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EXPERIMENTAL STUDY ON THE EFFECTS OF MELATONIN ADMINISTRATION ON THE OVARY AND THE OVIDUCT IN THE ADULT MICE

(With 2 Tables and 22 Figures)

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دراسة تجريبية عن تأثير إعطاء الميلاتونين على المبيض وقناة البيض في الفئران البالغه

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الهدف من هذه الدراسة إيضاح تأثير إعطاء الميلاتونين لفترات زمنية مختلفة على حويصلات المبيض والنسيج الطلائى لقناة البيض في الفئران البالغة. وكذلك دراسة ارتداد تأثير الميلاتونين فى محاولة لتقييم تأثيره على خصوبة الإناث. استخدم فى هذا البحث جرعة عالية من الميلاتونين تساوى ٨ ملى جرام لكل كيلوجرام وجرعة منخفضة تساوى ٤ ملي جرام لكل كيلو جرام. وأعطي الميلاتونين للحيوانات عن طريق الحقن تحت الجلد يومياً فى وقت متأخر بعد العصر. وقد استخدم في هذا البحث أربعون من إناث الفئران حديثة البلوغ والتي يبلغ عمرها شهرين في بداية التجربة وتم تقسيمها الي ثمانية مجموعات. شملت المجموعة الأولي والثانية حيوانات ضابطة تبلغ من العمر ثلاثة اشهر وأربعة اشهر. وأعطيت حيوانات المجموعة الثالثة الجرعة المنخفضة من الميلاتونين لمدة شهر وأعطيت المجموعة الرابعة نفس الجرعة لمدة شهرين. وأعطيت حيوانات المجموعة الخامسة الجرعة العالية لمدة شهر وأعطيت المجموعة السادسة نفس الجرعة لمدة شهرين. وبهذا أصبح عمر الحيوانات التى عولجت بالميلاتونين لمدة شهر يساوى ثلاثة أشهر والتى عولجت به لمدة شهرين يساوى أربعة أشهر فى نهاية التجربة. وشملت المجموعة السابعة حيوانات ضابطة تبلغ من العمر سبعة أشهر وذلك لمقارنتها بحيوانات المجموعة الشفائية وهى المجموعة الثامنة وتتكون من حيوانات أبقيت بدون علاج لمدة ثلاثة أشهر بعد إعطاؤها الجرعة العالية لمدة شهرين وبهذا أصبح عمرها سبعة أشهر فى نهاية التجربة. تمت التضحية بالحيوانات وتجهيز عينات من المبيض وقناة البيض لدراستها هستولوجيا بعد صبغها بالهيماتوكسيلين والأيوسين وتم تجهيز بعض العينات من منطقة البوق فى قناة البيض لفحصها بالمجهر الاليكتروني النافذ. وكذلك شملت الدراسة قياس الكثافة العددية وقطر الحويصلات الأولية والحويصلات النامية والأجسام الصفراء فى مبايض حيوانات جميع المجموعات السابق

ذكـرها وإجراء التحليل الاحصائى لها. أظهرت نتائج هذه الدراسة زيادة حجم المبيض فى جميع الحيوانات المعالجة بالميلاتونين وأظهر قياس الكثافة العددية وقطر الحويصلات النامية والأجسام الصفراء في مبايض هذه الحيوانات وجود زيادة معنوية بها عن مقارنتها بمثيلاتها في الحيوانات الضابطة. أما الكثافة العددية للحويصلات الأولية فقد وجد بها نقصا معنويا في الحـيوانات المعالجـة بالميلاتونين مقارنا بالحيوانات الضابطة. وقد وجد أن حجم وتركيب المبـيض فـي حـيَوانات المجموعة الشفائية مماثل تقريبا للمبيض في المجموعة الضابطة. واظهـر قـياس متوسـط الكـثافة العددية وقطر الحويصلات الأولية والحويصلات النامية والأجسام الصفراء في حيوانات المجموعة الشفائية عدم وجود تغيير معنوي بها عن مقارنتها بمثيلاتها في المجموعة الضابطة المقابلة لها. واظهر فحص النسيج الطلائي لمنطقة البوق في الحيوانات المعالجة بالجرعة العالية من الميلاتونين أن الخلايا الإفرازية كان هي السائدة به. وأوضـح الفحـص الدقـيق لها أن سطح هذة الخلايا به العديد من الزغب وأن السيتوبلازم يحـتوي علـي العديـد مـن الحبيـبات الإفرازية. وقد استنتج من هذه الدراسة أن إعطاء الميلاتونين في وقت الليل له تأثير منشط لنمو حويصلات المبيض وأن هذا التأثير لا يتوقف علـي جرعة أو مدة إعطاء الميلاتونين ووجد أن هذا التأثير علي المبيض يكون مرتدا. وقد أتضـح أيضـا أن الخلايا الإفرازية كانت هي السائدة في النسيج الطلائي لقناه البيض وهذا يمكـن أن يعطـي في المستقبل اتجاهات جديدة لدراسة دور الميلاتونين في فسيولوجيا وعلم أدوية الخصوبة ومنع الحمل في كل من الحيوان والإنسان.

SUMMARY

The aim of the present work is to demonstrate the effect of the melatonin administration for different periods of time on the ovarian follicles and the oviductal epithelium in the adult mice. Also the reversibility of melatonim effect is studied in a trial for assessment of its effect on the fertility in females. In this work, a high dose (8.0 mg/kg) and a low dose (4.0 mg/kg) of melatonin were used. Melatonin was injected to the animals subcutaneously once daily in the late afternoon. A total number of 40 young adult female mice were used. At the beginning of the experiments, they were two months old. Animals were divided into eight groups. Groups I and II composed of three and four months old control animals. Group III received the low dose of melatonin for one month and group IV received the same dose for two months. Group V received the high dose of melatonin for one month and group VI received the same dose for two months. At the end of the experiments, animals received the treatment for one month became three months old and those received the treatment for two months became four months old. Group VII composed of seven months old control animals. Group VIII composed of recovered animals (animals allowed to survive three months without treatment after receiving the high dose for two months). At the end of the experiment, the recovered animals became seven

months old. Animals were sacrificed, their ovaries and the oviducts were removed and processed for histological examination after staining with Haematoxylin and Eosin. Some specimens of the ampulla of the oviduct were processed for ultrastructural study with transmission electron microscope. The numerical density and the diameter of the primary follicles, growing follicles and corpora lutea in the ovaries of all groups of animals were measured and statistically analyzed. The results of the present work demonstrated apparent increase in the size of the ovary in the melatonin treated animals. The numerical density and the diameter of the growing follicles and corpora lutea in all melatonin treated groups showed significant increase in comparison with their corresponding control. The primary follicles showed significant reduction in their numerical density in melatonin treated animals as compared with the control. In the recovered animals, the size and the structure of the ovary appeared nearly similar to the control. Also the numerical density and the diameter of the primary follicles, growing follicles and corpora lutea showed non significant change in comparison with their corresponding control. The oviductal epithelium of the ampulla in the high dose melatonin treated animals showed predominance of the secretory cells. The ultrastructual study of these cells revealed that the apical surface had many microvilli and their cytoplasm contained a lot of secretory granules. In conclusion, this study revealed that nighttime melatonin administration had a stimulatory effect on the ovarian follicular growth. This effect was independent to the dose or the duration of its administration. The oviductal epithelium showed predominance of secretory cells. Its effect on the ovary was reversible. This may provide in the future new insights and directions for the study of its role in the physiology and pharmacology of fertility and contraception in animals and humans.

Key words: *Melatonin, ovary, oviduct*

INTRODUCTION

Melatonin is one of several hormones secreted rhythmically from the pineal gland. In animals and humans, it is produced and secreted into the blood stream in response to darkness. It is involved in the regulation of many physiological processes (Sirotkin and Schaeffer, 1997). Dardes *et al.* (2000) reported that melatonin plays a decisive role in modulating the reproductive activity of polyestrus mammals (like humans and

laboratory rodents). It influences the age of sexual maturation, the timing of the ovulatory cycle and gonadal steroidogenesis.

Cassone *et al.* (1993) stated that there are several possible sites of melatonin action in the reproductive system. Melatonin has been shown to mediate the relationship between the hypothalamus and the pituitary. Vanecek *et al.* (1987) found many melatonin binding sites in different neuroendocrine tissues like the superachiasmatic nucleus, median eminence and anterior pituitary of either non-photoperiodically sensitive mammals or the seasonal breeders. Melatonin also had been shown to have several binding sites in the ovary, testis and the mammary glands which suggest the concept of multiple sites of melatonin action on the reproductive system (Pang *et al.*, 1998). Itoh *et al.* (1997 & 1999) reported that the rat and human ovaries, like the pineal gland may synthesize melatonin from its precursor, serotonin.

Recently, melatonin preparations are available in health food stores and pharmacies in many countries. It becomes an accepted therapy for many disorders, however, its cellular effects are still unknown (Cupp, 1997). Guerin *et al.* (2000) reported that exogenous melatonin produced either progondal or antigondal effects in mammals depending on the time, dose and mode of administration. The aim of the present work is to demonstrate the effect of melatonin administration for different periods of time on the ovarian follicles and the oviductal epithelium in the adult mice. Also, the reversibility of melatonin effect is studied in a trial for assessment of its effect on the fertility in females.

MATERIALS and METHODS

A total number of 40 young adult female mice were used in this work. At the beginning of the experiments, they were 2 months old and weighted 25 – 30 grams. They kept under light: dark cycles of 12 h. light: 12 h. dark.

Melatonin (Sigma chemical company) dissolved in saline with few drops of ethanol. The final solution contained 0.4% ethanol. The injected volume per mouse was 1.0 ml containing the calculated dose of melatonin. Two doses of melatonin were used in this study; a high dose (8.0 mg/kg) and a low dose (4.0 mg/kg). Melatonin was injected to the animals subcutaneously once daily in the late afternoon (4.30 pm).

Animals were divided into eight groups. Groups I and II composed of three and four months old control animals. Groups III received the low dose of melatonin for one month and group IV received

the same dose for two months. Group V received the high dose of melatonin for one month and group VI received the same dose for two months. At the end of the experiments, animals received the treatment for one month became three months old and those received the treatment for two months became four months old. Animals were sacrificed except some animals of group VI (melatonin treated group with the high dose for two months) allowed to survive for another three months without treatment and they considered the recovered group (group VIII). At the end of the experiment, the recovered animals became seven months old and they compared to another group of control animals (group VII) aged seven months.

Ovaries were removed from the animals and fixed in Bouin's fluid, dehydrated in ascending grades of ethanol, embedded in paraffin wax. Serial sections (10 μm) thickness were prepared and stained with Haematoxylin and Eosin. The oviducts also were removed and dissected in the animals of groups I, II, V and VI. Specimens of the ampulla were processed for histological examination after staining with Haematoxylin and Eosin. Other specimens from the ampulla were processed for ultrastructural study with transmission electron microscope.

Morphometric procedure:

This include estimation of the diameter and numerical density of the primary follicles, growing follicles and the corpora lutea in the above mentioned groups of animals. These parameters were measured from paraffin sections (10 um thickness) stained with Haematoxylin and Eosin. All follicles surrounded by squamous cells or a mixture of squamous and cuboidal granulosa cells without follicular cavity were considered as primary follicles. All follicles containing follicular cavities at any pattern were considered as growing follicles. The corpus luteum of its different stages was counted as a single entity. In the all groups of animals, ovaries were selected at random for counting the follicles. All follicles were counted by a single technician.

The previous parameters were measured using a digitizing set which formed of Digitizer KD 3040 B connected to integar IBM compatible personal computer, with a specially prepared program to measure lengths. The major diameter (a) which is the widest diameter and minor diameter (B) which is the widest diameter perpendicular over (a) of each ovarian follicle profile were measured. The diameter of equivalent circle (D) was obtained.

The numerical density of the primary follicles (PF) per unit volume of the ovary was calculated as follow:

$$NV_{(PF)} = \frac{N_{(PF)}/a}{D_{(PF)} + t}$$

Where $N_{(PF)}$: the number of the primary follicles per unit volume of the ovary.

a : the area in which the number of the primary follicles was measured.

$D_{(PF)}$: the corrected mean diameter of the nucleus of the primary follicles.

t : section thickness, which is equal to 10 μm.

Numerical density of the growing follicles (GF) and the corpora lutea (CL) per unit volume of the ovary were calculated by the same method.

Statistical analysis:

For all groups of animals, the mean and the standard deviation of the diameter and the numerical density of the primary follicles, the growing follicles and the corpora lutea were calculated. Unpaired student t-test was used to compare between the means of the different groups.

RESULTS

I- The ovary:

Control animals:

Group I (3 months old mice) and Group II (4 months old mice):

Light microscopic examination demonstrated that the ovary is formed of outer cortex and inner medulla. The cortex showed the presence of follicles at different stages of maturation. They are separated by thin layer of stroma. The follicles include primordial follicles which formed of oocyte surrounded by a single layer of follicular cells, primary follicles in which the oocyte is surrounded by two or more layers of granulosa cells, growing follicles which include all the follicles formed of oocyte surrounded by several layers of granulosa cells in which fluid-filled spaces appear among the cells and mature (Graafian) follicles. It also shows the presence of corpora lutea in various stages of its formation. The medulla is formed of connective tissue and many dilated blood vessels. The ovary is surrounded by a layer of simple cubodial

epithelial cells and dense connective tissue layer called the tunica albuginea (Figs. 1 & 4).

Morphometric study of the three months old control mice shows that the means of the diameter of the primary follicles, growing follicles and corpora lutea are (0.236 ± 0.002, 0.327 ± 0.005 and 0.503 ± 0.003) (Table 1 and Fig. 17). The means of their numerical density are (136.5 ± 5.4, 105.2 ± 4.9 and 21.8 ± 1.26) respectively (Table 2 and Fig. 19).

In the 4 months old control mice, the means of the diameter of the primary follicles, growing follicles and corpora lutea are (0.232 ± 0.016, 0.334 ± 0.028 and 0.509 ± 0.006) (Table 1 and Fig. 18). The means of their numerical density are (137.0 ± 4.3, 105.8 ± 6.8 and 23.57 ± 4.4) respectively (table 2 and Fig. 20).

Low dose melatonin treated animals:

Group III (3 months old mice) received the low dose for one month and group IV (4 months old mice) received the low dose for two months:

Microscopic examination shows an increase in the growing follicles and the corpora lutea than that in the corresponding control animals (Figs. 2 & 5).

Morphometric study of the three months old low dose melatonin treated mice revealed that the mean diameter of primary follicles is (0.239 ± 0.004) which shows non significant increase as compared to the corresponding control (0.236 ± 0.002). In this group of animals, there is highly significant increase ($P < 0.01$) in the diameter of the growing follicles and corpora lutea where their means of diameter are (0.367 ± 0.017 and 0.554 ± 0.022) and in the corresponding control animals are (0.327 ± 0.005 and 0.503 ± 0.003) respectively (table 1 and Fig. 17). The mean numerical density of the primary follicles in this group of treated mice is (119.2 ± 5.9) which shows highly significant decrease ($P < 0.01$) as compared to the corresponding control (136.5 ± 5.4) while the numerical density of the growing follicles and corpora lutea shows highly significant increase ($P < 0.01$) where the means of their density are (185.6 ± 7.3 and 34.3 ± 4.68) and in the corresponding control are (105.2 ± 4.9 and 21.8 ± 1.26) respectively (Table 2 and Fig. 19).

In the four months old low dose melatonin treated mice, the mean diameter of the primary follicles is (0.250 ± 0.019) which shows non significant increase as compared to the corresponding control (0.232 ± 0.016). The diameter of the growing follicles and corpora lutea in these animals show mild significant increase ($P < 0.05$) where the means of their diameter are (0.385 ± 0.004 and 0.557 ± 0.033) and in the

corresponding control are (0.334 ± 0.028 and 0.509 ± 0.006) respectively (Table 1 and Fig. 18).

In this group, the numerical density of the primary follicles shows highly significant decrease ($P < 0.01$) where the mean of their density is (118.2 ± 2.1) and in the corresponding control is (137.0 ± 4.3) while the means of the numerical density of the growing follicles and the corpora lutea are (167.0 ± 4.9 and 37.84 ± 2.2) which show highly significant increase ($P < 0.01$) as compared to the corresponding control (105.8 ± 6.8 and 23.57 ± 4.4) respectively (Table 2 and Fig. 20).

High dose melatonin treated animals:

Group V (3 months old mice) received the high dose for one month and group VI (4 months old mice) received the same dose for two months.

In these groups of animals, the ovaries show apparent increase in size with marked increase in the growing follicles and corpora lutea (Figs. 3 & 6).

In the three months old high dose melatonin treated mice, the means of the diameter of the primary follicles and growing follicles are (0.243 ± 0.01 and 0.347 ± 0.034) which show non significant increase as compared with the corresponding control (0.236 ± 0.002 and 0.327 ± 0.005) respectively.The mean diameter of the corpora lutea shows very highly significant increase ($P<0.001$) where the mean is (0.574 ± 0.011) and in the corresponding control is (0.503 ± 0.003) (Table 1 and Fig. 17).In this group, the numerical density of the primary follicles shows mild significant decrease ($P < 0.5$) where the mean of their density is (123.6 ± 4.8) and in the corresponding control is (136.5 ± 5.4). The means numerical density of the growing follicles and corpora lutea are (163.7 ± 8.4 and 30.5 ± 1.56) show very highly significant increase ($P<0.001$) as compared with corresponding control (105.2 ± 4.9 and 21.8 ±1.26) respectively (Table 2 and Fig. 19).

In the four months old high dose melatonin treated mice, the means of the diameter of the primary follicles and growing follicles are (0.253 ± 0.014 and 0.369 ± 0.023) which show non significant increase as compared with the corresponding control (0.232 ± 0.016 and 0.334 ± 0.028) respectively. The mean diameter of the corpora lutea shows mild significant increase ($P<0.05$) where the mean is (0.546 ± 0.02) and in the corresponding control is (0.509 ± 0.006). (Table 1 and Fig. 18). In this group of treated mice, the mean numerical density of the primary follicles is (123.2 ± 8.7) shows mild significant decrease ($P < 0.05$) as compared with the corresponding control (137.0 ± 4.3). The numerical

density of the growing follicles and corpora lutea show highly significant increase ($P < 0.01$) where the means of their density are (137.3 ± 4.9 and 37.35 ± 1.51) and in the corresponding control are (105.8 ± 6.8 and 23.57 ± 4.4) respectively (Table 2 and Fig. 20).

Group VII (Seven months old control mice):

The histological picture of this age is similar to that of the three and four months old control mice (Fig. 7).At this age, the means of the diameter of the primary follicles, growing follicles and corpora lutea are (0.241 ± 0.024, 0.328 ± 0.0016 and 0.511 ± 0.008) (Table 1 and Fig. 21). The means of their numerical density are (134.0 ± 2.1, 136.4 ± 3.6 and 32.3 ± 1.5) respectively (Table 2 and Fig. 22).

The recovered group:

Group VIII (Seven months old mice):

Light microscopic examination demonstrates that the ovary restores its normal size and appearance and becomes nearly similar to the control (Fig. 8).Morphometric study shows that the means of the diameter of the primary follicles, growing follicles and corpora lutea in the recovered animals are (0.235 ± 0.027, 0.286 ± 0.099 and 0.499 ± 0.018) which show non significant difference from that of the corresponding control (0.241 ± 0.024, 0.328 ± 0.0016 and 0.511 ± 0.008) respectively (Table 1 and Fig. 21).In the recovered mice, the mean numerical density of the primary follicles is (124.9 ± 4.2) which shows mild significant decrease ($P<0.05$) as compared with that of the control (134.0 ± 2.1). The means of the numerical density of the growing follicles and corpora lutea in this group are (138.2 ± 4.4 and 30.1 ± 2.1) show non significant difference as compared with the control (136.4 ± 3.6 and 32.3 ± 1.5) respectively (Table 2 and Fig. 22).

II- The oviduct:

Control animals (Three and four months old mice):

Light microscopic examination of the ampulla of the oviduct demonstrates that the wall of the duct consists of mucous membrane which is formed of simple columner partially ciliated epithelium resting on a loose connective tissue called the lamina propria, smooth muscle layer and the serosa. The mucous membrane is thrown into folds that project into the lumen (Figs. 9 & 10). The ultrastructural study of the epithelial lining of the ampulla shows that the cells have ovoid nuclei. The cytoplasm is rich with mitochondria and shows the presence of some vesicles. There are abundant cilia and some microvilli associated with their apical surface (Figs. 13 & 14).

Melatonin treated mice with the high dose for one month (three months old mice):

In these animals, light microscopic examination of the cells of the mucous membrane of the ampulla demonstrates that these cells have numerous microvilli and some cilia (Fig. 11). The ultrastructural study of these cells shows that their cytoplasm contains mitochondria and electron dense secretory granules. Their apical surface shows the presence of many short microvilli (Fig. 15).

Melatonin treated mice with the high dose for two months (four months old mice):

Light microscopic examination of the epithelial lining cells of the ampulla of the oviduct shows the presence of few cilia associated with their apical surface (Fig. 12). The ultrastructural study of these cells demonstrates that their surface has long broad microvilli and a thick cilium (solitary cilium). Their cytoplasm contains Golgi complex, a lot of mitochondria and many electron lucent and electron dense secretory granules (Fig. 16).

LEGENDS FOR FIGURES

Fig. 1: A photomicrograph of transverse section in the ovary of 3 months old control mice. The cortex of the ovary shows the presence of primordial follicles (PF), primary follicles (PrF), growing follicles (GF) and corpora lutea (CL) in various stages of its formation. The medulla (m) is formed of connective tissue and many blood vessels. The ovary is surrounded by a layer of simple cubodial epithelial cells and the tunica albuginea (arrow). (Hx. & E. X 40)

Fig. 2: A photomicrograph of transverse section in the ovary of 3 months old melatonin treated mice with the low dose. The cortex of the ovary shows the presence of primordial follicles (PF), Primary follicles (Pr F), growing follicles (GF) and corpora lutea (CL). Note the relative increase in the number of growing follicles and corpora lutea in comparison with the corresponding control. (Hx. & E. X 40)

Fig. 3: A photomicrograph of transverse section in the ovary of 3 months old melatonin treated mice with the high dose. The cortex of the ovary appears to be rich with the growing follicles (GF) and corpora lutea (CL) in addition to the primordial follicles (PF)

and primary follicles (Pr F). Note the apparent increase in size of the ovary in comparison with the corresponding control. (Hx. & E. X 40)

Fig. 4: A photomicrograph of transverse section in the ovary of 4 months old control mice. The cortex of the ovary shows the presence of primordial follicles (PF), Primary follicles (Pr F), growing follicles (GF) and corpora lutea (CL). The medulla (m) is formed of connective tissue and blood vessels. (Hx. & E. X 40)

Fig. 5: A photomicrograph of transverse section in the ovary of 4 months old melatonin treated mice with the low dose. The cortex of the ovary is rich with the growing follicles (GF) and corpora lutea (CL). The cortex also shows the presence of primordial follicles (PF) and primary follicles (Pr F). (Hx. & E. X 40)

Fig. 6: A photomicrograph of transverse section in the ovary of 4 months old melatonin treated mice with the high dose. The cortex shows marked increase in the number of the growing follicles (GF) and corpora lutea (CL) in comparison with the corresponding control. The cortex contains also primordial follicles (PF) and primary follicles (Pr F). The medulla (m) has many dilated blood vessels. Note the apparent increase in the size of ovary as compared with the corresponding control. (Hx. & E. X 40)

Fig. 7: A photomicrograph of transverse section in the ovary of 7 months old control mice. The cortex of the ovary shows the presence of primordial follicles (PF), primary follicles (PrF), growing follicles (GF) and corpora lutea (CL). The medulla (m) is formed of connective tissue and many blood vessels. (Hx. & E. X 40)

Fig. 8: A photomicrograph of transverse section in the ovary of the recovered mice (7 months old). The cortex of the ovary shows the presence of primordial follicles (PF), Primary follicles (PrF), growing follicles (GF) and corpora lutea (CL). The medulla (m) shows the presence of many dilated blood vessels. Note that the size of the ovary and its general appearance is nearly similar to the corresponding control. (Hx. & E. X 40)

Fig. 9 & 10: Photomicrographs of sections in the ampulla of the oviduct in 3 & 4 months old control mice showing that the wall of the duct is formed of simple columner partially ciliated epithelium

(CE) [arrows point to cilia (C)] resting on a loose connective tissue, smooth muscle layer (M) and the serosa (S). Note that the mucous membrane is thrown into folds that project into the lumen. (Hx. & E. X 250)

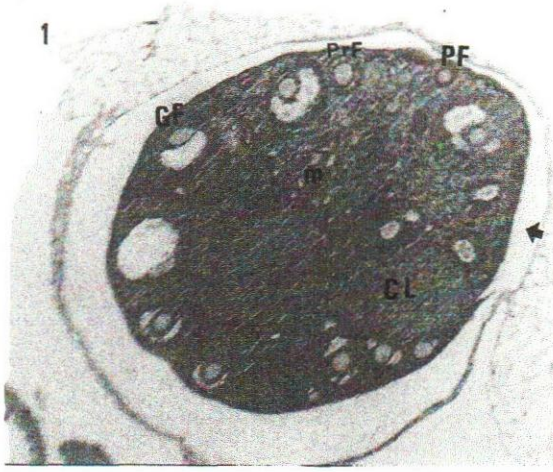
Fig. 11: A photomicrograph of a section in the ampulla of the oviduct in high dose melatonin treated animals for one month (3 months old). It shows that the mucous membrane of the ampulla have numerous microvilli (mv) and some cilia (c). (Hx. & E. X 250)

Fig. 12: A photomicrograph of a section in the ampulla of the oviduct in melatonin treated amimals with the high dose for 2 months (4 months old mice). The epithelial lining cells of the ampulla show the presence of few cilia (c) associated with their apical surface. (Hx. & E. X 250)

Fig. 13 & 14: Electron photomicrographs showing the epithelial lining cells of the ampulla of the oviduct in 3 and 4 months old control mice. The cells have ovoid nuclei (N) and their cytoplasm is rich with mitochondria (M) and shows the presence of some vesicles (V). There are both cilia (c) and microvilli (mv) associated with their apical surface. (X 5000)

Fig. 15: Electron photomicrograph showing the lining epithelium of the ampulla of the oviduct in melatonin treated animals with the high dose for one month (3 months old mice). The apical surface of the cells shows the presence of many short microvilli (mv). The cells have ovoid nuclei (N). Their cytoplasm contains mitochondria (M) and many electron dense secretory granules (thick arrow). (X 5000)

Fig. 16: Electron photomicrograph showing the lining epithelium of the ampulla of the oviduct in melatonin treated animals with the high dose for two months (4 months old mice). The apical surface of these cells shows the presence of long broad microvilli (mv) and a solitary cilium (c). Their cytoplasm contains Golgi complex (G), a lot of mitochondria (M), many electron dense secretory granules (thick arrow) and electron lucent secretory granules (thin arrow). (X 5000)


1
PrF
PF
GF
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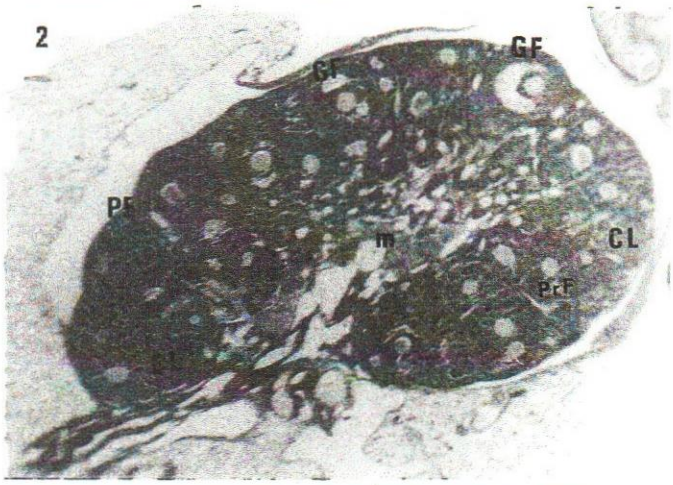
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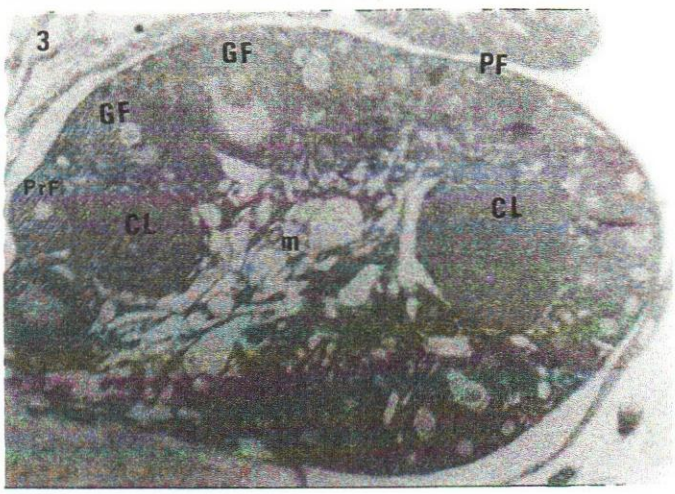
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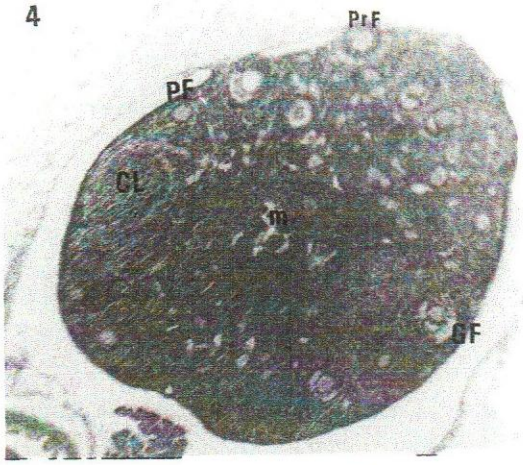
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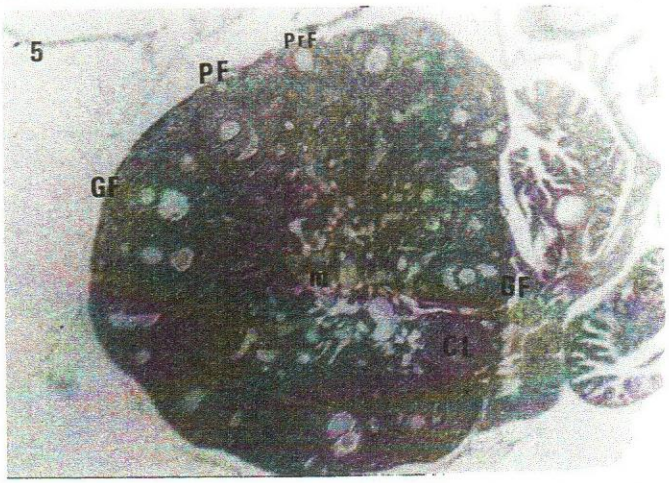
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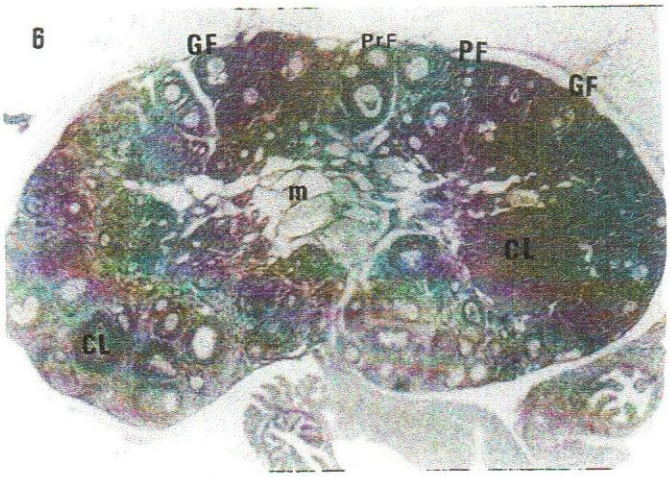
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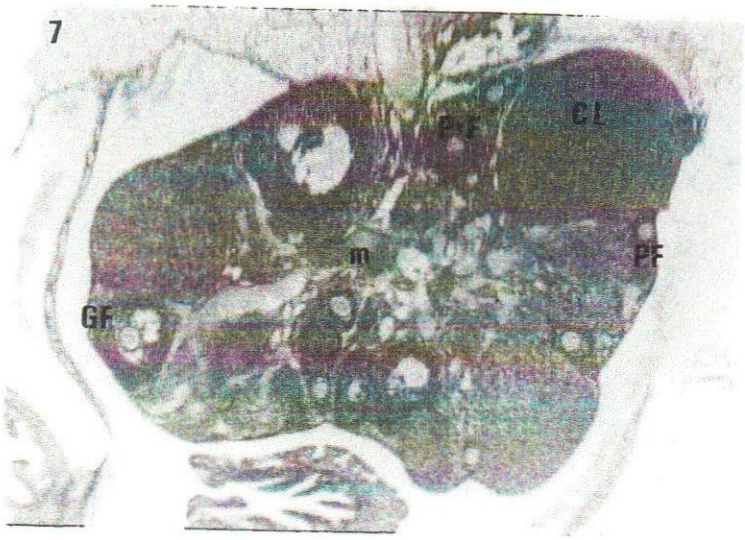
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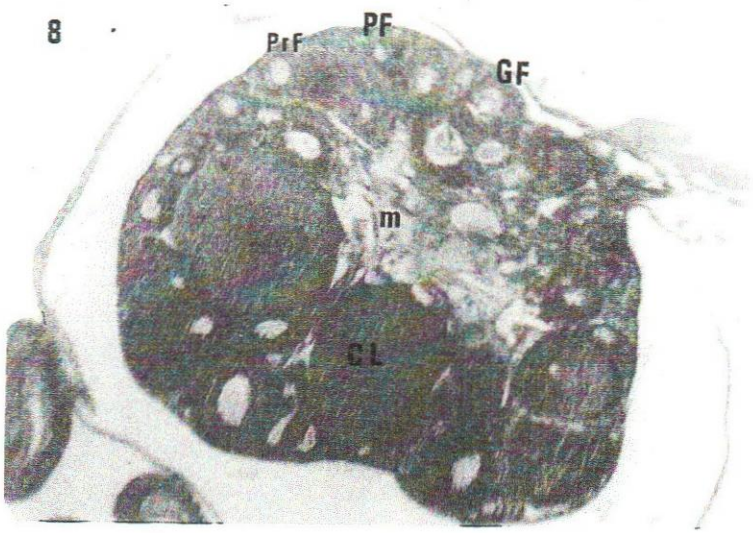
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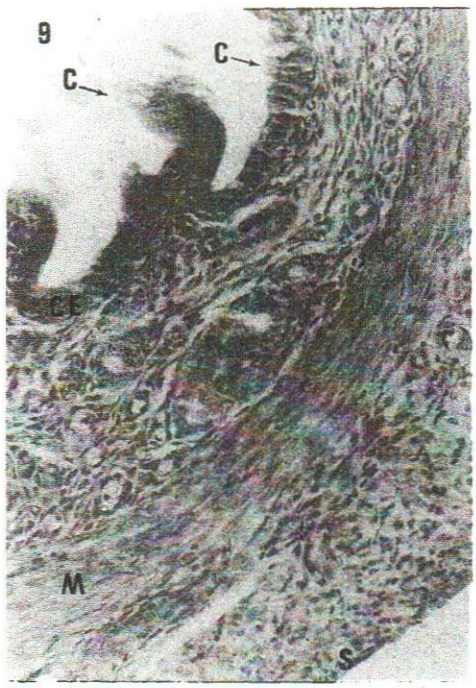
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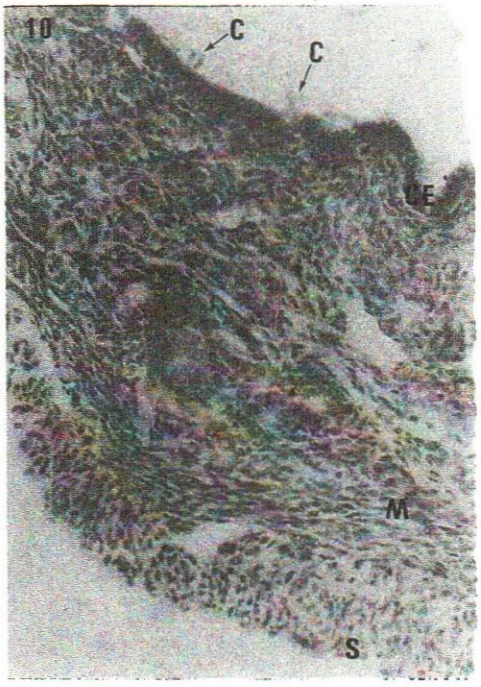
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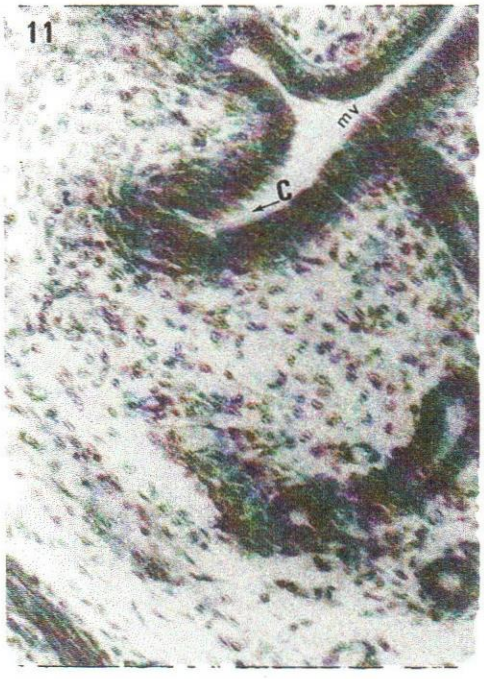
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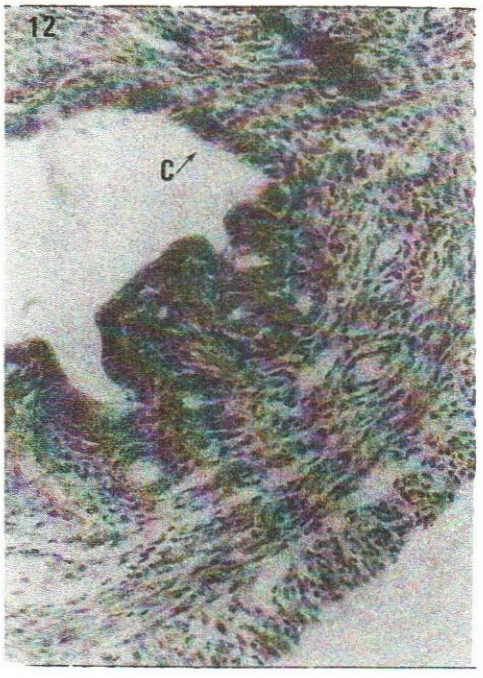
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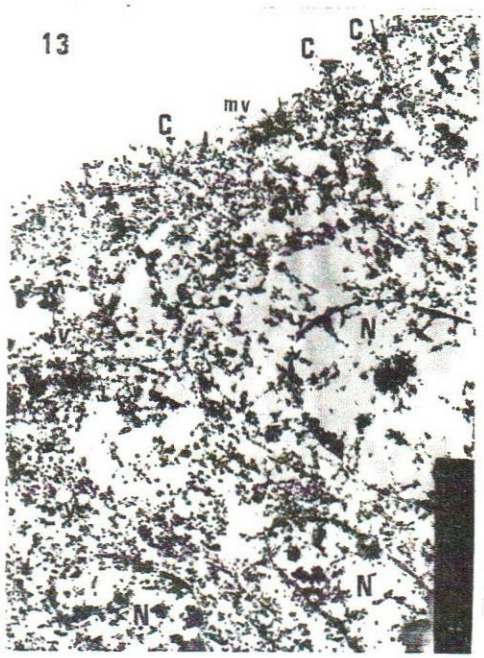
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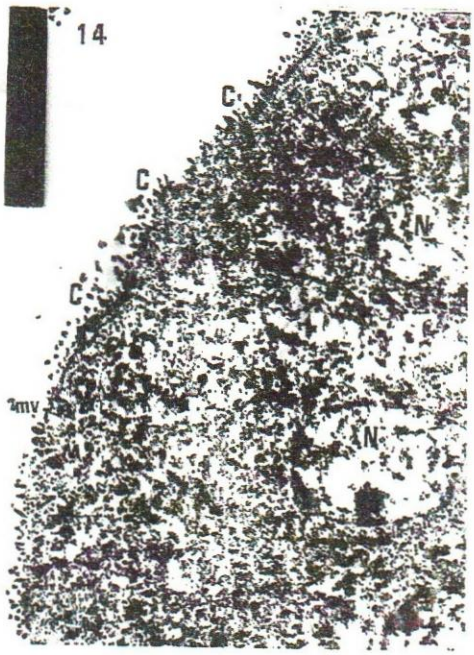
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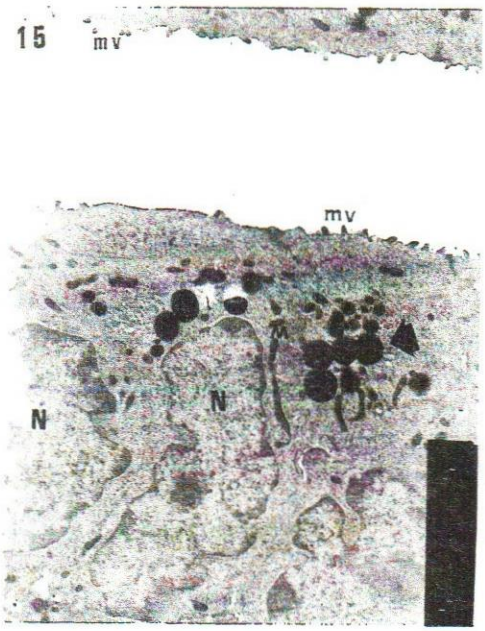
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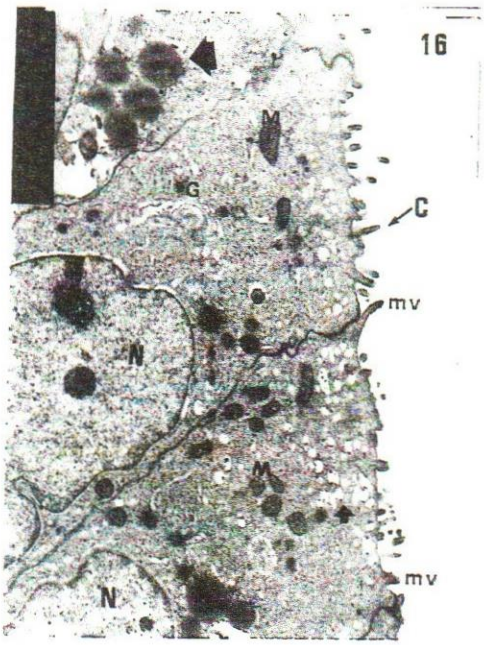
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Table (1): The mean diameter ± SD of primary follicles (PF), growing follicles (GF) and corpora lutea (CL) in the ovaries of the control mice (C) compared to that of the low dose (LD) and high dose (HD) melatonin treated mice and recovered mice(R).

		PF	GF	CL
3 Month	C	0.236 ±0.002	0.327 ±0.005	0.503 ±0.003
	LD	0.239 ±0.004	0.367 ±0.017	0.554 ±0.022
	HD	0.243 ±0.01	0.347 ±0.034	0.574 ±0.011
	P (C^LD)	0.19 ns	0.0096**	0.008**
	P (C^HD)	0.16 ns	0.19 ns	0.0002 ***
4 Month	C	0.232 ±0.016	0.334 ±0.028	0.509 ±0.006
	LD	0.250 ±0.019	0.385 ±0.0036	0.557 ±0.033
	HD	0.253 ±0.014	0.369 ±0.023	0.546 ±0.02
	P (C^LD)	0.14 ns	0.18*	0.034*
	P (C^HD)	0.08 ns	0.09ns	0.03*
7 Month	C	0.241 ±0.024	0.328 ±0.0016	0.511 ±0.008
	R	0.235 ±0.027	0.286 ±0.099	0.499 ±0.018
	P	0.39 ns	0.19 ns	0.19ns

^: i,e versus

P value	Wording	Summary
>0.05	Not significant	ns
0.01 to 0.05	Mild significant	*
0.001 to 0.01	High significant	**
<0.001	Extremely significant	***

Table (2):-The mean numerical density ± SD of primary follicles (PF), growing follicles (GF) and corpora lutea (CL) in the ovaries of the control mice (C) compared to that of the low dose (LD) and high dose(HD) melatonin treated mice and recovered mice(R).

		PF	GF	CL
3 Month	C	136.5 ±5.4	105.2 ±4.9	21.8 ±1.26
	LD	119.2 ±5.9	185.6 ±7.3	34.3 ±4.68
	HD	123.6 ±4.8	163.7 ±8.4	30.5 ±1.56
	P (C^LD)	0.01 **	<0.0001***	0.0055**
	P (C^HD)	0.018 *	0.0002***	0.0008***
4 Month	C	137.0 ±4.3	105.8 ±6.8	23.57 ±4.4
	LD	118.2 ±2.1	167.0 ±4.9	37.84 ±2.2
	HD	123.2 ±8.7	137.3 ±4.9	37.35 ±1.51
	P (C^LD)	0.001**	0.0001***	0.004**
	P (C^HD)	0.03*	0.0019**	0.003**
7 Month	C	134.0 ±2.1	136.4 ±3.6	32.3 ±1.5
	R	124.9 ±4.2	138.2 ±4.4	30.1 ±2.1
	P	0.014 *	0.3ns	0.12ns

^: i,e versus

P value	Wording	Summary
>0.05	Not significant	ns
0.01 to 0.05	Mild significant	*
0.001 to 0.01	High significant	**
<0.001	Extremely significant	***

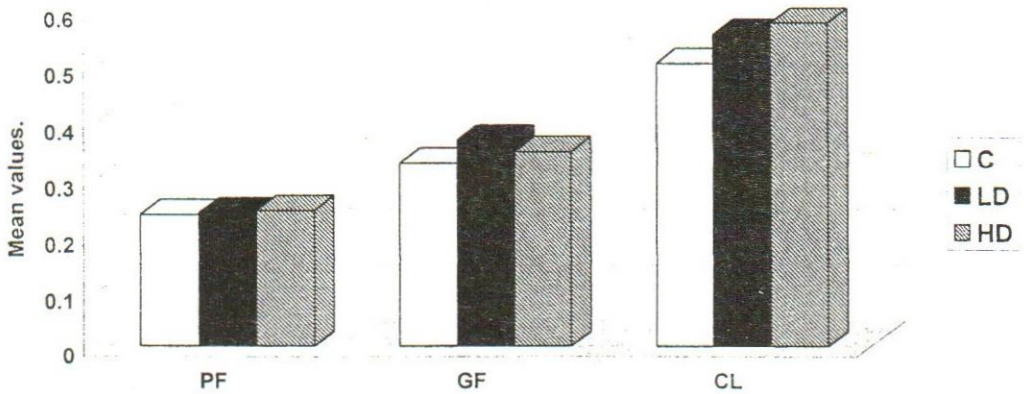

Fig. 17: Shows the relation between the mean diameter of primary follicles (PF), growing follicles (GF) and corpora lutea (CL) in the ovary of the three months old control mice (C), and that of the three months old treated mice with the low dose (LD) and high dose (HD) of melatonin.

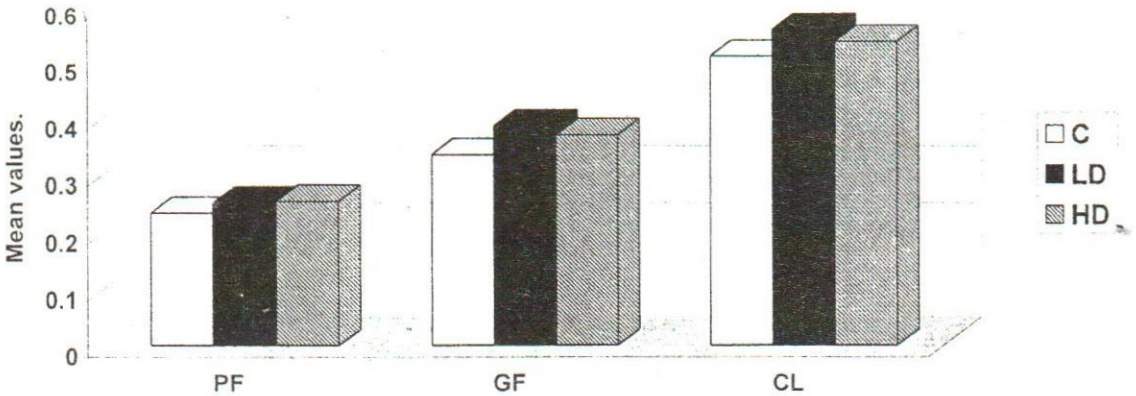

Fig. 18: Shows the relation between the mean diameter of primary follicles (PF), growing follicles (GF) and corpora lutea (CL) in the ovary of the four months old control mice (C), and that of the four months old treated mice with the low dose (LD) and high dose (HD) of melatonin.

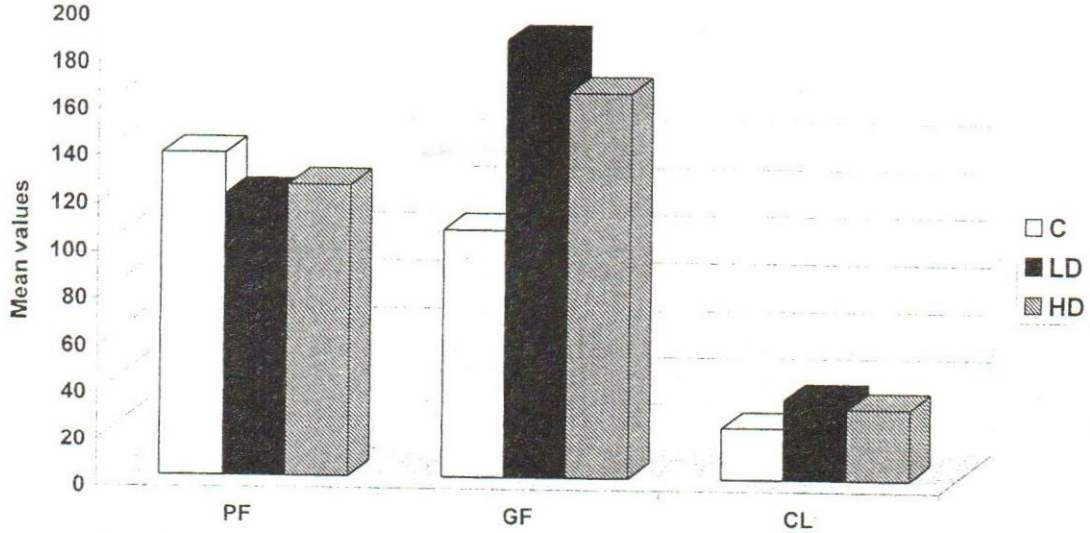

Fig. 19: Shows the relation between the mean numerical density of primary follicles (PF), growing follicles (GF) and corpora lutea (CL) in the ovary of the three months old control mice (C), and that of the three months old treated mice with the low dose (LD) and high dose (HD) of melatonin.

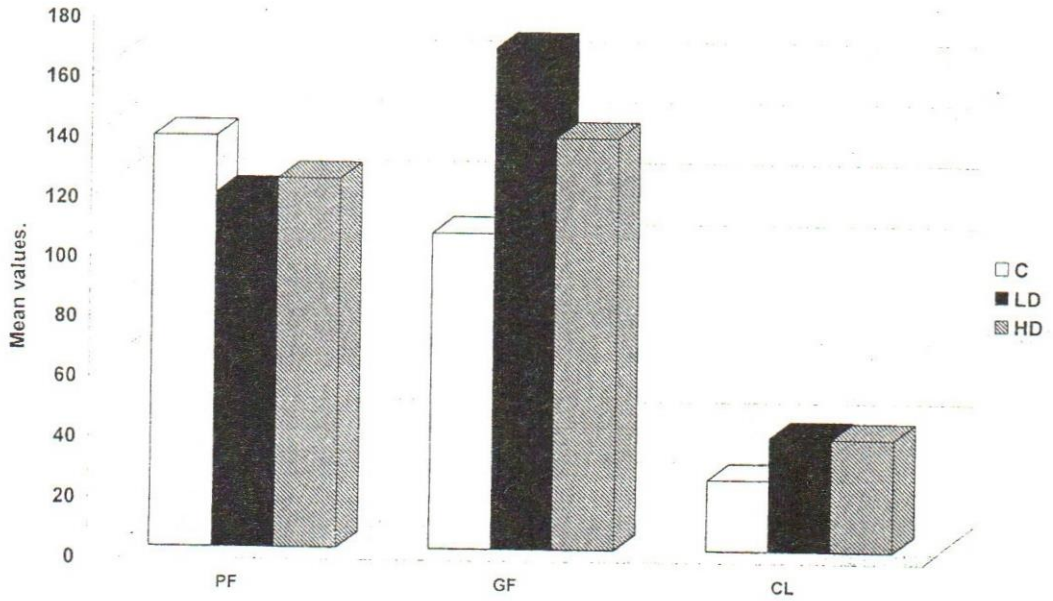

Fig. 20: Shows the relation between the mean numerical density of primary follicles (PF), growing follicles (GF) and corpora lutea (CL) in the ovary of the four months old control mice (C), and that of the four months old treated mice with the low dose (LD) and high dose (HD) of melatonin.

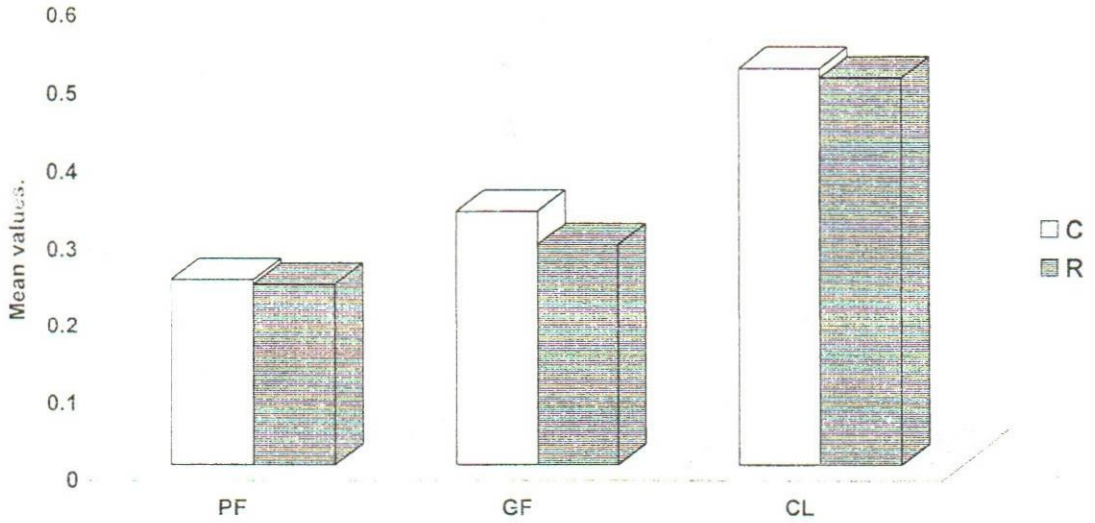

Fig. 21: Shows the relation between the mean diameter of primary follicles (PF), growing follicles (GF) and corpora lutea (CL) in the ovary of the seven months old control mice (C), and that of the recovered (R) mice.

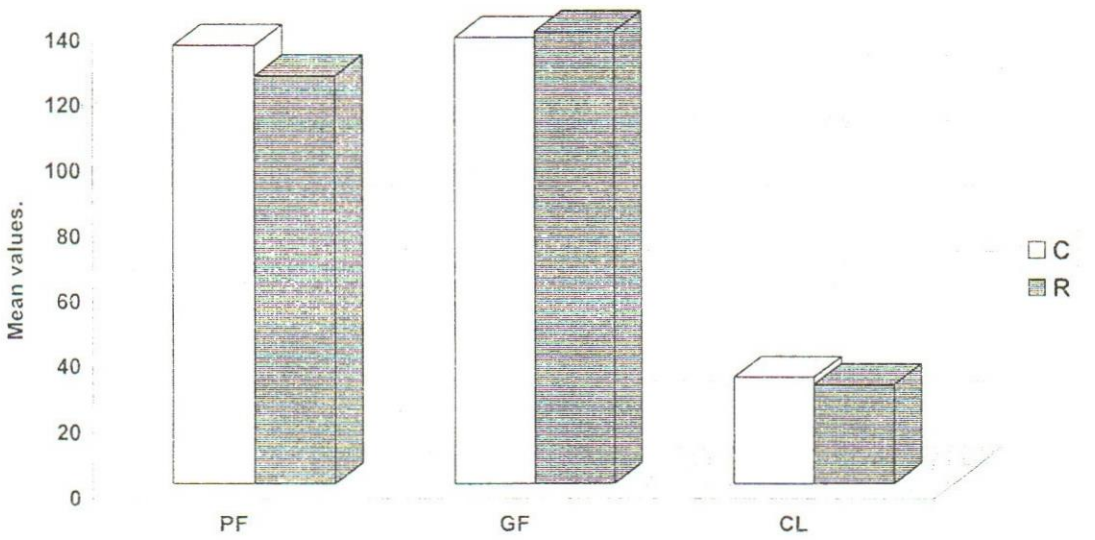

Fig. 22: Shows the relation between the mean numerical density of primary follicles (PF), growing follicles (GF) and corpora lutea (CL) in the ovary of the seven months control mice (C), and that of the recovered (R) mice.

DISCUSSION

In this work, a high dose (8.0 mg/kg) and a low dose (4.0 mg/kg) of melatonin were used. Each dose was used for a period of one and two months. Melatonin was injected to the animals subcutaneously once daily in the late afternoon. Histological study demonstrated apparent increase in the size of the ovary together with an increase in the number of growing follicles in the low dose and high dose melatonin treated animals in comparison to the control. Morphometric analysis confirmed these observations as it revealed significant increase in the mean density of the growing follicles and corpora lutea in the three and four months old treated mice with the low dose and high dose of melatonin as compared to their corresponding control. This data showed that the stimulatory effect of melatonin on the ovarian folliculogenesis in mice was independent to the dose or the duration of its administration.

These results were in agreement with the study of Sinhasane and Joshi (1997) in which they found that melatonin treatment increased ovarian weights and increased all types of growing follicles especially antral and Graafian follicles. They suggested that melatonin is involved in the growth of ovarian follicles. The present results were in consistent with Bister *et al.* (1999) who observed that the pretreatment of the ewes with melatonin reduced the atresia rate of large follicles and resulted in an increased ovulation rate. In contrast to the present results Ooi and Ng (1989) stated that treatment of female hamsters with pineal indoles resulted in a decreased number of Graafian follicles and corpora lutea and a proliferation of interstitial tissue in the ovary. However, Chan and Ng (1995) reported that exogenous melatonin may have an inhibitory, a stimulatory or no effect on the reproductive system of rodents, depending on the model system and species used. Hadley (1996) found that the time of administration of melatonin was a determing factor of its effect.

In the present work, morphometric study revealed significant reduction in the density of the primary follicles in the low dose and high dose melatonin treated animals. These findings could be attributed to the stimulatory effect of melatonin towards increasing growing follicles number. These results were in accordance with Sinhasane and Joshi (1997) who observed that melatonin and darkness reduced the number of small preantral follicles. On the contrary, Meredith *et al.* (2000) found that nighttime, but not continuous supplementation with melatonin, delayed reproductive senescence without any effect on the number of

primordial follicles. The difference might be attributed to the methedology.

Morphometric analysis in the present work showed significant increase in the mean diameter of the growing follicles and corpora lutea in three and four months old melatonin treated mice with the low dose in comparison with the control. These findings were in agreement with the hisological study of the Joshi *et al.* (1994) for the ovaries of the Indian desert gerbil. They observed elargement of follicles and corpora lutea with hypertrophied granulosa cells in melatonin treated gerbils in comparison to their control. The present study also showed that the mean diameter of the corpora lutea was significantly increased in the three and four months old melatonin treated mice with the high dose while the mean diameter of their growing follicles showed non significant increase in comparison with the control. This might be explained by the dose dependant effect of melatonin. This observation supported by the study of Witt-Enderly *et al.* (2000) in which they reproted that the number of cells in the ovary showing the altered shape is dependent on melatonin concentration.

In the present work, histological examination of the ovary in the recovered mice showed that the size and the structure of the ovary is nearly similar to the correspoding control. Morphometric analysis of the recovered animals revealed that the mean diameter and the mean density of the primary follicles, gorwing follicles and corpora lutea had no significant difference as compared with the corresponding control. This indicated the reversible effect of melatonin on the ovary. In consistent with the present results, Guerin and Matthews (1998) found that the alteration of the estrous activity in the ewe induced by their exposure to extended darkness would be reversed on their return to the natural photoperiod.

The mammalian oviduct provides the necessary environment for maturation of gametes, fertilization and embryonic development (Archibong *et al.*, 1989).

In the present work, the oviducal epithelium of the ampulla in the animals treated with the high dose of melatonin for one month showed the predominance of the secretory cells. These cells had many short microvilli. The ultrastructural study showed the presence of many electron dense secretory granules in their cytoplasm. In the animals treated with the high dose of melatonin for two months, the ultrastrucutral study of the secretory cells demonstrated that the apical surface had long broad microvilli and solitary or single cilium. Their

cytoplasm showed the presence of both electron lucent and electorn dense secretory granules together with a lot of mitochondria and Golgi complex. Similar changes in the oviductal epithelium would be described in the luteal phase of the estrous cycle in women (Verhage *et al.*, 1979), in the Chinese Meishan Pig (Abe and Oikawa, 1992) and in the rat (Hasanin *et al.*, 2001).

The results of the present work were in accordance with Rivest (1987) who found that chronic melatonin administration increases the number of patterns typical of luteal phases and reduces those of the follicular type, resulting in a decrease frequency of lutenizing hormone pulses and longer intervals between estrous cycles in the rat. The changes in the oviductal epithelium could be attributed to the highly significant increase in the density and diameter of the corpora lutea in the high dose melatonin treated animals which demonstrated by the morphometric analysis of the present work. In consistent with the present results, Brzezinski *et al.* (1992) and Durotoye *et al.* (1997) reprorted that melatonin could act directly on the corpus luteum to increse progesterone production. Its mechanism of action appears to be different from that of human chorionic gonadotrophin or luteinizing hormone.

In conclusion, this study revealed that nighttime melatonin administration had a stimulatory effect on the ovarian follicular growth. This effect was independent to the dose or the duration of its administration. It effect on the ovary was reversible. The oviductal epithelium showed predominance of secretory cells. This may provide in the furture new insights and directions for the study of its role in the physiology and pharmacology of fertility and contraception in animals and humans.

REFERENCES

Abe, H. and Oikawa, T. (1992): Examination by scanning electorn microscopy of oviductal epithelium of the Prolific Chinese Meishan pig at follicular and luteal phases. The anatomical record, 233: 399– 408.

Archibong, A.E.; Petters, R.M. and Johnson, B.H. (1989): Development of porcine embryos form one and two cell stages to blastocyts in culture medium supplemented with porcine oviductal fluid. Biol. Reprod., 41: 1076 – 1083.

Bister, J.L.; Noel, B.; Perrad, B.; Mandiki, S.N.; Mbayahaga, J. and Paquary, R. (1999): Control of ovarian follicles activity in the ewe. Domest Anim. Endocrinol., 17 (2 -3): 315 – 328.

Brzezinski, A.; Fibich, T.; Cohen, M.; Schenker, J.G. and laufer, N. (1992): Effects of melatonin on progesterone production by human granulosa lutein cells in cultrure.Fertil Steril., 58(3): 526 – 529.

Cassone, V.M.; Warren, W.S.; Brooks, D.S. and Lu, J. (1993): Melatonin, the pineal gland and circadian rhythms. Journal of Biological Rhythms, 8 (suppl) 573 – 581.

Chan, W.Y. and Ng, T.B. (1995): Effects of pineal indoles on ovarian response to gonadotropin-induced ovulation in mice. J. Neural Transm. Gen. Sect. 100: 239 – 246.

Cupp, M.J. (1997): Melatonin. Clinical pharmacology, 56(5):1421-1425.

Dardes, R.C.; Baracat, E.C. and Simoes, M.J. (2000): Modulation of estrous cycle and LH, FSH and melatonin levels by pinealectomy and sham-pinealectomy in female rats. Prog neuropsychopharmacol Biol Psychiatry, 24(3): 441 – 453.

Durotoye, L.A.; Webley, G.E. and Rodway, R.G. (1997): Stimulation of the production of progesterone by the corpus luteum of the ewe by the perfusion of melatonin in vivo and by treatment of granulosa cells with melatonin in vitro. Res. Vet. Sci., 62(2): 87 – 91.

Guerin, M.V. and Matthews, C.D. (1998): Alterations of estrous activity in the ewe by circadian-based manipulation of the endogenous pacemaker. Journal of Biological Rhythms, 13(1): 60 – 69.

Guerin, M.V.; Deed, J.R. and Matthews, C.D. (2000): The coincidence of light and melatonin with a specific phase of the circadian pacemaker is important for the timing of seasonal breeding in the ewe. J Biol Rhythms, 15(6): 514 – 523.

Hadley, M.E. (1996): Endocrine role of the pineal gland. In (Endocrinology) Fourth edition, Chapter 20, P. 458 – 476, Prentice-Hall international. USA.

Hasanin, N.A.; Sakkara, Z.A. and Hadidy, A.R. (2001): Scanning and transmission electorn microscopic study of the oviductal epithelium of female albino rat during follicular and luteal phases of the estrous cycle. Egypt. J. Anat., 24(1): 59 – 79.

Itoh, M.T.; Ishizuka, B.; Kudo, F.; Fusama, S.; Amemiya, A. and Sumi, Y. (1997): Detection of melatonin and serotonin N-acetyltransferase and hydroxyindole-o-methyltransferase activities in rat ovary. Mol cell endocrinol., 136(1): 7 – 13.

Itoh, M.T.; Ishizuka, B.; Kuribayashi, Y.; Amemiya, A. and Sumi, Y. (1999): Melatonin, its precursors, and synthesizing enzyme activities in the human ovary. Mol Hum. Reprod., 5(5): 402 – 408.

Joshi, B.N.; Manepalli, S.K. and Saibaba, P. (1994): Melatonin in the control of the estrous cycle of the Indian desert gerbil (Meriones hurrianae: Jerdon). Biol. Signals, 3(6): 288 – 295.

Meredith, S.; Jackson, K.; Dudenhoeffer, G.; Graham, L. and Epple, J. (2000): Long-term supplementation with melatonin delays repoductive senescence in rats, without an effect on number of primordial follicles. Experimental Gerontology, 35: 343 – 352.

Oai, V.E. and Ng, T.B. (1989): Hisological studies on the effects of pineal 5-methoxyindoles on the reprorductive organs of the male golden hamster.J. Pineal Res. 7: 315 – 324.

Pang, S.F.; Li, L.; Ayre, E.A.; Pang, C.S.; Yu, Z.H. and Shiu, S.Y. (1998): Neuroendocrinology of melatonin in reproduction: recent developments. J chem Neuroanat, 14 (3 – 4): 157 – 166.

Rivest, R.W. (1987): The female rat as a model describing patterns of pulsatile LH secretion during puberty and their control by melatonin. Gynecol. Endocrinol. 1(3): 279 - 293.

Sinhasane, S.V. and Joshi, B.N. (1997): Melatonin and exposure to constant light/darkness affects ovarian follicular kinetics and estrous cycle in Indian desert gerbil Meriones hurrianae. Gen Comp. Endocrinol., 108(3): 352 – 357.

Sirotkin, A.V. and Schaeffere, H.J. (1997): Direct regulation of mammalian reproductive organs by serotonin and melatonin-Journal of Endocrinology, 154: 1 – 5.

Vanecek, J.; Pavlik, A. and Illnerova, H. (1987): Hypothalamic melatonin receptor sites revealed by autoradiography. Brain Res. 435: 359 – 363.

Verhage, H.G.; Bareither, M.L.; Jaffe, R.L. and Akba, M. (1979): Cyclic changes in ciliation, secretion and cell height of the oviductal epithelium in women. Am. J. Anat., 156: 505 – 521.

Witt-Enderby, P.A.; Mackenzie, R.S.; Mckeon R.M.; Carroll, E.A.; Bordt, S.L. and Melan, M.A. (2000): Melatonin induction of filamentous structures in non-neuronal cells that is dependent on expression of the human mt 1 melatonin receptor. Cell Motility and the cytoskeleton, 46: 28 – 42.