# The Role of Different Photoperiods in the Activation of the Thyroid Gland and Ovaries of Adult and Aged Rats ( *Rattus norvegicus* )

**Gabry M.S., El-Hennamy R.E., Ibrahim M.A., and Fathalla A.S.**
Zoology and Entomology Department, Faculty of Science, Helwan University, Egypt.

## Abstract

**Back ground**: Many animals use the length of the day (photoperiod) to predict and adjust to seasonal changes in the environment through predictive changes in physiology and behaviour. The present work is designed to study the effect of different photoperiod regimes, i.e., short photoperiod 10L:14D, long photoperiod 14L:10D and 12L:12D light-dark cycle (LD) on the structure and hormonal secretion of the thyroid gland and ovaries in adult and aged female rats.

**Material and methods**: The rats were divided into 6 groups, each group contained 6 adults (6 months old) or aged rats (18 months old) and maintained for one month under different photoperiod regimes.

**Results:** Short and long photoperiods increased the activity of the thyroid gland of the adult rats. This gland was full of small follicles lined with simple columnar epithelium. Serum thyroxin levels were extremely higher in long photoperiod group than that observed in 12:12LD and short photoperiod groups. In contrast short photoperiod induced an increase in serum thyroxin level in the aged rats. Short photoperiod and 12:12 LD cycle enhanced gonadal activity of the adult rats. The ovary contained clusters of primordial follicles, different stages of ovarian follicles, and, few numbers of atretic follicles. In the groups of the aged rats all the stages of the ovarian follicles exhibited signs of atresia in such groups. LH, FSH, progesterone and estrogen were increased mainly in the control group in adult and aged rats compared to the photoperiod groups. From the present result we could conclude that: 12:12 light dark cycle enhanced the structural and functional activities of ovaries in adult rats, while aging decreased their activities. In contrast, long photoperiod induced thyroid gland activities in adult rats while short photoperiods activated the gland of aged rats.

**Key words: Photoperiod, Thyroid gland, Thyroxin, Ovary, Female hormones, Rat.**

## Introduction

Photoperiodism is the ability of plants and animals to measure environmental day length to a certain time of year. In mammals, melatonin provides the hormonal signal transducing day length. Duration of pineal melatonin is inversely related to day length and its secretion drives enduring changes in many physiological systems, including the HPA, HPG, brain-gut axes, the autonomic nervous system and the immune system [1].

Many animals use day length (photoperiod) to predict and adjust seasonal changes in the environment through predictive changes in physiology and behavior [2]. Most the physiological processes in mammals exhibit daily rhythms generated by a system of cell autonomous circadian oscillators located in the brain and in peripheral organs and tissues. A master clock in the hypothalamic suprachiasmatic nucleus provides circadian output signals that are essential for maintaining synchrony of oscillators within organs and between organ systems and for coupling circadian physiology to environmental light-dark cycles [3].

The gonadotrophs (LH and FSH-secreting cells) are an important link in the pituitary-gonadal axis and thus in the regulation of reproduction. However, these cells do not work alone. The pituitary homeostasis and adaptation to reproductive needs requires a coordinated action among cells that are part of different hypothalamic-pituitary-target organs axes [4].

Photoperiod affects reproduction in a number of rodent species. The effects of photoperiod in adults are manifested by maintenance of reproductive function on long photoperiods and regression of reproductive structures on short photoperiods. The pineal gland and its hormone melatonin have been implicated in the photoperiodic regulation of reproduction in all mammals studied to date [5].

Studies of thyroid physiology in rats support the view that the pineal gland has an anti-thyrotropic action. An inhibitory action of the pineal gland on hypothalamic -pituitary-thyroid axis response to TSH in the presence of elevated



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melatonin levels has been evidenced in hamsters and rats and *in vitro* [6].

Aging may affect thyroid function and regulation differently depending on gender and thus be modulated by gonadal hormones [7]. So the present work is aimed to study the effect of different photoperiods on the structure and function of thyroid gland and ovaries in adult and aged female rats.

## Material and methods:

### Experimental Animals:

Adult ( 6 months) and aged (18 months) female Wistar rats "*Rattus norvegicus*" were used in this study. Rats were obtained from the Animal House of the Holding Company for Biological Products and Vaccines, VACSERA, Cairo-Egypt. The rats were maintained under normal environmental conditions of temperature and humidity and were supplied with food and water *ad libitum*. Before the beginning of the experiments the rats were left to acclimatize for 1 week under 12 hours of light and 12 hours of darkness.

### Experimental Design:

The rats were divided into 6 groups, each contained 6 female rats, and maintained for one month under different experimental conditions as follows:

1- The first group (control group): Adult female rats housed under 12:12 light-dark cycle (12L:12D).

2- The second group: Adult female rats housed under short photoperiod (l0L: 14D).

3- The third group contained adult female rats housed under long photoperiod (l4L:10D).

4- The fourth group (control group): Aged female rats housed under (12L: 12D).

5- The fifth group: Aged female rats housed under short photoperiod (l0L: 14D).

6- The sixth group: Aged female rats housed under long photoperiod (14L: 10D).

At the end of the experiments, the rats were sacrificed and dissected; the thyroid glands and ovaries were removed from the rats of all the groups for histological examinations. Blood samples were collected, allowed to clot, and then centrifuged at 3000 rpm for 15 minutes to obtain clear sera which were stored at –20 ºC for hormonal analyses.

### Light microscopic preparations:

The thyroid glands and ovaries were fixed in Bouin's fluid for 24 hours, dehydrated in ascending series of alcohol, cleared in xylene and embedded in paraffin wax. Sections were cut at the thickness of 5 µm and were stained with Haematoxylin & Eosin, mounted in DPX [8], examined and photographed by using light microscopy (Zeiss), fitted with 10x - 100x objective lenses. Images were captured using digital camera (Cannon).

### Hormonal analyses:

Hormonal analyses of $T_4$, FSH, LH, estrogen and progesterone are done by using VIDAS device. The test used is an automated quantitative test that measured any hormone in serum or plasma, using the Enzyme-Linked Fluorescent Assay (ELFA) [9-11].

Hormonal analyses are done during diestrus as indicated by histological observations.

### Statistical analyses:

The statistical analyses were carried out between the means of the control and the other groups by one way analysis of variance (ANOVA) according to **Duncan's test** [12] using the statistical package for the social science (SPSS) version 8.

## Results

### Histological observations of the thyroid gland of adult female rats:

The thyroid gland of animals of the control group is formed of many spheres called thyroid follicles. In the microscopical sections, the follicles have an extremely variable diameter and are distended with colloid. The gland is covered with loose connective tissue capsule that sends septa into the parenchyma. As these septa become thinner they reach all the follicles separating them from one another by fine, irregular connective tissue. The follicular epithelium ranges from simple squamous to low cuboidal (Fig. 1a).

On the other hand, the thyroid gland of rats subjected to short photoperiod embodied small follicles that occupied the central region and contained little or no colloid. The follicular epithelium ranged from simple cuboidal to simple columnar. The interfollicular spaces were very narrow with very few connective tissue (Fig. 1b).

Large number of variable-sized follicles with colloid were observed in the thyroid gland of the long photoperiod group. The majority of follicles were small in size. The follicles were lined with cuboidal epithelium or low columnar cells. The interfollicular spaces were very narrow (Fig. 1c).

**Histological observations of the thyroid gland of aged female rats:**

In aged rats there was a greater incidence of inactive large follicles when compared with the adult rats. Almost all the follicles embodied colloid and were lined with simple squamous to low cuboidal epithelium. Generally, the interfollicular stroma increased. The connective tissue between the follicles contained dilated blood vessels. A number of parafollicular cells - which associated with follicles - was observed (Fig. 2a).

Unlike the control aged rats, the thyroid gland of rats exposed to short photoperiod contained large number of active follicles with few colloids. The follicles were lined with cuboidal or columnar epithelium. Parafollicular cells were observed between the follicles (Fig. 2b).

The thyroid gland of the aged rats subjected to long photoperiod was composed of follicles of variable sizes. The connective tissue between the thyroid follicles increased if compared to the control group. In aged rats there were greater incidences of inactive large follicles compared to the thyroid of the control adult rats. Almost all the follicles have colloid and were lined with simple squamous to low cuboidal epithelium (Fig. 2c).

**Histological observations of the ovary of the adult rats:**

The ovary of the rats of the control group is lobulated, covered with coelomic epithelium followed by germinal epithelium (Fig. 3b) and divided into an outer cortex and inner medulla (Fig. 3a). The cortical region contains a number of follicles exhibiting a wide range of sizes. Primordial follicles, tertiary follicle (Fig. 3b), and a Graafian follicle (Fig. 3c) were also observed. The ovary also contains degenerated follicles (Fig. 3b) and corpora lutea (Fig. 3a).

Rats subjected to short photoperiod had more lobulated ovary than those of the rats of the control group. The cortex appeared as cords of cuboidal interstitial cells and contained different stages of ovarian follicles and numerous corpora lutea (Fig. 5a). Clusters of primordial follicles, secondary follicle (Fig. 5b), and Graafian follicle (Fig. 5a) were observed. Few numbers of atretic follicles appeared in the cortex (Fig. 5c).

The ovary of long photoperiod group, contained few primordial follicles (Fig. 7a, 7b), large number of atretic follicles (Fig. 7a, 7b) and a Graafian follicle (Fig. 7a).The early and late stages of atretic follicles were found (Fig. 7b).The number of corpora lutea was fewer than that observed in the ovary of short photoperiod group (Fig. 7a) Corpora albicanses with some blood vessels in between were observed in the medulla region (Fig. 7c).

**Histological observations of the ovary of the aged rats:**

The ovary of aged female rat subjected to 12/12 light-dark cycle, contains different stages of ovarian follicles exhibiting signs of atresia (Fig. 4a). Atretic preantral follicle (Fig. 4b) and atretic Graafian follicles (Fig. 4c) are observed. The theca layer around the late atretic follicle is hypertrophied (Fig. 4b). No primordial follicles were found in the ovary. The medulla contained numerous congested blood vessels (Fig. 4a).

All the stages of the ovarian follicles of the aged rats subjected to short photoperiod exhibited signs of atresia (Fig. 6a). Atretic follicles contained degenerated oocytes and granulosa layer (Fig. 6b). The atretic Graafian follicles contained degenerated granulosa cells with pyknotic nuclei (Fig. 6c). Corpus luteum was observed (Fig. 6a).The medulla contained a number of blood vessels (Fig. 6a).

The ovary of the aged female rats subjected to long photoperiod, contained a lot of atretic follicles (Fig. 8a, 8b, 8c), corpora lutea and corpora albicans (Fig. 8c). Atretic follicles in late stage of atresia with degenerated granulosa cells and degenerated oocytes were found in the cortical region (Fig. 8b).The medulla contained a lot of blood vessels (Fig. 8a).

**The effect of different photoperiods on hormonal concentrations in adult and aged female rats.**

**-Thyroxin analysis:**

In the adult rats, thyroxin levels increased significantly in long photoperiod group ($p<0.05$) compared to the short photoperiod and control groups (Fig. 9). In contrast, the aged rats maintained under short photoperiod showed the highest significant value of thyroxin ($p<0.05$) compared to the long photoperiod and the control groups.

Thyroxin level in the aged rats maintained under long photoperiod decreased significantly, compared with that found in adult ones ($p<0.05$). In contrast, its levels were significantly higher in aged rats subjected to short photoperiod than those of adults.

**-FSH analysis:**

FSH concentration in adult and aged rats significantly decreased ($p<0.05$) in short and

long photoperiod groups compared with the levels observed in the control group (Fig. 10).

**-LH analysis:**

There was a significant decrease in LH level in short and long photoperiod groups compared with the control group of adult rats. LH levels in adult rats subjected to long photoperiod were significantly higher ($p<0.05$) than those of the short photoperiod group (Fig. 11).

In aged rats, the highest value of LH levels was recorded in the control group followed by the value observed in the short photoperiod group (Fig.11).

**-Estrogen analysis:**

The adult female rats subjected to short or long photoperiods showed a significant decrease ($p<0.05$) in estrogen concentrations compared with that found in rats maintained under 12:12 light-dark cycle. On the other hand, estrogen concentration was significantly higher ($p<0.05$) in the long photoperiod group than in the short photoperiod group (Fig. 12).

Estrogen concentration in aged rats kept under short and long photoperiods decreased significantly ($p<0.05$) compared with that observed in the control group.

**-Progesterone analysis:**

A significant decrease in progesterone level ($p<0.05$) was found in adult rats subjected to short and long photoperiods compared with the level observed in the control group. Progesterone concentration showed significant increase in long photoperiod group in comparison with the level observed in the short photoperiod group (Fig. 13).

Progesterone levels decreased significantly ($p<0.05$) in aged rats maintained under long photoperiod compared with that found in the control group.

**Discussion**

Thyroid gland of adult rats subjected to short and long photoperiods in this study showed signs of activity. Their follicles are lined with epithelium that ranges from simple cuboidal to simple columnar. In contrast, rats subjected to 12:12 LD had inactive large follicles lined with simple squamous or low cuboidal epithelium. **Rao-Rupanagudi et al.** [13] studied the structure of the thyroid gland of rats during different ages. They found that by 17 weeks of age, there was a greater incidence of inactive large follicles that present in the periphery and deeper in the gland. Some of the follicles had columnar epithelium, but the majority had cuboidal epithelium. Almost all follicles contained colloid.

Serum thyroxin levels were higher in adult rats kept under long photoperiod than that observed in rats maintained under 12:12 LD and short photoperiod. Similar to our finding, previous studies indicated that peak plasma thyroid hormones concentrations have been recorded during periods of increasing day length in spring, and minimal levels during decreasing photoperiod in late summer-early autumn, in rams [14]. These changes coincide with the end and the onset of the sexual season, respectively. Results of artificial photoperiod stimulation indicate the dependence of the thyroid gland activity and thyroid hormone metabolism on day length. Photoperiod affects thyroxin ($T_4$) and triiodothyronin ($T_3$) plasma concentration in male goats, with different lighting regimes resulting in different profiles of both thyroid hormones. Bearing in mind the pivotal role that thyroid hormones play in stimulating the metabolic activity of the whole body; it is possible that light-induced increase in circulating hormones could sustain and possibly improve animal production. The suitability and viability of supplementary light treatments needs to be considered [15].

Hypothalamic secretion of the thyrotropin (TSH) may be influenced by melatonin [16]. **Zieba et al.** [17] reported that melatonin is able to modulate the function of the hypothalamic-pituitary-thyroid axis. Melatonin plays either a stimulatory or an inhibitory role in the regulation of thyroid gland activity, depending on the season. It has been reported that injection of melatonin inhibits the secretion of thyrotropin releasing hormone "TRH" from the hypothalamus, TSH from the anterior pituitary gland and thyroid hormone secretions [18]. Structural and physiological findings in this study clarified that the thyroid gland of adult rat is activated mainly by long photoperiod.

In the present study, the thyroid gland of aged rats maintained under long photoperiod and 12:12 LD were inactive. Almost all the follicles have colloid and were lined with simple squamous to low cuboidal epithelium. An increase in the connective tissue between follicles was observed. These observations agree with **Rao-Rupanagudi et al.** [13] who found marked differences in the morphologic appearance of rat thyroid gland at 108 weeks. The gland is characterized by a further decrease in epithelial height, an increase in interstitial

fibrous connective tissue, and increase in the numbers and diameters of the follicles. Unlike the long photoperiod and control groups, the thyroid gland of aged rats exposed to short photoperiod contained large number of active follicles. The follicles were lined with cuboidal or columnar epithelium. Scanty interstitial connective tissue was observed in this group.

In contrast to the result obtained in adult thyroid of the present study, high level of serum thyroxin was observed in aged rats maintained under short photoperiod followed by that observed in long photoperiod group. This result agree in part with **Seidel *et al.*** [19] who found that the circulating levels of $T_4$ and free thyroxin did show an annual cycle in both adult and senescent Djungerian hamsters. During spring, higher concentrations were found as compared to fall and winter. The pattern of the $T_4$-cycle was similar in both groups of hamsters; however, the cycle found in senescent hamsters seemed to be shifted about two months as compared to the adult groups. The patterns of free thyroxin-concentrations were different in both groups. In senescent hamsters levels of $T_4$ were increased during winter, whereas in adult hamsters the pattern closely resembled the annual cycle of of $T_4$. Moreover, during winter the plasma titers were higher in senescent hamsters as compared to adult animals. These findings suggest that aging does modify the phase of the seasonal cycle rather than the amplitude or the hormone level.

Parafollicular cells were seen clearly in all groups of aged rats in the present study. **Rao-Rupanagudi *et al.*** [13] found that male and female rats showed discrete area(s) of C-cell hyperplasia in aged rats. These areas contained increased numbers of C-cells in clusters, or scattered in the interfollicular spaces with no apparent compression of the surrounding tissue. This explains the finding of **Kurosawa *et al.*** [20] who demonstrated that both the concentration of immunoreactive calcitonin (iCT) in the systemic blood circulation and the secretion of iCT from the thyroid gland increase with age in anesthetized male Wistar rats. A possible reason for the increased level of iCT is that the sensitivity of the parafollicular cells in the thyroid gland for secreting CT in response to the circulating calcium concentration is increased with age.

The thyroid gland structure and thyroxin secretion in aged rats of the present study are activated mainly by the short photoperiod regime.

It seems that 12:12 light-dark cycle and short photoperiod regimes in this study induced reproductive function in adult rats, which indicated by the presence of clusters of primordial follicles, different stages of ovarian follicles, well-developed Graafian follicle, few numbers of atretic follicles and a number of corpora lutea. In contrast, the ovary of adult rats maintained under long photoperiod contained few numbers of primordial follicles, and corpora lutea and large number of atretic follicles.

In contrast to our findings, the long-day (LD) seasonal breeders, such as Siberian hamsters (Phodopus sungorus), exposure to long photoperiods induced and maintaind reproductive function, whereas exposure to short photoperiods results in the cessation of reproductive function [21]. Ovaries from Siberian hamsters maintained under LD were typical of normal folliculogenesis and were characterized by the presence of follicles in all stages of development, atretic follicles and corpora lutea. Six weeks of SD exposure continued to promote the formation of advanced atretic follicles. By 9 weeks of SD exposure, advanced atretic follicles appeared to be in transition to terminal-stage atretic follicles. By week 12, SD ovaries were highly regressed, contained few follicles and no corpora lutea, and consisted mainly of terminal-stage atretic follicles [22]. They found that apoptosis is an initial process in SD-induced ovarian regression.

In the Arctic charr, *Salvelinus alpinus*, as in other salmonids that spawn during fall or early in the winter, exposure to short days from the middle of summer accelerated complete ovary development and facilitated ovulation [23]. Conversely, exposure of Arctic charr to a long day (LD) photoperiod regime in fall and winter did not completely inhibit ovulation, but markedly delayed it and prolonged the ensuing ovulation period [24].

The effect of environmental light on the retina is the predominant external cue for the endogenous timing system that regulates the rhythmicity of seasonal and daily reproductive mechanisms [25]. The light information is processed by the suprachiasmatic nucleus which then signals the pineal gland to begin nocturnal production of melatonin [26]. Melatonin has been shown to transduce the effects of photoperiod on reproduction in seasonally breeding mammals [27]. In many rodents, exposure to short

photoperiods augmented melatonin secretion and suppressed gonadal growth and function [27]. However **Soares *et al.*** [28] detected an increase in the number of ovarian interstitial and stromal cells as well as a reduction in the number of corpora lutea in pineal-ectomized female rats. Moreover, **Steinman *et al.*** [29] showed that pineal-ectomized animals suffered a reduction in fertility with a decrease in the number of oocytes during ovulation, had upsets during the gestation period, and had diminished serum melatonin. Sheep and goats are short-day breeders; their reproductive activity takes place during fall and winter (i.e., as length of day light decreases) and therefore, in these species, melatonin can be considered as "pro-gonadotrophic" stimulus. In fact, melatonin stimulates GnRH and LH secretion during anestrus in ewes, by reducing tyrosine hydroxylase activity and, therefore, the secretion of dopamine in the median eminence [30]**;** as the dopaminergic system is clearly involved in the suppression of LH secretion by estradiol during seasonal anestrus [31], a low dopamine secretion during anestrus is associated with an improvement of the reproductive activity. Oocyte quality, evaluated by *in vitro* developmental kinetics and blastocyst output, has also been found to be increased after melatonin administration in ewes and goats [32,33]. In long-day breeders, the increase in the photoperiod during spring stimulates the secretion of the gonadotropin-releasing hormone (GnRH) from the hypothalamus and the subsequent release of gonadotropins, leutinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland. By contrast, the decrease in the photoperiod during autumn stimulates the secretion of these hormones in short-day breeders [16]. GnRH is normally secreted in episodic bursts, and these bursts produce the circhoral peaks of LH secretion. Fluctuations in the frequency and amplitude of the GnRH bursts are important in generating the other hormonal changes. Frequency is increased by estrogens and decreased by progesterone. The frequency increases late in the follicular phase of the cycle, culminating in the LH surge. During the secretory phase, the frequency decreases as a result of the action of progesterone; but when estrogen and progesterone secretion decrease at the end of the cycle, the frequency once again increases [34].

In the present study, the highest values of FSH, LH, estrogen and progesterone were found in the control group in adult female rats. However long photoperiod induced a little increase in the levels of LH, estrogen and progesterone when compared to that observed in the short photperiod group. The slight differences in hormonal levels between long and short photoperiods may result from a change in the phase of the hormones circadian rhythm rather than a change in their concentration.

California mice (*Peromyscus californicus*) are not photoperiodic breeders; yet they appear to have annual rhythms in breeding activity. Field observations show that although most pups are born in the winter [35], breeding can occur throughout the year [35]. Photoperiod had no effect on estradiol levels in female California mice [36]. The effects of photoperiod on the female reproductive axis are limited under ad libitum conditions [29]**.** In contrast the LD photoperiod, in fall and winter, reduced LH plasma levels in females Arctic charr while they were ovulating, but did not modify the responsiveness of the pituitary to GnRH stimulation compared to a short photoperiod group [24]**.** Photoperiod influences the transcription of estrogen-responsive genes and estrogen receptors in zebrafish. Gene transcription levels were higher as temperature and photoperiod length increased [37]**.**

The presence of melatonin receptors in the ovarian follicular cells [28] and the presence of high concentrations of melatonin in follicular fluid [38] suggest a direct role for melatonin on folliculogenesis. Furthermore, the *in vitro* culture of isolated mice secondary follicles in follicle-stimulating hormone (FSH)-based medium supplemented with melatonin increases the secretion of androstenedione and progesterone, eliciting an effect of melatonin on steroidogenesis [39]**.** Treatment with melatonin and FSH increased significantly the follicular and oocyte diameters [40]. So, melatonin exerts a role on the maintenance of a proper follicular function, and is thus important for ovulation and progesterone production [41]**.** The modulation of growth factor expression by melatonin might also support follicular development. For example, melatonin stimulates insulin-like growth factor-I (IGF-I) production in cultured human granulosa cells [42]**.** IGF-I is a mitogenic peptide that stimulates DNA synthesis and estradiol and progesterone secretion by human granulosa cells [43].

Injected melatonin has effects on the gonads, but at least in some species these effects are sometimes stimulating and sometimes inhibitory, depending on the time of day the hormone is injected. This observation led to the hypothesis that the diurnal change in melatonin secretion functions as a timing signal that coordinates endocrine and other internal events with the light-dark cycle in the environment [34].

Recent molecular analyses have revealed that local thyroid hormone activation in the hypothalamus plays a critical role in the regulation of the neuroendocrine axis involved in seasonal reproduction in both birds and mammals. Furthermore, functional genomics analyses have revealed a novel function of the hormone thyrotropin. This hormone plays a key role in signaling day-length changes to the brain, and thus triggers seasonal breeding [16]. Long day (LD)-induced thyrotropin (TSH) in the pars tuberalis (PT) of the pituitary gland acts as a master factor regulating seasonal reproduction on the ependymal cells (ECs) within the mediobasal hypothalamus (MBH) to induce expression of type 2 deiodinase (Dio2), a thyroid hormone (TH)-activating enzyme in both LD and short day (SD) breeders. Locally activated TH in the MBH is believed to trigger GnRH secretion from the hypothalamus in LD breeders, while it terminates reproductive activity in SD breeders. Dio3 metabolizes THs under SD conditions, while Dio2 converts prohormone thyroxin ($T_4$) to bioactive triiodothyronin ($T_3$) under LD conditions in birds and mammals. Photoperiodic regulation of type 2 deiodinase (Dio2) and/or type 3 deiodinase (Dio3) has been confirmed in birds (e.g., the tree sparrow [44]) as well as mammals such as the Siberian hamster [45], and rat [46]. In quail, LD-induced $T_3$ causes morphological changes in the GnRH nerve terminals and glial processes, thereby causing GnRH secretion into the hypophyseal portal blood. Melatonin mediates transmission of photoperiodic information in mammals. LD-induced TSH acts on the ependymal cells (EC) to induce Dio2 expression and reduce Dio3 expression in both mammals and birds [47].

Taken together all the previous data, we could postulate that both melatonin and thyroid hormones affect reproduction in seasonally breeding animals differently through different pathways.

All the stages of ovarian follicles exhibit signs of atresia in aged rats of all groups studied. In Siberian hamsters, *Phodopus sungorus*, raising females in short days (SD) was associated with a delay in reproductive aging. Additionally, SD females had significantly more ovarian primordial follicles than LD hamsters at 3 and 6 months of age [48], and this advantage persisted through 12 months of age. Primordial follicles represent the resting pool of germ cells in the mammalian ovary, and their numbers decline with age because the activation of follicular growth is irreversible and occurs throughout a female's lifetime [49]. The ovaries of SD female hamsters were developmentally younger in terms of primordial follicle number [48]. Similarly, hamsters raised in LD, then transferred to SD as young adults and remained there for 6 months were found to have greater numbers of ovarian primordial follicles at advanced ages. A robust response to SD in juvenile and adult hamsters is associated with decelerated reproductive aging, which may result in greater reproductive success in older females as compared to age matched individuals demonstrating a more modest response to SD [50]. In this study, photoperiods did not affect the reproductive aging like that observed in Siberian hamster perhaps because we subjected rats lately when they were already aged to different photoperiods.

In aged female rats of the present study, the highest concentrations of FSH, LH and estrogen were observed in the control group. However, rats maintained under short photoperiod exhibited slightly higher values of LH, FSH, estrogen and progesterone compared to long photoperiod group. In female rice rats, the reproductive response to a short photoperiod did not decline with age. Older females retained responsiveness to a short photoperiod and underwent reproductive organ regression [5]. Serum FSH, estrogen and progesterone were decreased in the control group of the old rats compared to the adult ones in the present study. However, short and long photoperiods increased the levels of serum FSH, LH, and progesterone in aged rats compared to adult ones. In aging humans, night levels of melatonin decline progressively. Also, thyroid and gonadal functions decline during aging, while gonadotropins luteotropic hormone (LH) and follicle stimulating hormone (FSH)) steadily increase. Melatonin administration produced a significant diminution of LH in the women of age 43 to 49 years-old, while no effect was seen in the older women (50-62 years old). A

decrement of FSH was observed in Melatonin-treated women with low basal melatonin levels [51].

**Maganhin** [41] postulated that estrogen and progesterone regulate and stabilize circadian systems in animals. Decreased hypothalamic sensitivity to estrogen at the menopause may culminate in circadian rhythm disturbances. Aging is also associated with loss of this regularity and stability [52]. Elderly women show a greater range of circadian rhythm phases and amplitudes [53]. Melatonin possibly changes GnRH (gonadotropin-releasing hormone) secretion by modifying the proportion of gonadotropins released, with the consequent predominance of luteinizing hormone (LH) over follicle-stimulating hormone (FSH) [54].

In hamsters, age related changes in pineal melatonin content have been reported [55] In Syrian hamsters, for example, melatonin rhythms are severely dampened in 18 month-old males and females compared with 2-month-old animals, whereas in old gerbils (19 month of age) there is no nocturnal rise in melatonin [56]. In voles, on the other hand, no detectable differences in pineal melatonin content in older vs. younger males were observed [57]. Reproductive responsiveness to photoperiod has been shown to decline with age in several species of rodents housed on short photoperiods, such as the Siberian (Djungarian) hamster, Syrian hamster, prairie vole, and meadow vole [58].

From the present result, it could be concluded that thyroid gland of adult and aged female rats is affected mainly by photoperiod. Short and long photoperiods increase the activity of the gland of adult rats. Long photoperiod rose serum thyroxin levels compared to that observed in the control and short photoperiod groups. Short photoperiod and 12:12 light dark cycle enhanced structural activities of ovaries in adult rats. Highest values of reproductive hormones were observed in control 12:12LD group in adult and aged rats. However, aging decreased the activities of ovaries of rats in all the groups perhaps as a result of decreasing melatonin secretion. Photoperiod induced a slight change in the levels of reproductive hormones in old rats compared to the adult ones.

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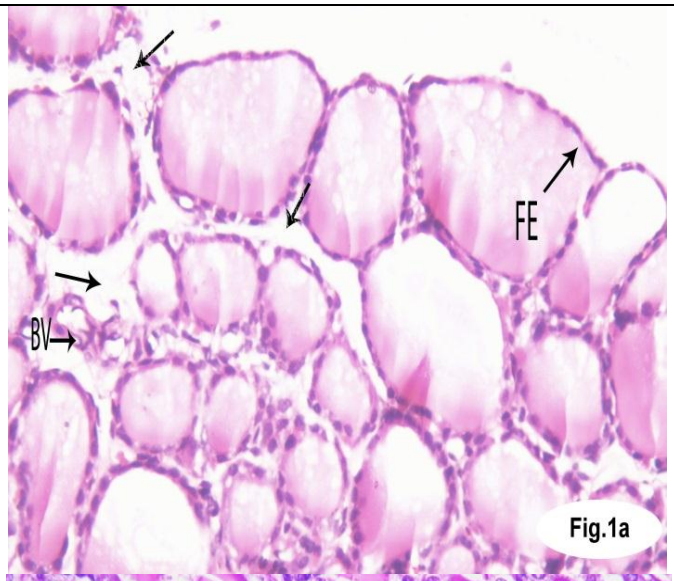
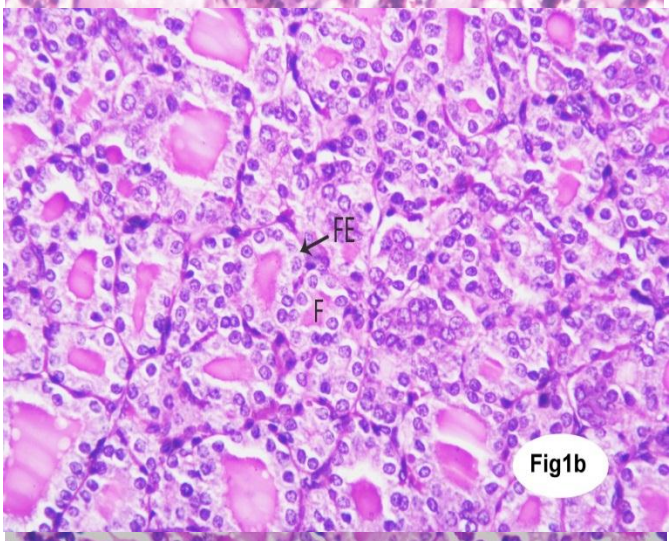


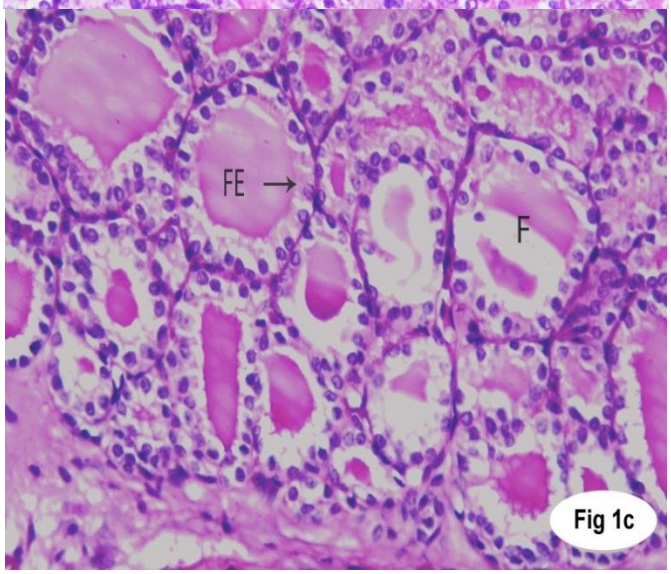
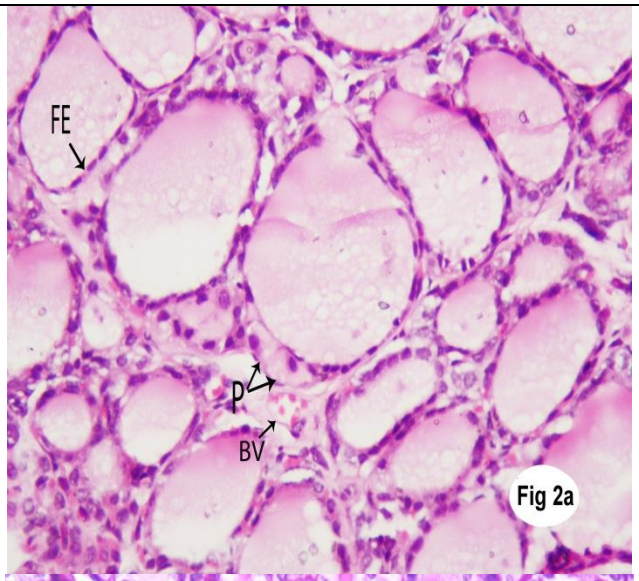
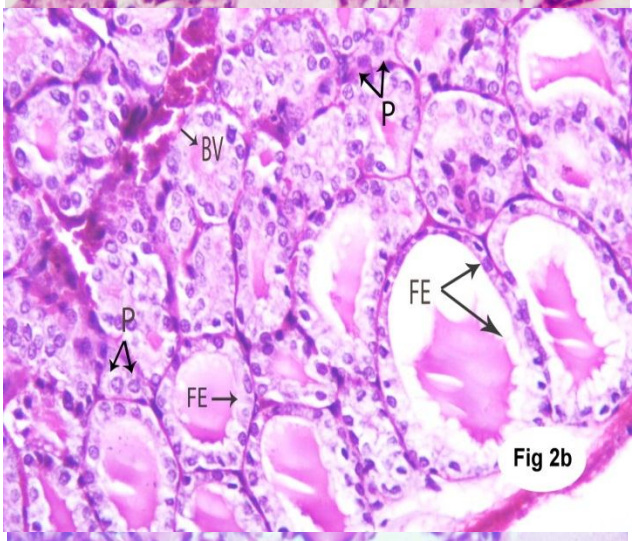




**Figure 1: Photomicrographs of sections of the thyroid gland from adult female rat subjected to 12/12 light-dark cycle (1a), short photoperiod (1b), and long photoperiod (1c).Thyroid follicles (F) lined with follicular epithelium (FE) and interfollicular spaces filled with connective tissue (arrows). (400 X)**



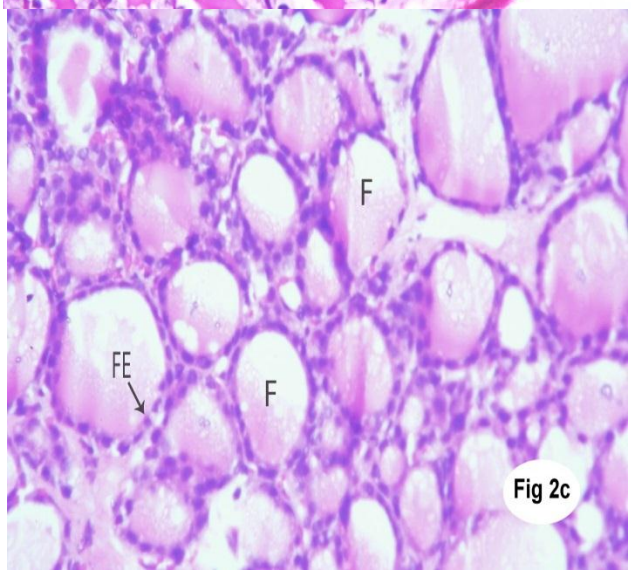
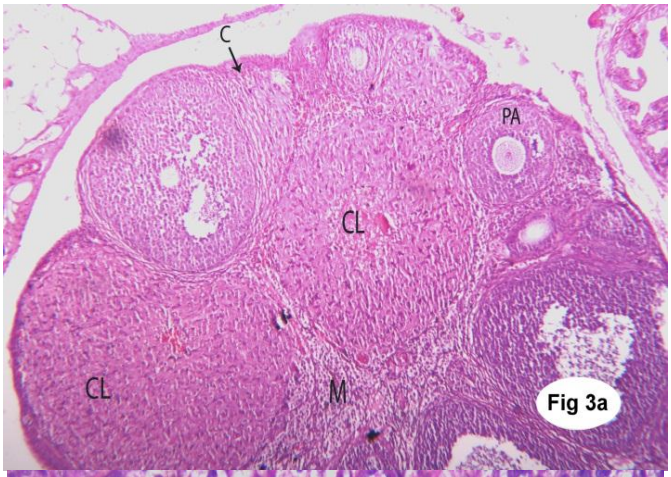
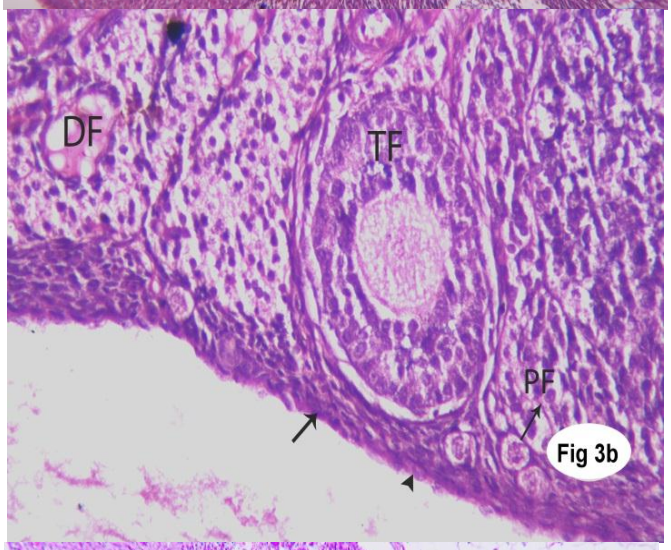




**Figure 2: Photomicrographs of sections of the thyroid gland from aged female rat subjected to 12/12 light-dark cycle (1a), short photoperiod (1b), and long photoperiod (1c).Thyroid follicles (F) lined with follicular epithelium (FE), parafollicular cells (P) and blood vessels (BV) in the connective tissue stroma. (400 X)**



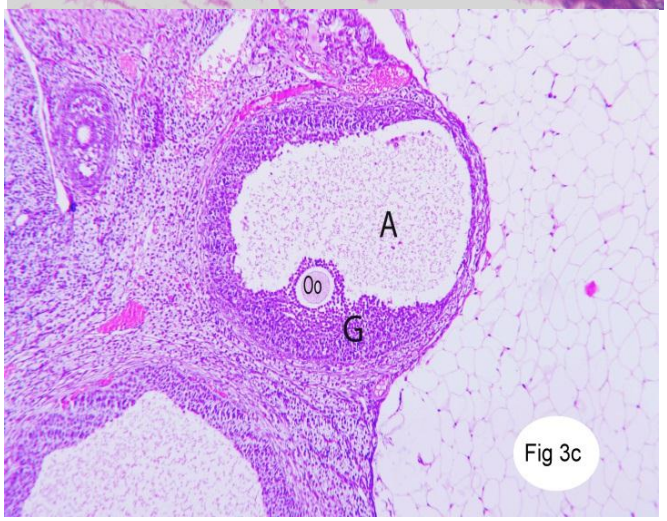
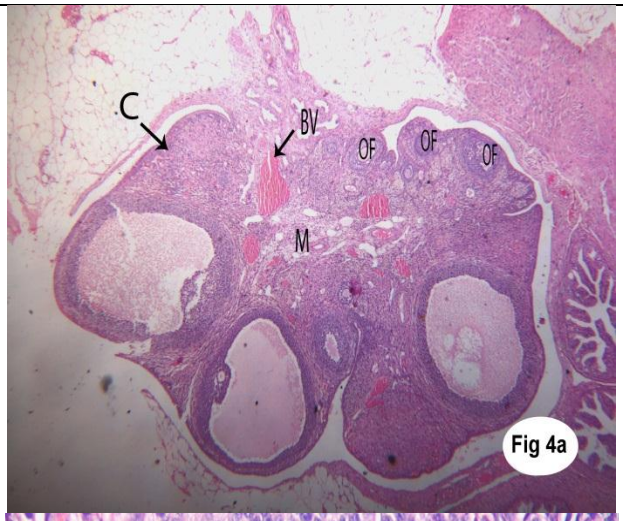
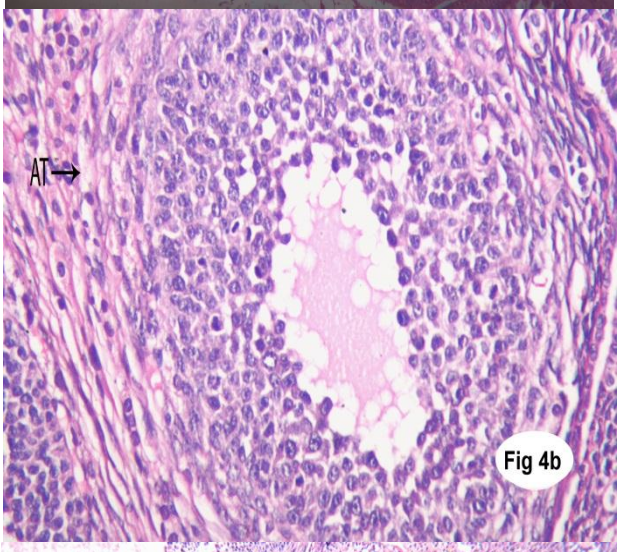




**Figure 3: Photomicrographs of sections of the ovary from adult female rat subjected to 12/12 light-dark cycle. The photomicrograph (3a) showing 2 layers, the cortex (C), containing preantral follicle (PA) and corpora lutea (CL) and the inner layer, medulla (M). The photomicrograph (3b), showing the coelomic epithelia (arrowhead), germinal epithelia (arrow), primordial follicles (PF), tertiary ovarian follicles (TF). Degenerated follicle (DF) is also observed. photomicrograph (3c), showing Graafian follicle (GF) with oocyte (Oo), granulosa layer (G) and large antrum (A). (400 X)**



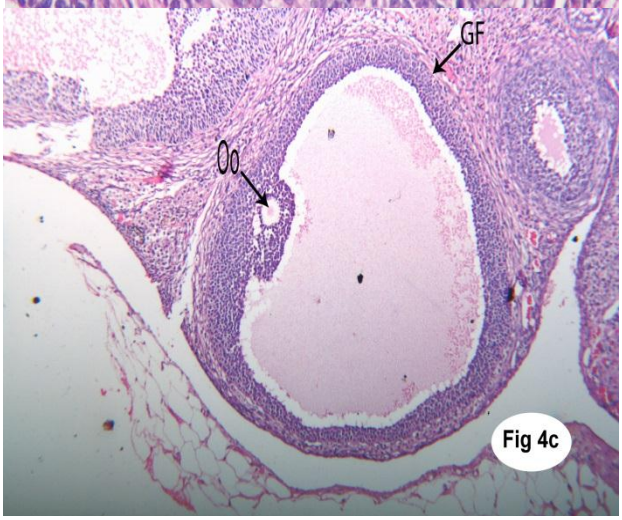
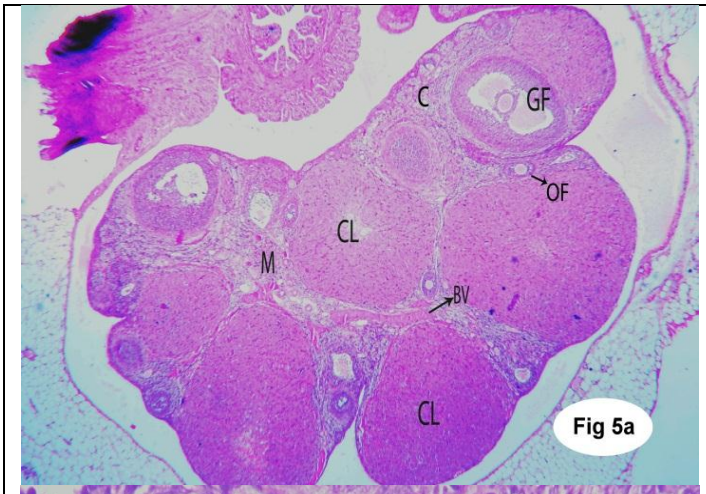
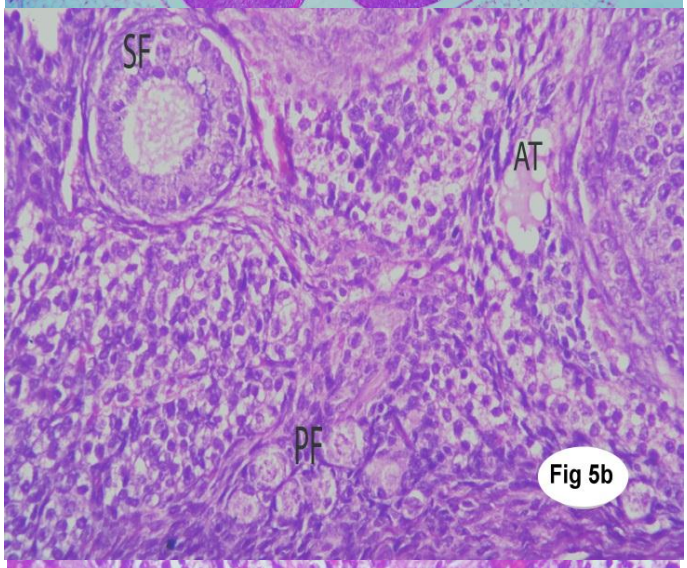




**Figure 4: Photomicrographs of sections of the ovary from aged female rat subjected to 12/12 light-dark cycle. The photomicrograph (4a) showing the cortex (C) containing a number of ovarian follicles (OF) and the medulla (M) contains numerous congested blood vessels (BV). (100 X). The photomicrograph (4b), showing late stage of atretic follicles (AT) with degenerated oocyte. The theca layer around the follicle is hypertrophied (arrow). The photomicrograph (4c), showing early stage of atretic Graafian follicle (GF) with degenerated oocyte (Oo). (400 X)**



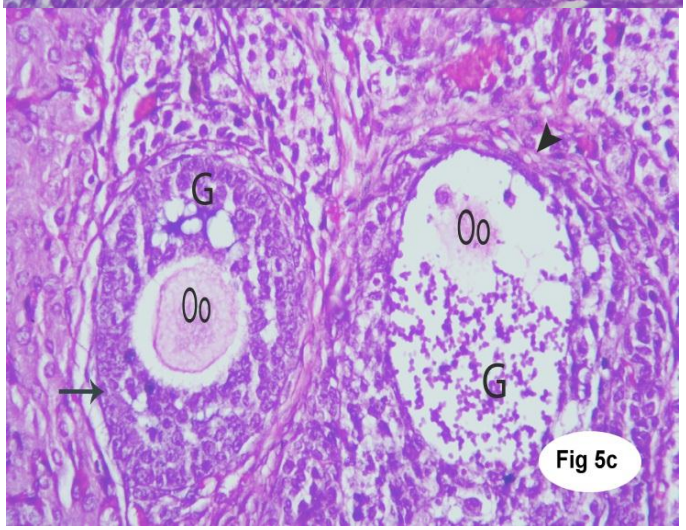
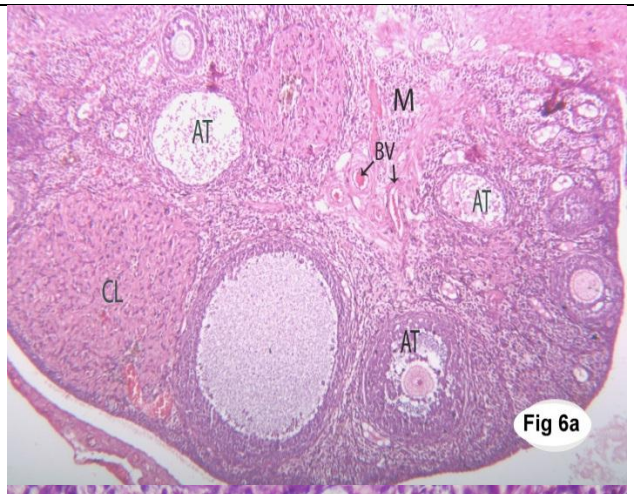
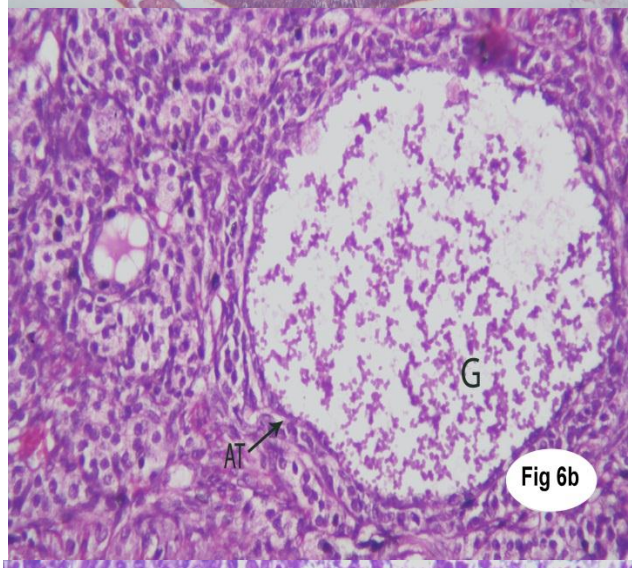




**Figure 5: Photomicrographs of the ovary of adult female rat subjected to short photoperiod. The ovary (5a) is lobulated, the cortex (C) contains numerous ovarian follicles (OF), Graafian follicle (GF) and numerous corpora lutea (CL). The medulla (M). (100 X).**

**The cortex of photomicrograph (5b) contains clusters of primordial follicles (PF), a secondary follicle (SF) and an atretic follicle (AT) with degenerated cells. The photomicrograph (5c), showing early stage (arrow) and late stage (arrowhead) of atretic follicles with degenerated granulosa cells (G) and degenerated oocyte (Oo). (400 X)**



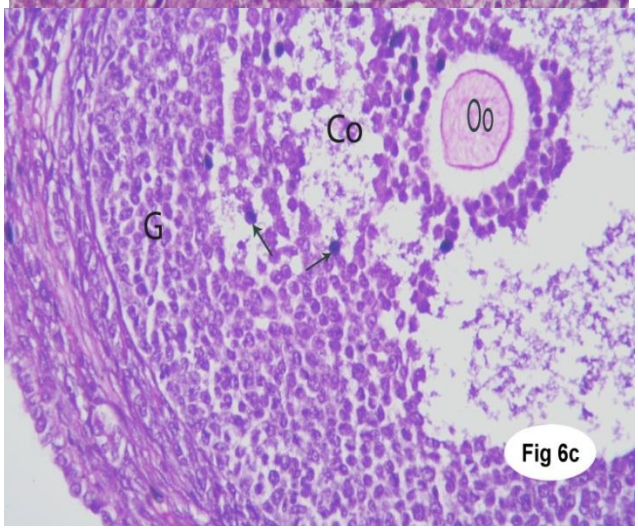
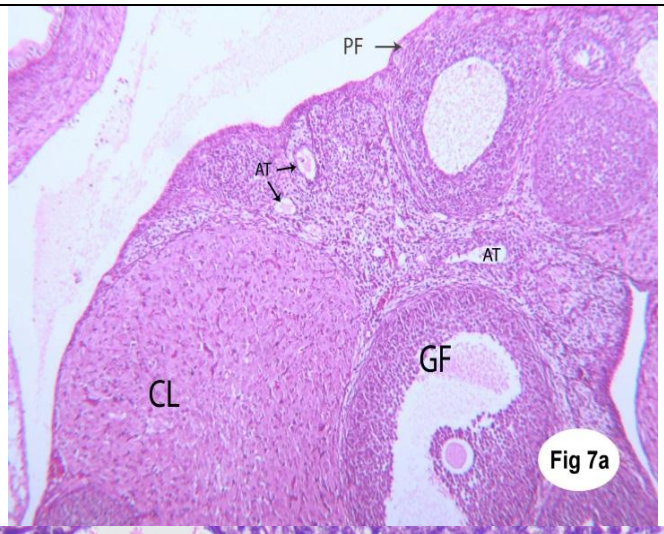
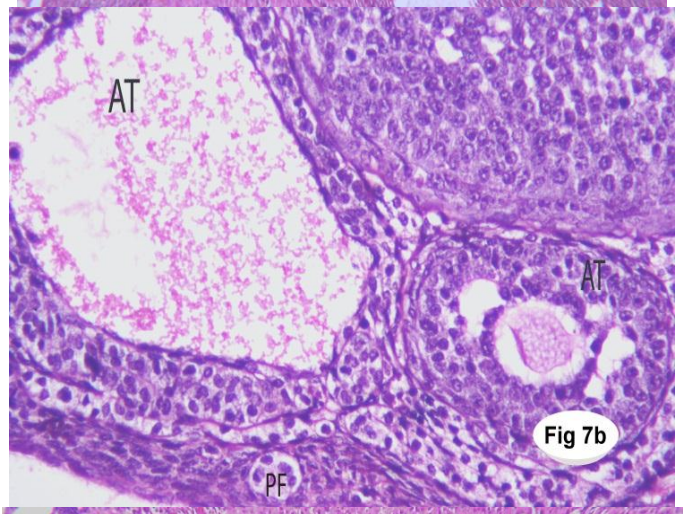




**Figure 6: Photomicrographs of the ovary of aged female rat subjected to short photoperiod. Photomicrograph (6a) showing large number of atretic follicles (AT), corpus luteum (CL). The medulla (M) contains a number of blood vessels (BV) (400X). Late stage of atretic follicles (AT) appear in the photomicrograph (6b) with degenerated oocyte and degenerated granulosa cells (G). (400 X). Atretic Graafian follicle (GF) appears in this section (6c). Granulosa cells (G) contain pyknotic nuclei (arrow). Detached and degenerated cumulus oophorous (Co). The oocyte (Oo) shows signs of shrinkage. (1000X)**



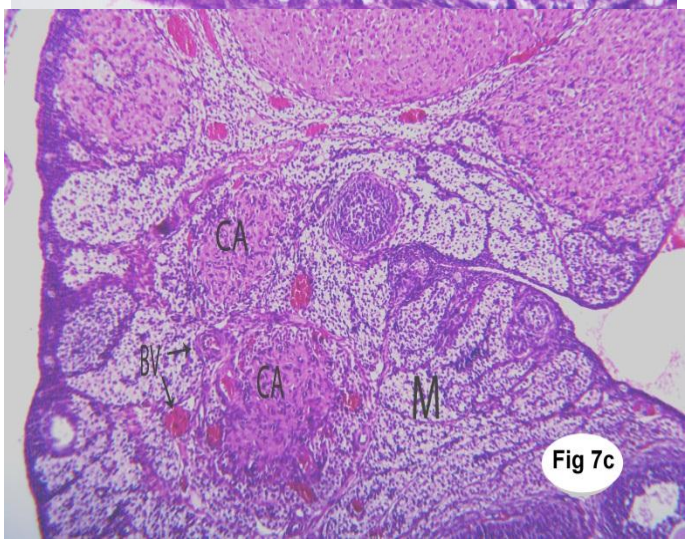
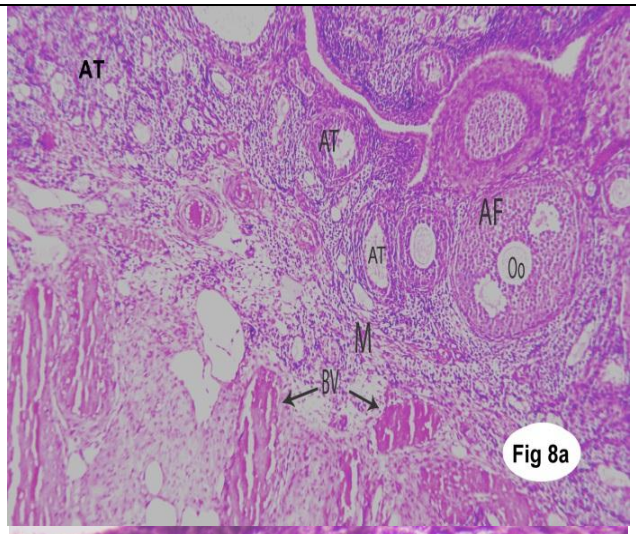
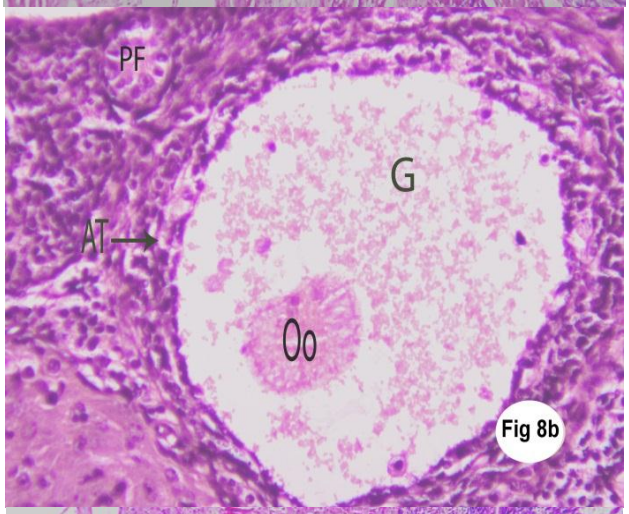




**Figure 7: Photomicrographs of the ovary of adult female rat subjected to long photoperiod. Few number of corpora lutea (CL), atretic follicles (AT), and Graafian follicle (GF) are shown in photomicrograph (7a). (100 X). Primordial follicle (PF) and atretic follicles (AT) are shown in photomicrograph (7b). (400 X). The photomicrograph (7c), showing 2 corpora albicanses (CA) that appear in the medulla (M) region with some blood vessels (BV) in between. (400 X)**



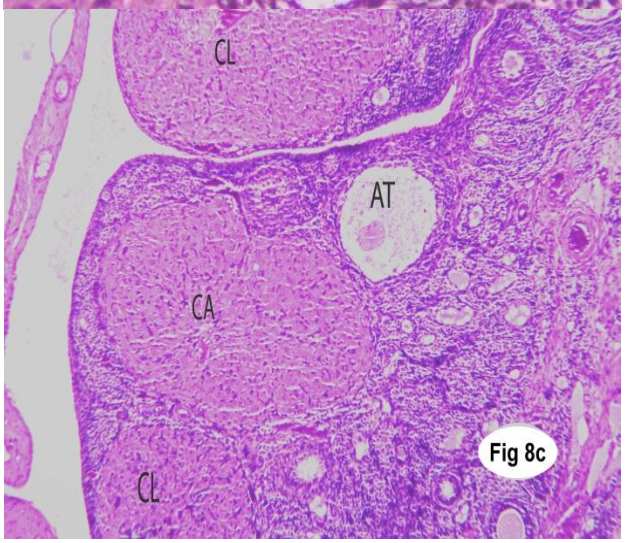




**Figure 8: Photomicrographs of the ovary of aged female rat subjected to long photoperiod. The photomicrograph (8a), showing antral follicle (AF) with oocyte (Oo) and atretic follicles (AT). The medulla (M) contains numerous congested blood vessels (BV). (400 X). Primordial follicle (PF), late stage of atretic follicle (AT) with degenerated granulosa cells (G) and degenerated oocyte (Oo) are shown in photomicrograph (8b). (1000 X). Photomicrograph (8c), showing corpora lutea (CL), corpus albicans (CA) and atretic follicle (AT). (400 X)**

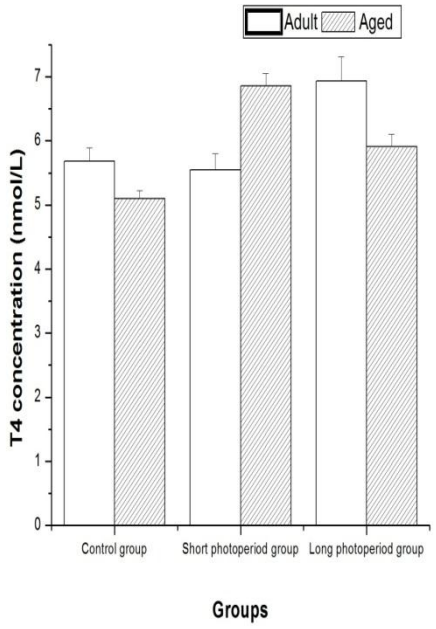



Fig. "9": Levels of thyroxin in adult and aged female rats subjected to different photoperiods

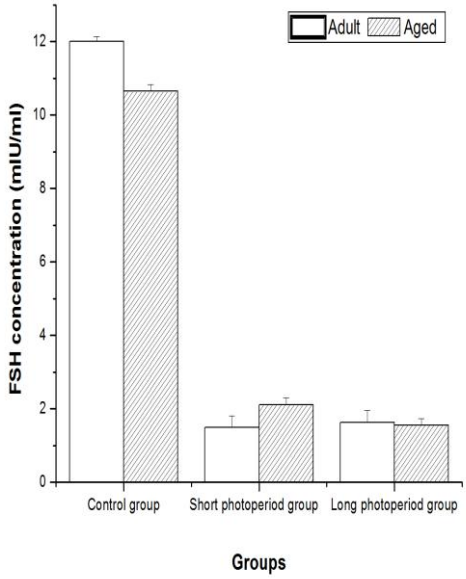



Fig. "10": Levels of FSH in adult and aged female rats subjected to different photoperiods.

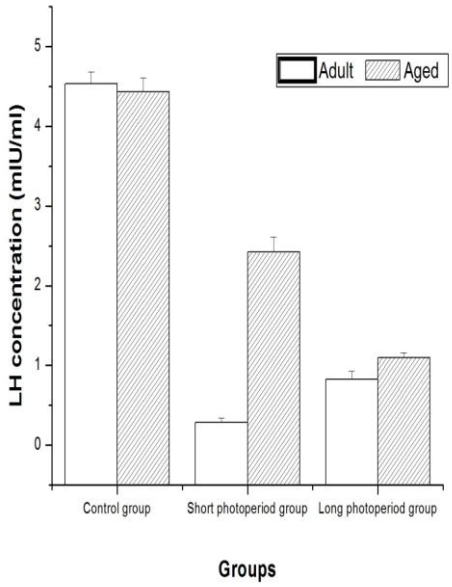



Fig. "11": Levels of LH in adult and aged female rats subjected to different photoperiods.

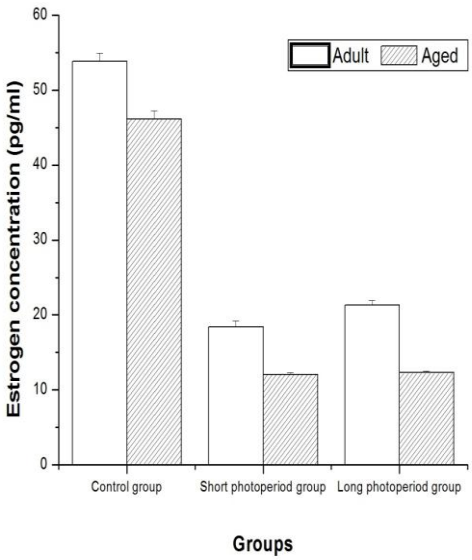



Fig. "12": Levels of estrogen in adult a aged female rats subjected to different photoperiods.

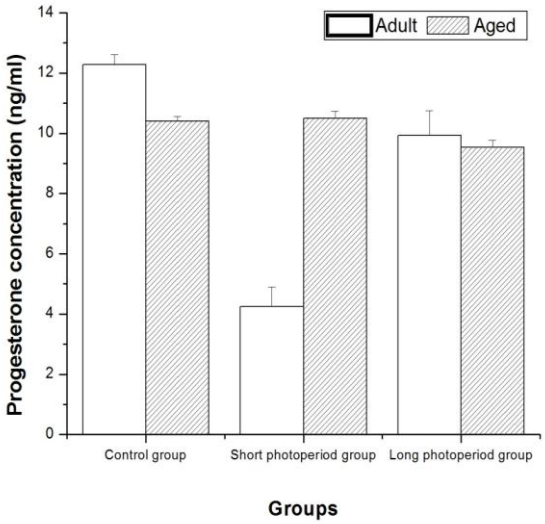



Fig. "13": Levels of progestrone in adult and aged female rats subjected to different photoperiods.