Examination of the effect of melatonin use in Pomeroy method of tubal ligation on ovarian histology in rats

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Summary

Objective: To examine the effects of melatonin use in the unilateral Pomeroy method of tubal ligation on ovarian histology in rats. *Setting:* Fırat University Medical School, Obstetrics and Gynecology Department, Elazığ. *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 abdomen was opened and closed. G2 (n: 10): The group where the abdomen was opened, and the Pomeroy method of tubal ligation was performed. G3 (n: 10): The group where the abdomen was opened, and Pomeroy method of tubal ligation was performed 15 min after 10 mg/kg/ip melatonin administration. Abdomens of all rats were opened six months later and left oophorectomy was performed. 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. An atretic follicle count was made. The corpus luteum and corpus albicans were counted, and the number of total corpuses were calculated. Regression of angiogenesis within the corpus luteum was examined. Presence of fibrosis on ovarian stroma was examined. An ordinal scale was formed for the regression of angiogenesis within the corpus luteum and presence of fibrosis (none: 0p, present: 1p, markedly present: 2). Follicle cysts in the ovary were counted. Kruskal Wallis variance analysis was used in the statistical analysis of data; $p < 0.05$ were considered significant. *Results:* The comparison between G1 and G3 showed that all values were similar ($p > 0.05$, Kruskal Wallis variance analysis). When G2 was compared with G1 and G3, regression of angiogenesis in the corpus luteum was found to be significantly lower ($p < 0.05$, Mann Whitney U test), while atretic follicle count and fibrosis were significantly higher in G2 ($p < 0.05$, Mann Whitney U test). *Conclusion:* The Pomeroy method of tubal ligation reduces regression of angiogenesis in the corpus luteum, and increases atretic follicles and fibrosis development. Melatonin use restores these harmful effects. Melatonin can be used to refrain from this negative effect of the Pomeroy method of tubal ligation on the ovary.

Key words: Melatonin; Ovarian histology; Pomeroy tubal ligation; Rat.

Introduction

Tubal sterilization is the most commonly used contraceptive method among women over 30 years of age in the United States. In both humans and rats, ovarian blood flow is impaired when the fallopian tube is destroyed, and this may damage ovarian tissue [1-4].

Zackrisson *et al*. [5] established in their rat study that ovarian artery ligation (OL) or uterine artery ligation (UL) had negative effects on ovarian blood flow and functions.

A procedure conducted on the fallopian tube (tubal lig., salpingectomy, etc.) may theoretically 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 in the mesosalpinx, and are necessary for the feeding of the ovary [6].

Ischemia and/or reperfusion injury leads 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 [7]. Consequently, cell membrane, lysosome membrane, and membranes of such cell organelles as endoplasmic reticulum, etc. are destroyed, cells break down, and necrosis results [8]. This event is called lipid peroxidation. Sugino [9] reported that oxygen radicals and the antioxidant system in the ovary have 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 the reactive oxygen species (ROS) inhibited oocyte development, and increased degenerated oocyte count and apoptosis [9].

However, lipid peroxidation stimulates collagen gene transcription in cell culture [10, 11].

Melatonin, a pineal secretory product, modulates ovarian function and reproduction in mammals [12]. Melatonin is present in human pre-ovulatory follicular fluid concentrations 3-fold higher than in peripheral serum [13].

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 [14]. Ishizuka *et al*. found that melatonin supported fertilization and early embryo development after in vitro fertilization because melatonin is a ROS scavenger [15].

Takasaki *et al*. [16] used oral melatonin in infertile

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women with poor quality oocytes and found a high amount of intrafollicular melatonin and a low amount of lipid peroxide. Melatonin use reduced degenerated oocyte count and increased fertilized oocyte count.

Melatonin is effective on hydroxyl radicals, singlet oxygen, peroxyl radicals and superoxide anion, among oxygen radicals. It protects nucleus DNA, membrane lipids, and cytosolic proteins against oxidative stress [17]. Moreover, it supports SOD, GSH-Px, glutathione reductase, and glyose-6-phosphate dehydrogenase of the antioxidant system [18]. It has an inhibitor effect on nitric oxide synthetase [19]. In addition, 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 and penetrates against oxidative stress. Furthermore, it has a protective effect on mitochondrion, which is a cell organelle [20].

Our Pub Med scan (melatonin, Pomeroy tubal ligation, rat) did not show any experimental study on this topic.

We attempted to examine the effects of melatonin use in the unilateral Pomeroy method of tubal ligation on ovarian histology in rats.

Material and Method

This study was conducted in the experimental Animals Laboratory of Fırat University Medical School. Thirty 12-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 and 12-hour dark photo period, 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. Oral feeding was stopped 18 hours before the experiment, and only water was allowed. The rats, which 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 and 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 the left Pomeroy method of tubal ligation was performed.

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

Layers of the abdomen and skin were closed with 3/0 silk. 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 left ovary was taken out. Ovarian tissue was fixed in 10% formaldehyde for histological examination, and placed into 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 [21]. 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 the corpus luteum was examined. Presence of fibrosis on the ovarian stroma was also 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. The Bonferroni correction Mann-Whitney U test was carried out for parameters; level of significance was set at $p < 0.05$.

Results

The experiment was successful in all rats. All values were similar in the comparison between G1 and G3, (Kruskal Wallis variance analysis).

The comparison of G2 with G1 and G3 showed that regression of angiogenesis in the corpus luteum was significantly lower in G2 ($p < 0.05$, Mann-Whitney U test). Atretic follicle count and fibrosis were significantly higher ($p < 0.05$, Mann-Whitney U test). Ovarian follicle reserve was high in G1 and G3, but there was no statistically significant difference (Kruskal Wallis variance analysis).

All examined parameters are presented in Table 1.

Table 1. — *Examined parameters of all groups. Values are expressed as mean ± SD.*

Parameter	G1	G2	G3	p
Primordial follicle (no.)	9.2 ± 3.3	9.2 ± 4.7	9.5 ± 2.5	NS
Primary follicle (no.)	8.9 ± 3	7.7 ± 4.7	9 ± 3	NS
Secondary follicle (no.)	0.4 ± 0.5	0.2 ± 0.4	0.5 ± 0.7	NS
Tertiary follicle (no.)	3.5 ± 0.7	3.7 ± 2.5	3.3 ± 0.6	NS
Ovarian follicle reserve (no.)	22 ± 4.9	21 ± 10	22.5 ± 4.3	NS
Corpus luteum (no.)	6.8 ± 1.3	6.7 ± 1.2	7 ± 0.9	NS
Corpus albicans (no.)	0.3 ± 0.4	0.3 ± 0.4	0.4 ± 0.5	NS
Total	7.1 ± 1.2	7 ± 1.4	7.3 ± 0.9	NS
Atretic follicle (no.)	0.2 ± 0.42	4.4 ± 1.81	0.01 ± 0.32	*
CL angiogenesis (points)	2 ± 01	1 ± 0.02	2 ± 01	*
Stromal fibrosis (points)	0 ± 02	1 ± 01	0 ± 02	*
Cystic follicle (microscopic)	0.1 ± 0.3	0.1 ± 0.3	0 ± 0	NS

NS = non significant.
* = $p < 0.05$ (Kruskal Wallis variance analysis).
Means are placed in descending order in the numbering process.

Discussion

The left Pomeroy method of tubal ligation performed by laparotomy in rats reduces regression of angiogenesis in the corpus luteum, and increases atretic follicles and fibrosis development in the sixth month. Melatonin can be used to avoid the negative effect of the Pomeroy method of tubal ligation on the ovary.

According to our Pub Med search (melatonin, rat, Pomeroy tubal ligation), our study is the first of its kind, and original in this respect.

The Pomeroy of method tubal ligation may lead to damage such as ischemia reperfusion. Melatonin reduces neutrophil infiltration and destructive tissue effects of

neutrophils during ischemia reperfusion, and particularly in reperfusion [17-20]. The fact that the damage in G3 was less than the damage in G2 can be attributed to the effect of melatonin. Our results are consistent.

It was found that changes in angiogenesis in the corpus luteum did not regress in G2. In normal rat ovaries, capillaries that emerge in the corpus luteum regress. Vascular endothelial growth factor (VEGF) has a major role in the emergence of these structures in the corpus luteum. One of the main stimulants of VEGF is hypoxia [21, 22]. As we impaired the blood flow in utero-ovarian anastomosis during the left Pomeroy method of tubal ligation, hypoxia resulted in the ovary [5], and this probably led to an increase in angiogenesis in the corpus luteum, and a decrease in regression of angiogenesis through VEGF. This may explain why regression of angiogenesis was lower in G2, relative to G1 and G3.

Hypoxia-induced factor-1 (HIF-1) is activated in both the ovary and other organs in case of acute or chronic hypoxia [23, 24]. HIF-1 alpha and hypoxic environments bring about regression and apoptosis in follicles, and result in an increase in atretic follicles and a decrease in follicular reserve [23]. The increase in atretic follicles and fibrosis observed in G2 may be associated with the apoptotic and degenerative effects of chronic hypoxia and lipid peroxidation products [23]. Our results are consistent.

Melatonin has a favorable effect on microvascular perfusion as it supports the endothelium [25]. Restoration of microvascular perfusion will reduce the effect of hypoxia (HIF-1 alpha, VEGF). This may be one of the reasons why there was no damage in the melatonin group.

HIF-1 alpha also increases VEGF secretion. VEGF helps angiogenesis, increases vascular permeability and 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 [21, 22, 26, 27]. Furthermore, VEGF directly stimulates collagen synthesis [27, 28]. The increase in fibrosis and follicle cysts in G2 may be explained by VEGF.

Although there was no ovarian fibrosis in G1 and G3, it was found to be significantly higher in G2. In case of blood or lymphatic circulation impairment, collagen neoformation is stimulated [29]. Uterine and tubal lymphatics are very close to each other on the broad ligament [29]. Lymphatic circulation may be damaged during the left Pomeroy method of tubal ligation, which may cause an increase in collagen formation. Our results are consistent with G2.

Melatonin can 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 caused the opposite effect [30]. Fibrosis caused by lipid peroxidation and its products decreases after the administration of antioxidants [melatonin, vit E) in animal models [31-33]. Therefore, the melatonin group has no fibrosis. The protective action of melatonin may be related with its antioxidant activity.

Conclusion

The left Pomeroy method of tubal ligation reduces regression of angiogenesis in the corpus luteum, and increases atretic follicle and fibrosis development. Melatonin use restores these harmful effects. Melatonin can be used to refrain from the negative effect of the Pomeroy method of tubal ligation on the ovary.

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