H&E.100x

The histological findings of the progesterone-treated group at 60 days revealed severe testicular degeneration, with pronounced atrophy of seminiferous tubules, a marked reduction and necrosis of spermatogonia, and extensive expansion of the interstitial tissue, suggestive of fibrosis or edema. These advanced changes reflect near-complete suppression of spermatogenesis and confirm the cumulative detrimental impact of prolonged progesterone exposure. Our findings are in agreement with (**Hasan et al., 2022**), who reported that chronic hormonal fertility control induces extensive testicular degeneration, including necrosis of spermatogenic cells and stromal fibrosis. (**Amr et al., 2024**) similarly observed that prolonged hormonal manipulation in tom cats leads to severe testicular atrophy and near-complete disruption of the seminiferous epithelium. (**Cosso et al., 2021**) also emphasized that endocrine alterations can cause profound impairment of reproductive function, particularly under long-term treatment conditions. In contrast, (**Lue et al., 2013**) noted that while progesterone has strong suppressive effects on spermatogenesis, the extent of degeneration may vary, with some studies reporting partial rather than complete spermatogenic arrest, likely depending on treatment protocol, dose, and duration. These discrepancies suggest that while the present study demonstrates profound and largely irreversible degenerative changes, differences in hormonal regimens and animal variability may explain milder outcomes in other reports.

IV. CONCLUSIONS

Progesterone and melatonin implants caused clear testicular regression in tomcats. While testicular size remained stable in controls, both treated groups showed significant reductions—appearing later with melatonin and earlier, more markedly with progesterone. Histology confirmed smaller seminiferous tubules, thinner germinal epithelium, and fewer Leydig cells, most severe in the progesterone group. Thus, both hormones suppress gonadal structure and function, with progesterone acting faster and stronger, and melatonin providing a milder, reversible effect.

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The authors of this article worked on it and declared that they had no conflicts of interest.

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