Melatonin use in unilateral total salpingectomy in rats

E. Sapmaz[1], A. Kale[2], N. Akpolat[3]

[1]Obstetrics and Gynecology Department, Firat University School of Medicine, Elaziğ
[2]Obstetrics and Gynecology Department, Numune Educatıonal and Research Hospital, Adana
[3]Pathology Department, Firat University School of Medicine, Elaziğ (Turkey)

Summary

Objective: To investigate the effects of melatonin use in unilateral total salpingectomy on ovarian histology in rats. *Setting:* Fırat University, Medical School, Obstetrics and Gynecology Department, Elaziğ. *Material and Method:* Thirty adult, female rats of Wistar albino species with regular cycles were randomly allocated to three groups in the estrus phase. G1 (n: 10): The group where the abdomen was opened and closed, and left oophorectomy was performed six months later. G2 (n: 10): The group where left total salpingectomy was performed and followed by left oophorectomy six months later. G3 (n: 10): The group where the abdomen was opened, left total salpingectomy was performed 15 min after 10 mg/kg/IP melatonin administration, and left oophorectomy was performed six months later. Samples of the left ovary were fixed in formaldehyde. The preparations were stained with hematoxylin-eosin, and primordial, primary, secondary and tertiary follicles were counted. All the numbers were added up to determine the ovarian follicle reserve. Atretic follicles were counted. Corpus luteum and corpus albicans were counted. Number of total corpuses was calculated. Regression of the presence of angiogenesis within the corpus luteum was examined. Presence of fibrosis on the ovarian stroma was examined. An ordinal scale was formed for the presence of regression of angiogenesis within the corpus luteum and presence of fibrosis (none: 0p, present: 1p, markedly present: 2p). Follicle cysts in the ovary were counted. Kruskal Wallis variance analysis was used in the statistical analysis of data; $p < 0.05$ was considered significant. *Results:* Primordial follicle count, ovarian follicle reserve and regression of angiogenesis in the corpus luteum were found to be significantly lower ($p < 0.05$, Mann-Whitney U test), and atretic follicle count, microscopic follicle cyst and fibrosis development were found to be significantly higher in G2, when compared to G1 and G3 ($p < 0.05$, Mann-Whitney U test). None of the rats in G1 and G3 had ovarian cysts, whereas five rats in G2 were identified as having macroscopic follicle cysts. Other data were found to be similar in G1, G2, and G3 (Kruskal Wallis variance analysis). *Conclusion:* Left total salpingectomy reduces primordial follicles, ovarian follicle reserve and regression of angiogenesis in the corpus luteum, while increasing atretic follicles, microscopic ovarian cysts and fibrosis development. It leads to the development of macroscopic follicle cysts in the ovary at a high rate (50%) in the sixth month. Melatonin use eliminates these harmful effects. Melatonin can be used to avoid the unfavorable effect of total salpingectomy on the ovary.

Key words: Melatonin; Ovarian histology; Rat; Total salpingectomy.

Introduction

IVF-ET results (implantation, pregnancy rates etc.) are adversely affected in hydrosalpinx cases [1-3]. The most common method used in the treatment of hydrosalpinx cases is salpingectomy. Both retrospective and prospective studies have found that implantation and pregnancy rates increase in hydrosalpinx cases that undergo salpingectomy [4-6].

The effects of the salpingectomy procedure on the ovary are still debatable. Dar *et al*. [7] did not find any negative effect on the ovaries of cases who had laparoscopic salpingectomy due to ectopic pregnancy. Chan *et al*. [8], on the other hand, reported that salpingectomy by laparotomy did not have any negative effect on ectopic pregnancy cases, but that laparoscopic salpingectomy adversely affected the ovary.

Theoretically, salpingectomy can lead to the impairment (hypoxia and/or ischemia) of ovarian perfusion in humans. Branches of the uterine artery are located in the blood vessel network of the mesosalpinx, and are necessary to feed the ovary [9]. Moreover, uterine artery ligation (UL) in rats reduces ovarian blood flow, and the resulting hypoxia and/or ischemia impairs ovulation [10].

Ischemia and/or reperfusion injury lead to the formation of oxygen radicals (superoxide, hydroxyl, peroxyl, alkoxyl, and singlet oxygen radicals). These oxygen radicals have a destructive effect on lipids in all membranes. The most effective radical is hydroxyl [11]. Consequently, cell membrane, lisosome membranes, and membranes of such cell organelles as endoplasmic reticulum etc. are destroyed, cells break down, and necrosis results [12, 13]. This event is called lipid peroxidation.

However, lipid peroxidation stimulates collagen gene transcription in cell culture [14, 15].

Sugino noted that the oxygen radicals and antioxidant system in the ovary had a part in many events of reproductive physiology (follicle development, oocyte maturation, ovulation, C. luteum function, and follicular atresia development). Oxygen radicals in the ovary are produced by neutrophils and macrophages, and reside in C. luteum and follicles. Furthermore, it was shown that oxygen radicals (ROS) inhibited oocyte development and increased degenerated oocyte count as well as apoptosis [16].

Melatonin, a pineal secretory product, modulates ovarian function and reproduction in mammals [17].

Revised manuscript accepted for publication April 22, 2011

Table 1. — *Parameters examined in all groups (values are presented as mean ± SD).*

Parameter	G1	G2	G3	*p*
Primordial follicle (no.)	9.5 ± 2.6[1]	4.3 ± 2[2]	9.7 ± 2[1]	*
Primary follicle (no.)	8 ± 1.8	9 ± 2.6	8 ± 2	NS
Secondary follicle (no.)	1.8 ± 0.8	1.8 ± 0.9	1.8 ± 0.7	NS
Tertiary follicle (no.)	2.2 ± 0.8	2.2 ± 0.8	1.9 ± 0.6	NS
Ovarian follicle reserve (no.)	21 ± 2.6[1]	17 ± 3.3[2]	21 ± 3.4[1]	*
Corpus luteum (no.)	6.4 ± 1.41	3.7 ± 1.72	6.6 ± 1.31	*
Corpus albicans (no.)	0 ± 0	0 ± 0	0 ± 0	NS
Total	6.4 ± 1.4[1]	3.7 ± 1.7[2]	6.6 ± 1.3[1]	*
Atretic follicle (no.)	0.1 ± 0.3[2]	1.8 ± 0.8[1]	0.2 ± 0.4[2]	*
Corpus luteum angiogenesis (point)	2 ± 0[1]	0.9 ± 0.3[2]	1.9 ± 0.3[1]	*
Stromal fibrosis (point)	0 ± 0[2]	1.9 ± 0.3[1]	0 ± 0[2]	*
Cystic follicle (microscopic)	0 ± 0[2]	1.3 ± 1.4[1]	0.2 ± 0.6[2]	*

NS = $p > 0.05$, Kruskal Wallis variance analysis.
* = $p < 0.05$, Kruskal Wallis variance analysis.
Different superscripted letters over the means show that the difference among groups was significant ($p < 0.05$ Mann-Whitney U test). Means were placed in descending order in the numbering process.

Melatonin is present in human pre-ovulatory follicular fluid at concentrations 3-fold higher than in peripheral serum [18].

The ampullar ends of mammalian fallopian tubes, where fertilization occurs, are bathed by follicular fluid. Thus melatonin in follicular fluid may play a physiological role in fertilization and early embryo development [19]. Ishizuka *et al*. found that melatonin supports fertilization and early embryo development after in vitro fertilization. That is because of the fact that melatonin is a reactive oxygen species (ROS) scavenger [20].

Takasaki *et al*. [21] used oral melatonin in infertile women with poor quality oocytes and found a high intrafollicular melatonin amount and a low lipid peroxide amount. Melatonin use reduced degenerated oocyte count, and increased fertilized oocyte count.

Melatonin is effective on hydroxyl radicals, singlet oxygen, peroxyl radical, and superoxide anion, among oxygen radicals. It protects nucleus DNA, membrane lipids, and cytosolic proteins against oxidative stress [22]. Moreover, it supports SOD, GSH-Px, glutathione reductase, and glyose-6-phosphate dehydrogenase of the antioxidant system [23]. It has an inhibitor effect on nitric oxide synthetase [24]. Besides, melatonin is easily absorbed, and rapidly passes through the morphophysiological barriers (blood-brain barrier, placenta, etc.), by whichever route it is administered. It protects the cells of the organ it penetrates against oxidative stress. Furthermore, it has a protective effect on mitochondria, which is a cell organelle [25].

A PubMed search (melatonin, salpingectomy, rat) did not show any experimental study on this topic. Thus, we attempted to examine the effects of melatonin use in unilateral total salpingectomy on ovarian histology in rats.

Materials and Methods

This study was conducted in the experimental animal laboratory of Fırat University Medical School. Thirty 14-week-old adult female rats of Wistar albino species, weighing 190-220 g and with regular cycles, were kept in a room with a 12-hour light (08-22) and 12-hour dark photoperiod, at 21-23°C fixed temperature, and fed with standard pellet feed and tap water. Permission of the Ethics Committee of Fırat University Medical School was given for the study. Feeding was interrupted 18 hours before the experiment, and only water was allowed. The rats that were found to be in the estrus phase by vaginal cytology follow-up were administered 400 mg/kg/IP chloral hydrate to induce anesthesia. The animals were laid on the operation table on their backs. The abdomen was opened with a midline incision. The rats were randomly allocated to three groups.

G1 (n:10): The group where the abdomen was opened and closed.

G2 (n:10): The group where the abdomen was opened, and left total salpingectomy was performed.

G3 (n:10): The group where the abdomen was opened, and left total salpingectomy was performed 15 min after 10 mg/kg/IP melatonin (melatonin 1 g flacon, N-acetyl-5-methoxytryptamine; Sigma Chemicals Co.) administration.

Layers of the abdomen and skin were closed with 3/0 silk suture. The rats were monitored throughout the study with blood pressure, heartbeat and body temperature measurements. They were kept in different cages in groups of five. On the postoperative 180th day, the animals were anesthetized in the same way. The abdomens were opened, and the ovaries were taken out. Ovarian tissue was fixed in 10% formaldehyde for histological examination, and placed in paraffin blocks, from which 4 µm cross sections were prepared. The cross sections were stained with hematoxylin-eosin. Primordial, primary, secondary and tertiary follicles were counted in the preparations examined under light microscopy. Total follicle reserve was calculated by the sum of all [26]. An atretic follicle count was made. Corpus luteum and corpus albicans were counted, and the total number of corpuses was calculated. Regression of angiogenesis in corpus luteum was examined. Presence of fibrosis on the ovarian stroma was examined. An ordinal scale was formed for regression of angiogenesis in the corpus luteum and presence of fibrosis (none = 0p, present = 1p, markedly present = 2p). Follicle cysts in the ovary were counted.

SPSS 9.0 computer software was used for the statistical analysis. Kruskal Wallis variance analysis was employed in the statistical analysis of continuous and ordinal data. Bonferroni correction Mann-Whitney U test was carried out for parameters for which the level of significance was set at $p < 0.05$.

Results

The experiment was successful in all rats.

Comparison between G1 and G3 showed that all values were similar (Kruskal Wallis variance analysis).

Comparison of G2 with G1 and G3 revealed that primordial follicle count, ovarian follicle reserve, corpus luteum count, and regression of angiogenesis in the corpus luteum were significantly lower in G2 ($p < 0.05$, Mann-Whitney U test), and atretic follicle count, fibrosis, and microscopic follicle cysts were found to be significantly higher ($p < 0.05$, Mann-Whitney U test). There were no macroscopic follicle cysts in G1 and G3, whereas macroscopic follicle cysts were found in five rats (50%) in G2 ($p < 0.05$, X^2 test).

Ovarian follicle cysts that are formed due to total salpingectomy lead to a decrease in ovarian follicle reserve elements and corpus luteum count, while increasing fibrosis. In other words, cystic degeneration occurs in the ovary.

Discussion

Left total salpingectomy performed on rats by the laparotomy method reduces primordial follicles, ovarian follicle reserve and regression of angiogenesis in the corpus luteum, while increasing atretic follicles, microscopic follicle cysts and fibrosis development in the sixth month. Moreover, it leads to macroscopic cyst development in the ovary (G1 = G3 = 0%, G2 = 50%). These cysts have a negative effect on ovarian follicle reserve and the corpus luteum, and a positive effect on fibrosis and atretic follicle development. Melatonin use restores these harmful effects. Melatonin can be used to avoid the negative effects of total salpingectomy on the ovary.

According to a Pub-Med search (melatonin, rat, total salpingectomy), our study is the first of its kind, and thus original in this respect.

Primordial follicles in the left total salpingectomy group were significantly reduced.

That is because oocytes are surrounded by cumulus cells in follicles. It has been reported that cumulus cells have a close connection with oocytes during maturation. Strong immunostaining of Cu/Zn-SOD was found in cumulus cells [27]. Interestingly, there is a report showing that cumulus cells protect oocytes against oxidative stress by increasing the glutathione content, an antioxidant, in the oocytes in gilts [28]. Primordial follicle does not have cumulus cells. Therefore, it is the most susceptible follicle to lipid peroxidation.

However, Suzuki et al. reported that Cu, Zn-SOD was detected in theca interna cells of preantral, non dominant and dominant follicles, and in granulosa cells of only dominant follicles in human [29]. In addition, Mn-SOD expression is detected in both granulosa cells and theca interna cells in human follicles [29]. Cu, Zn-SOD and Mn-SOD protect granulosa cells and theca interna cells by scavenging superoxide radicals for normal steroidogenesis and follicular development. Primordial follicles do not have theca interna and granulosa cells. Therefore, they are the most susceptible follicles to lipid peroxidation. Granulosa and theca cells emerge in the primary follicle, and cumulus cells in the secondary follicle. Thus, primordial follicles were found significantly lower, and primary and tertiary follicles were found similar in G2. Our results are consistent.

An increase was established in macroscopic and microscopic follicle cysts in our left total salpingectomy cases in the sixth month. The ovarian cysts identified macroscopically in G2 were quite large, and were filled with a yellow fluid. Microscopic examination showed small follicle cysts. Ovarian structures (ovarian follicle reserve, corpus luteum) were atrophied, and fibrosis and atretic follicle count were significantly higher. As the number of macroscopic cysts increased, a decrease was found in ovarian follicle reserve and corpus luteum count, with an increase in fibrosis and atretic follicle count. This may be proof that the follicle cysts that develop in the ovary after salpingectomy adversely affect ovarian reserve. This event, called cystic degeneration [30], is consistent with our results.

Anderson *et al*. [31] found that there were luteolytic agents in the uterus (which affect mitochondria and lisosomes in the luteal cells, and ensure regression of the corpus luteum), and that regression in the corpus luteum was delayed when these substances could not be transported to the ovary due to the impairment of blood flow. Mean corpus luteum value in G2 was lower than that in G1 and G3. There seems to be a contradiction in the corpus luteum results of our study. Corpus luteum that is expected to increase in G2 was found lower, relative to G1 and G3. This may be explained by the fact that the corpus luteum structure in the ovaries of rats with macroscopic follicle cysts in G2 was found lower, since in the presence of an ovarian cyst, cystic degeneration is seen in the ovary. Our results are consistent with those of Tenney *et al*. [30]. The highest corpus luteum value was found in G3, which had fewer ovarian cysts. Our results are also consistent with those of Anderson *et al*. [31].

It was found that changes associated with angiogenesis in the corpus luteum did not regress in G2. In a normal rat ovary, capillary structures that are formed in the corpus luteum regress. Vascular endothelial growth factor (VEGF) plays the largest part in the formation of these structures in the corpus luteum. One of the major stimulants of VEGF is hypoxia [32, 33]. As we impair the blood flow in the utero-ovarian anastomosis during left total salpingectomy, hypoxia results [9, 10], which in turn possibly leads to an increase in angiogenesis in the corpus luteum, and a decrease in the regression of angiogenesis, through VEGF; this is how regression in G2 was lower than in G1 and G3. This may be attributed to hypoxia in the acute phase. The effect of hypoxia may relatively decrease because of anastomoses that develop in the long-term, as the effect of ovarian artery ligation (OL) on ovulation increases in time, whereas the effect of uterine artery ligation (UL) decreases in time [10]. This event may lead to damage like the one in ischemia-reperfusion. Melatonin curtails neutrophil infiltration and tissue destructive effects of neutrophils during ischemia-reperfusion, and particularly in reperfusion [34]. The fact that the damage in G3 was less than the damage in G2 can be attributed to this effect of melatonin. Our results are consistent.

Increase in VEGF secretion under hypoxic conditions has been shown in both human ovaries and fallopian tubes. VEGF increases vascular permeability, and regulates lumen secretion in the fallopian tube. The increase in vascular permeability in the ovary causes fluid formation in ovarian cysts [32, 33, 35]. VEGF secreted in the hypoxic environment may be responsible for the follicle cysts found in G2 and G3. Especially, the higher number of follicle cysts in G2 indicates that hypoxia is more effective in this group. Melatonin reduces this hypoxic effect through various mechanisms [22-25, 34]. The lower cystic development in the melatonin group can be explained by melatonin reducing the hypoxic effect. Our results are consistent.

Hypoxia-induced factor-1 (HIF-1) is activated in both the ovary and other organs in case of acute or chronic

hypoxia [36-38]. HIF-1 alpha and hypoxic environment bring about regression and apoptosis in follicles, and result in an increase in atretic follicles and a decrease in follicular reserve [36]. The increase in atretic follicles and fibrosis, observed in G2, may be associated with the apoptotic effect of chronic hypoxia [38]. Our results are consistent. Melatonin has a favorable effect on microvascular perfusion, as it has a supportive effect on endothelium [39]. Restoration of microvascular perfusion will reduce the effect of hypoxia (HIF-1 alpha, VEGF). This may be one of the reasons why there is less damage in the melatonin group. Our results are consistent.

HIF-1 alpha also increases VEGF secretion. VEGF helps angiogenesis, increase in vascular permeability, normal functioning of folliculogenesis in the ovaries, development of follicle cysts in the ovary, and in the long-term, development of fibrosis via fibroblast growth factor-2 from the third week on [32, 33, 35, 40]. Moreover, VEGF directly stimulates collagen synthesis [41]. The increase in fibrosis and follicle cysts in G2 may be explained by VGEF.

Ovarian fibrosis and atretic follicle count were found low in G1 and G3, but significantly high in G2. Ovarian stroma contains collagen, contractile and interstitial cells [42]. In case of blood or lymphatic circulation impairment, collagen neoformation is stimulated [26]. Uterine and tubal lymphatics are very close to each other on the broad ligament [43]. Lymphatic circulation may be damaged during left total salpingectomy, which may cause an increase in collagen formation. Our findings are consistent with G2, but contradict G3. This suggests that other factors may be influencing fibrosis in the ovary. Actually, VEGF, the secretion of which increases under hypoxic conditions, boosts collagen synthesis both directly and through fibroblast growth factor 2 [40, 41]. The increase in fibrosis may be a mechanism compensating the decrease in ovarian follicle reserve [26]. Mean ovarian follicle reserves in G1 and G3 alike were higher than that in G2. Our results are consistent.

These data may explain why fibrosis was lower in the melatonin group. Our results are consistent.

Melatonin may reduce fibroblast proliferation and collagen synthesis. Increased collagen levels in both intact skin and wounds have been observed following pinealectomy, whereas exogeneous application of melatonin has caused the opposite effect [44]. Fibrosis caused by lipid peroxidation and its products decreases after the administration of antioxidants (melatonin, Vit E) in animal models [45-47]. That is why the melatonin group has no fibrosis. The protective action of melatonin may be related with its antioxidant activity.

Conclusion

Left total salpingectomy reduces primordial follicles, ovarian follicle reserve and regression of angiogenesis in the corpus luteum, while increasing atretic follicles, microscopic ovarian cysts and fibrosis development. It causes a high rate (50%) of macroscopic follicle cyst development in the ovary in the sixth month. Melatonin use corrects these harmful effects. Melatonin can be used to avoid the adverse effect of total salpingectomy on the ovary.

References

[1] Camus E., Poncelet C., Goffinet F., Wainer B., Merlet F., Nisand I. *et al*.: "Pregnancy rates after in vitro fertilization in cases of tubal infertility with and without hydrosalpinx: a meta-analysis of published comparative studies". *Hum. Reprod*., 1999, *14,* 1243.

[2] Vandromme J., Chasse E., Lejeune B., Rysselborge M.V., Delvigne A., Leroy F.: "Hydrosalpinges in in-vitro fertilization: an unfavorable prognostic feature". *Hum. Reprod*., 1995, *10*, 579.

[3] Nackley A.C., Muasher S.J.: "The significance of hydrosalpinx in in vitro fertilization". *Fertil. Steril*., 1998, *69,* 373.

[4] Strandell A., Lindhard A., Waldenstrom U., Thorburn J., Janson P.O., Hamberger L.: "Hydrosalpinx and IVF outcome: a prospective randomized multicentre trial in Scandinavia on salpingectomy prior to IVF". *Hum. Reprod*., 1999, *14*, 2762.

[5] Murray D.L., Sagoskin A.W., Widra E.A., Levy M.J.: "The adverse effect of hydrosalpinges on in vitro fertilization pregnancy rate and the benefit of surgical correction". *Fertil. Steril*., 1998, *69* 41.

[6] Bredkjaer H.E., Ziebe S., Hamid B., Zhou Y., Loft A., Lindhard A. *et al*.: "Delivery rates after in-vitro fertilization following bilateral salpingectomy due to hydrosalpinges: a case control study". *Hum. Reprod*., 1999, *14*, 101.

[7] Dar P., Sachs G.S., Strassburger D., Bukovsky I., Arieli S.: "Ovarian function before and after salpingectomy in artificial reproductive technology patients". *Hum. Reprod*., 2000, *15,* 142.

[8] Chan C.C., Ng E.H., Li C.F., Ho P.C.: "Impaired ovarian blood flow and reduced antral follicle count following laparoscopic salpingectomy for ectopic pregnancy". *Hum. Reprod*., 2003, *18,* 2175.

[9] San Filippo J.S., Lincoln S.R.: "Surgical treatment of diseases of the ovary". In: Keye W.R., Chang R.J., Rebar R.W., Soules M.R. (eds.). Infertility: Evaluation and Treatment. Philadelphia, WB Saunders, 539.

[10] Zackrisson U., Mikuni U., Peterson M.C., Nilsson B., Janson P., Brannstrom M.: "Evidence for the involvement of blood flow-related mechanisms in the ovulatory process of the rat". *Hum. Reprod*., 2000, *15*, 264.

[11] Richard M.J., Arnaud J., Jurkovitz C., Hachache T., Meftahi H., Laporte F. *et al*.: "Trace elements and lipid peroxidation abnormalities in patients with chronic renal failure". *Nephron*., 1991, *57,* 10.

[12] Welbourn C.R., Goldman G., Paterson I.S., Valeri C.R., Shepro D., Hechtman H.B.: "Pathophysiology of ischaemia reperfusion injury: central role of the neutrophil". *Br. J. Surg*., 1991, *78,* 651.

[13] Grace P.A.: "Ischaemia-reperfusion injury". *Br. J. Surg*., 1994, *81,* 637.

[14] Bedossa P., Houglum K., Trautwein C., Holstege A., Chojkier M.: "Stimulation of collagen alpha 1(I) gene expression is associated with lipid peroxidation in hepatocellular injury: a link to tissue fibrosis?". *Hepatology*, 1994, *19,* 1262.

[15] Lee K.S., Buck M., Houglum K., Chojkier M.: "Activation of hepatic stellate cells by TGF alpha and collagen type I is mediated by oxidative stress through c-myb expression". *J. Clin. Invest*., 1995, *96*, 2461.

[16] Sugino N.: "Reactive oxygen species in ovarian physiology". *Reprod. Med. Biol*., 2005, *4,* 31.

[17] Wurtman R.J., Axelrod J., Chu E.W.: "Melatonin, a pineal substance: effect on the rat ovary". *Science,* 1963, *141*, 277.

[18] Yie S.M., Brown G.M., Liu G.Y., Collins J.A., Daya S., Hughes E.G. *et al*.: "Melatonin and steroids in human pre-ovulatory follicular fluid: seasonal variations and granulosa cell steroid production". *Hum. Reprod*., 1995, *10,* 50.

[19] Odor D.L., Blandau R.J.: "EGG transport over the fimbrial surface of the rabbit oviduct under experimental conditions". *Fertil. Steril*., 1973, *24,* 292.

[20] Ishizuka B., Kuribayashi Y., Murai K., Amemiya A., Itoh M.T.: "The effect of melatonin on in vitro fertilization and embryo development in mice". *J. Pineal Res*., 2000, *28,* 48.

[21] Takasaki A., Nakamura Y., Tamura H., Shimamura K., Morioka H.: "Melatonin as a new drug for improving oocyte quality". *Reprod. Med. Biol.,* 2003, *2,* 139.
[22] Reiter R., Tang L., Garcia J.J., Munoz-Hoyos A.: "Pharmacological actions of melatonin in oxygen radical pathophysiology". *Life Sci.,* 1997, *60,* 2255.
[23] Pablos M.I., Reiter R.J., Ortiz G.G., Guerrero J.M., Agapito M.T., Chuang J.I., Sewerynek E.: "Rhythms of glutathione peroxidase and glutathione reductase in brain of chick and their inhibition by light". *Neurochem. Int.,* 1998, *32*, 69.
[24] Bettahi I., Pozo D., Osuna C., Reiter R.J., Acuna-Castroviejo D., Guerrero J.M.: "Melatonin reduces nitric oxide synthase activity in rat hypothalamus". *J. Pineal. Res.,* 1996, *20*, 205.
[25] Reiter R.J., Tan D.X., Qi W., Manchester L.C., Karbownik M., Calvo J.R.: "Pharmacology and physiology of melatonin in the reduction of oxidative stress in vivo". *Biol. Signals Recept.,* 2000, *9*, 160.
[26] Souza A.Z., Fonseca A.M., Izzo V.M., Clauzet R.M., Salvatore C.A.: "Ovarian histology and function after total abdominal hysterectomy". *Obstet. Gynecol*., 1986, *68,* 847.
[27] Tamate K., Ishikawa M., Sengoku K., Abe M., Nakata T., Shimizu T., Taniguchi N.: "Role of oxygen radical and superoxide dismutase in ovulation in the rat". *Nippon Sanka Fujinka Gakkai Zasshi*. 1992, *44,* 1.
[28] Tatemoto H., Sakurai N., Muto N.: "Protection of porcine oocytes against apoptotic cell death caused by oxidative stress during In vitro maturation: role of cumulus cells". *Biol. Reprod.,* 2000, *63,* 805.
[29] Suzuki T., Sugino N., Fukaya T., Sugiyama S., Uda T., Takaya R. *et al*.: "Superoxide dismutase in normal cycling human ovaries: immunohistochemical localization and characterization". *Fertil. Steril*., 1999, *72,* 720.
[30] Tenney B., Parker F., Robbins S.L.: "The effect of hysterectomy on ovarian function in the rabbit". *Am. J. Obstet. Gynecol.,* 1955, *70,* 889.
[31] Anderson L.L., Bland K.P., Melampy R.M.: "Comparative aspectes of uterine luteal relationship". *Recent. Progr. Horm. Res.,* 1969, *25*, 57.
[32] Abulafia O., Sherer D.M.: "Angiogenesis of the ovary". *Am. J. Obstet. Gynecol*., 2000, *182,* 240.
[33] Geva E., Jaffe R.B.: "Role of vascular endothelial growth factor in ovarian physiology and pathology". *Fertil. Steril.,* 2000, *74*, 429.
[34] Guerrero J.M., Reiter R.J., Ortiz G.G., Pablos M.I., Sewerynek E., Chuang J.I.: "Melatonin prevents increases in neural nitric oxide and cyclic GMP production after transient brain ischemia and reperfusion in the Mongolian gerbil (Meriones unguiculatus)". *J. Pineal Res*., 1997, *23*, 24.
[35] Gordon J.D., Mesiano S., Zaloudek C.J., Jaffe R.B.: "Vascular endothelial growth factor localization in human ovary and fallopian tubes: possible role in reproductive function and ovarian cyst formation". *J. Clin. Endocrinol. Metab*., 1996, *81*, 353.
[36] Ferrara N., Frantz G., LeCouter J., Dillard-Telm L., Pham T., Draksharapu A. *et al*.: "Differential expression of the angiogenic factor genes vascular endothelial growth factor (VEGF) and endocrine gland-derived VEGF in normal and polycystic human ovaries". *Am. J. Pathol.,* 2003, *162,* 1881.
[37] Hejmadi M.V., Dajas-Bailador F., Barns S.M., Jones B., Wonnacott S.: "Neuroprotection by nicotine against hypoxia-induced apoptosis in cortical cultures involves activation of multiple nicotinic acetylcholine receptor subtypes". *Mol. Cell. Neurosci.,* 2003, *24,* 779.
[38] Tanaka T., Hanafusa N., Ingelfinger J.R., Ohse T., Fujita T., Nangaku M.: "Hypoxia induces apoptosis in SV40-immortalized rat proximal tubular cells through the mitochondrial pathways, devoid of HIF1-mediated upregulation of Bax". *Biochem. Biophys Res Commun.,* 2003, *309,* 222.
[39] Bertuglia S., Marchiafava P.L., Colantuoni A.: "Melatonin prevents ischemia reperfusion injury in hamster cheek pouch microcirculation". *Cardiovasc. Res.,* 1996, *31,* 947.
[40] Rosmorduc O., Wendum D., Corpechot C., Galy B., Sebbagh N., Raleigh J. *et al*.: "Hepatocellular hypoxia-induced vascular endothelial growth factor expression and angiogenesis in experimental biliary cirrhosis". *Am. J. Pathol*., 1999, *155,* 1065.
[41] Corpechot C., Barbu V., Wendum D., Kinnman N., Rey C., Poupon R. *et al*.: "Hypoxia induced VEGF and collagen I expressions are associated with angiogenesis and fibrogenesis in experimental cirrhosis". *Hepatology,* 2002, *35,* 1010.
[42] Ross G.T., Schreiber J.R.: "The ovary, Reproduction Endocrinology". Edited by SSC Yen, RB Jaffe. Philadelphia, Saunders, 1979, 63.
[43] Barber H.R.K. (ed.): "Anatomy, Embriology, and Comparative Anatomy. Ovarian Carcinoma: Etiology, Diagnosis and Treatment". New York, Masson, 1978, 13.
[44] Drobnik J., Dabrowski R.: "Melatonin suppresses the pinealectomy-induced elevation of collagen content in a wound". Cytobios., 1996, *85,* 51.
[45] Poli G., Parola M.: "Oxidative damage and fibrogenesis". *Free Radic Biol Med.,* 1997, *22,* 287.
[46] Svegliati Baroni G., D'Ambrosio L., Ferretti G., Casini A., Di Sario A., Salzano R. *et al*.: "Fibrogenic effect of oxidative stress on rat hepatic stellate cells". *Hepatology,* 1998, *27,* 720.
[47] Tahan V., Ozaras R., Canbakan B., Uzun H., Aydin S., Yildirim B. *et al*.: "Melatonin reduces dimethylnitrosamine-induced liver fibrosis in rats". *J. Pineal. Res*., 2004, *37,* 78.

Address reprint requests to:
A. KALE, M.D.
Yeni baraj mah 68070
sok no: 11/4
Seyhan/Adana (Turkey)
e-mail: ahmetkale5@yahoo.com