

Effect of melatonin on frozen-thawed ovarian autograft in a rat model

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Summary

Objective: Fertility preservation is one of the most important issues in good prognosis oncologic patients of young age. Ovarian tissue cryopreservation is a method of fertility preservation suitable for prepubertal girls and for patients with high risk of oncologic therapy delay. To improve ovarian graft survival is one of the largest challenges in this field of medicine. **Materials and Methods:** Female Sprague Dawley rats were randomly assigned into four experimental groups: c14 – euthanized 14 days after ovarian tissue transplantation (OTT) without any therapy, c28 – euthanized 28 days after OTT without any therapy, m14 – euthanized 14 days after OTT, treated with melatonin, and m28 – euthanized 28 days after OTT treated with melatonin. Ovariectomy was carried out two months before OTT; ovarian cortical fragments were frozen by vitrification and after two months transplanted into rat back muscle. **Results:** Fourteen days after OTT no statistically significant difference was recorded in estrogen blood level, in percentage of live ovarian tissue, and in number of capillaries in ovarian transplant between the control (c14 and c28) and treated (m14 and m28) groups. A statistically significant difference ($p < 0.005$) was recorded in the number of capillaries in $250 \mu\text{m}^2$ (7.4 ± 2.1 vs. 10.5 ± 0.6 in c28 and m28, respectively) between the control and treated groups 28 days after transplantation. **Conclusion:** Cryoinjury and post-transplantation ischemic injury of ovarian graft are the most important problems of ovarian tissue transplantation, while ischemic injury seems to be more important than cryoinjury. Melatonin – a potent antioxidant and antiapoptotic factor – can improve transplanted ovarian tissue function via neoangiogenesis in ovarian graft. Further studies of these melatonin effects are needed.

Key words: Fertility preservation; Ovarian tissue cryopreservation; Melatonin.

Introduction

Today about 10% of cancers are diagnosed in young people who have not yet completed their family planning. Typical malignancies of young females are breast cancer, cervical cancer, haematological malignancies, melanoma and colorectal carcinoma [1]. The five-year survival rate in the age category between 15 and 49 years is today more than 80%, which is very positive [2]. Thus, preserving fertility for these young cancer survivors is one of the most important goals for reproductive medicine. Chemotherapy and radiotherapy are often gonadotoxic; according to treatment regimens, gonadotoxicity ranges between 20% and 80% risk [3].

Cryopreservation of ovarian tissue is a method of fertility preservation which can be performed without delaying oncological therapy, and, especially, it is the only method available for prepubertal patients. This procedure has been available as an experimental method for more than 15

years, and thus far 86 successful births and nine ongoing pregnancies worldwide have been published in the literature after transplantation of ovarian tissue [4].

Among the several factors that can affect the success of organ and tissue transplantation are immune intolerance and apoptotic/necrotic cell death due to ischemia/reoxygenation [5]. One of the largest challenges in ovarian tissue transplantation is tissue damage and follicular loss after transplantation. This is caused by the occurrence of free radicals after ischemia – reperfusion distress. Melatonin is an indolamine produced mainly by the pineal gland; it is a potent free-radical scavenger, with subsequent antioxidant and antiapoptotic functions [6]. It is a powerful antioxidant, the use of which can be beneficial after transplantation due to a reduction in transplant-rejection processes. It prevents both free-radical damage and induction of the apoptotic mitochondrial pathway by reducing Bcl2 expression and caspase-3 activity [7]. There is credible evidence to suggest that melatonin should be classified as a mitochondrial-tar-

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geted antioxidant [8]. Its capacity to prevent oxidative stress is well-documented in stroke and heart-attack patients. The direct free-radical scavenging activity of melatonin has been known for almost 25 years [9].

The aim of this study on a rat model was to determine whether the survival of frozen-thawed rat ovarian tissue can be improved by the melatonin therapy after transplantation.

Materials and Methods

Female Sprague Dawley rats ($n = 28$), six months of age, were used in the study. The animals were randomly assigned into the following experimental groups, each containing seven rats: (1) c14 – control rats, ovariectomized, transplanted, without therapy, euthanized 14 days after ovarian tissue transplantation, (2) c28 – control rats, ovariectomized, transplanted, without therapy, euthanized 28 days after ovarian tissue transplantation, (3) m14 – experimental rats, ovariectomized, transplanted, treated with melatonin, euthanized 14 days after ovarian tissue transplantation, and (4) m28 – experimental rats, ovariectomized, transplanted, treated with melatonin, euthanized 28 days after ovarian tissue transplantation.

The animals were housed in polycarbonate cages and maintained at constant temperature ($23 \pm 2^\circ\text{C}$), with a 12-hour light-dark cycle and free access to water and food. All procedures were carried out in accordance with European Union Regulations (Directive 86/609/CEE) for animal experiments.

The study was approved by the Ethics Committee of the Faculty of Medicine of Pavol Jozef Safarik University in Košice and by the State Veterinary and Food Administration of the Slovak Republic (Č.k. Ro-982/15-221).

Every surgery was performed under inhalation anesthesia with isoflurane. Tramadol was used for analgesia in a dose of 0.5 mg/kg intramuscularly. Atropin was administered subcutaneously as premedication in a dose of 0.05 mg/kg.

Ovariectomy was performed on all groups under standard aseptic conditions, as was described in the authors' previous study [10]. Ovarian samples were sent for further processing. Fat and medullary tissue were removed, and the cortical part was divided into fragments ($1 \times 1 \times 1$ mm) in HEPES buffered medium. These cortical fragments were frozen by vitrification and cryopreserved in liquid nitrogen for two months. Afterwards, the ovarian blocks were warmed and autotransplanted for the same rat.

Ovarian fragments were thawed and transplanted into the back muscles of the experimental animals. Two 3-cm long, parallel, full thickness skin incisions were made under aseptic conditions on the left and right side of each rat's dorsum. After the back muscle was dissected, ovarian slices were pushed inside with delicate forceps, 10 mm from the edge of the incision to a depth of 3–5 mm. The incisions were closed with stitches.

After transplantation, the rats in the experimental groups (m14 and m28) were treated with melatonin dissolved in drinking water at the highest dissolving concentration (240 mg/l) in light-proof bottles two weeks before transplantation and throughout the experiment. A fresh solution of melatonin was made every day, and water consumption was recorded. The melatonin solution was offered ad libitum. In the control group the consumption of normal water was also recorded on a daily basis.

On day 14 (groups c14 and m14) or 28 (groups c28 and m28) each animal was euthanized by inhalation of ether, after which the back muscle with ovarian tissue was extracted for histological evaluation, and a sample of animal blood was taken for estradiol evaluation.

The recovered ovaries after ovariectomy were transported to the laboratory immediately in a cell culture dish containing 2 mL of HEPES medium, and the ovarian cortex was then processed at room temperature (25°C) and cut into small sections (between $1 \times 1 \times 1$ mm in size). The ovarian cortical sections were transferred into cell culture dishes in a stepwise manner, with a solution containing 10% v/v ethylene glycol and 10% v/v Dimethyl sulfoxide (DMSO) in HEPES for 20 minutes at room temperature. They were then transferred into a solution containing 17% v/v ethylene glycol, 17% v/v DMSO and 0.75 M sucrose in HEPES for three minutes at room temperature.

The ovarian cortical sections were then loaded individually with minimal vitrification solution to the surface of 0.25 ml cryostraws cut angle-wise. The straws were inserted into precooled larger 0.5 ml cryostraws, sealed with an ultrasound sealer, and stored in liquid nitrogen (-196°C) for two months.

Prior to reimplantation, the straws containing the samples were removed from liquid nitrogen storage. The contents of the straws were exposed to room temperature for one minute. Then each ovarian cortical section sample was expelled into a cell culture dish submitted to three five-minute baths in a solution containing HEPES medium + 10% human serum albumin (HSA) and decreasing concentrations of sucrose (0.5, 0.25, and 0.0 M) at room temperature. These steps wash out the cryoprotectants.

Blood was collected from all animals immediately after euthanasia. The serum was prepared and kept frozen at -20°C until the serum hormone concentrations were determined. Serum estradiol concentrations were measured using 17β -estradiol enzyme-linked immunosorbent assay antibody-coated tube kits.

One slice measuring 1–2 mm from every ovarian sample was fixed immediately after ovariectomy and ovarian dissection (fresh ungrafted control) for evaluating pregrafting follicular density in every animal to ensure, on the basis of follicular counts in the fresh ungrafted controls, that only follicle-rich samples would be used for grafting. In addition, histological samples after transplantations (14 or 28 days) were processed routinely for light microscopy (fixation, dehydration, embedding, cutting, and staining with hematoxylin-eosin and immunohistology for Factor VIII and CD34).

The slides were viewed independently by two of the authors, and the following histopathological parameters were evaluated: angiogenesis and percentage of live tissue. Angiogenesis was evaluated as the number of capillaries in $250 \mu\text{m}^2$ of live ovary tissue. The percentage of live tissue was calculated as the ratio of live ovary tissue to all ovary tissue.

Data on the estrogen blood level, angiogenesis, and percentage of live tissue are presented as the mean \pm standard deviation. Analysis of variance followed by Tukey-Kramer multiple comparison tests were used to compare the differences in hormone serum levels and percentage of live tissue. To compare the data obtained from the angiogenesis measurement, the Kruskal-Wallis test was used. Significance was accepted at $p < 0.05$ for each test.

Results

No statistically significant difference was recorded in the results in groups 14 days after ovarian tissue transplantation. Estrogen blood level was 15.2 ± 2.6 pg/ml in the non-treated group (c14) and 16.3 ± 4.2 pg/ml in the treated group (m14) (Figure 1). The percentage of the live tissue was $16.8 \pm 6.2\%$ in the control group (c14) and eventually $15.6 \pm 9.3\%$ in the treated group (m14) (Figure 2). In the control group (c14) 6.3 ± 1.5 capillaries were recorded in

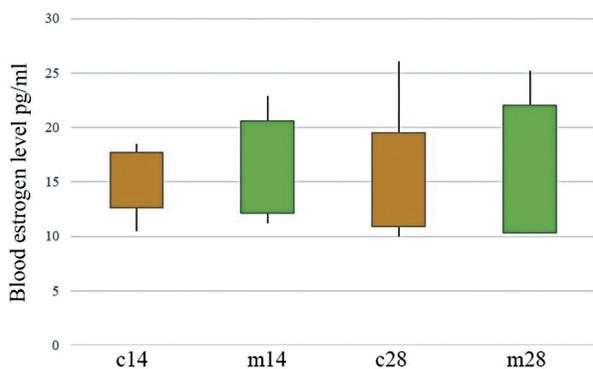


Figure 1. — Blood estrogen level in the groups.

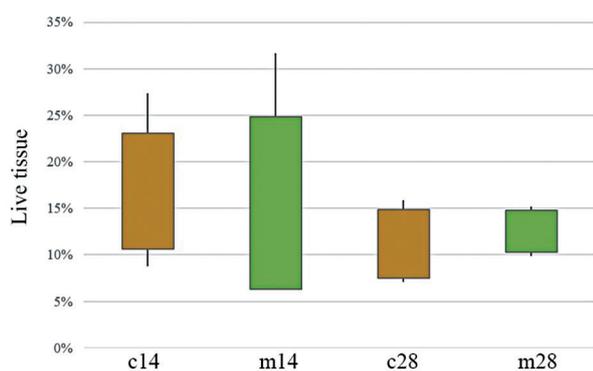


Figure 2. — Percentage of live ovarian tissue in the groups.

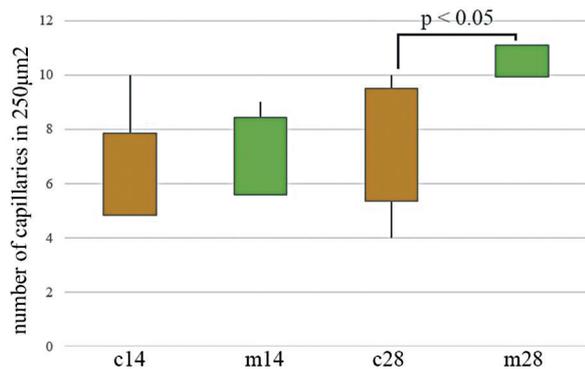


Figure 3. — Number of capillaries in 250 µm² in the groups.

250 µm² and in the treated group (m14) 7.0 ± 1.4 (Figure 3, Table 1).

In the groups euthanized 28 days after ovarian tissue transplantation the following results were recorded. Estrogen blood level in the control group was 15.2 ± 4.3 pg/ml (c28) and 16.2 ± 5.8 pg/ml in the melatonin group (m28) (Figure 1), and no statistically significant differences were recorded between the treated and control groups at the same time after transplantation. Likewise, no statistically significant difference was recorded in the percentage of live ovar-

Table 1. — Blood estrogen level, percentage of live tissue, and angiogenesis in the control and melatonin-treated groups (c14, c28, m14, m28) (**p* < 0.05).

	Control group	Treated group
Blood estrogen level (pg/ml)		
14 days after TX	15.2 ± 2.6	16.3 ± 4.2
28 days after TX	15.2 ± 4.3	16.2 ± 5.8
Live tissue (%)		
14 days after TX	16.8 ± 6.2	15.6 ± 9.3
28 days after TX	11.2 ± 3.7	12.5 ± 2.3
Angiogenesis (number of capillaries in 250 µm²)		
14 days after TX	6.3 ± 1.5	7.0 ± 1.4
28 days after TX	7.4 ± 2.1	10.5 ± 0.6*

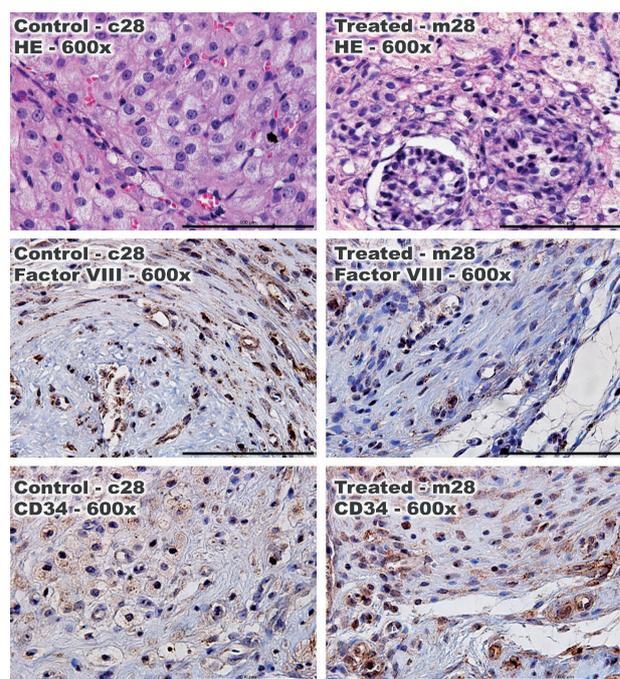


Figure 4. — Histological picture of neoangiogenesis in the control group (c28) and the melatonin-treated (m28) group 28 days after transplantation (coloured by haematoxylin eosin, factor 8 antibody, and CD34 antibody).

ian tissue 28 days after transplantation. The authors recorded 11.2 ± 3.7% live tissue in group c28 and 12.5 ± 2.3% live tissue in group m28 (Figure 2). A statistically significant difference (*p* < 0.005) in the number of capillaries in 250 µm² (7.4 ± 2.1 vs. 10.5 ± 0.6 in c28 and m28, respectively) was recorded between the control and the treated group 28 days after transplantation (Figures 3 and 4, Table 1).

Discussion

The present study of frozen-thawed ovarian graft recipients of melatonin treatment in a rat model yielded impor-

tant findings. The graft tissue showed better neo-vascularization after 28 days of treatment with melatonin. Freezing and transplantation led to accelerated follicular loss after transplantation. This follicular loss is essential to future graft functioning, and so the most important task is to reduce this graft follicular loss as much as possible. To date, several methods and substances have been tested in relation to ovarian graft protection after transplantation. One of these substances is melatonin, a hormone with documented beneficial effects attributed to almost every field of medicine.

Estradiol levels were measured in this study, but this does not seem to be a very good marker of ovarian transplant function. Follicle development in rats is divided into ten phases. Growth of the ovarian follicle to the eighth generation follicle takes more than 50 days and it takes another 2.5 days to reach ninth follicle generation, during which an antrum begins to form. Follicle recruitment and most atresia occurs in this phase. The final follicular maturation takes another three days [11]. The same process takes about 120 days in humans, so the return of endocrinological function of ovarian transplant in women is documented as four to five months after ovarian transplantation [12]. The period of 28 days in this study should not be sufficient for the estradiol level to increase, despite adequate ovarian graft function.

In the animal model, the route and dosage of melatonin are very important. This dosage can affect graft survival. In this study melatonin was orally administered, diluted in drinking water, as described in the study by Friedman *et al.* [7]. According to intramuscular transplantation of ovarian tissue, the present authors preferred oral administration, as described by previous authors. Back muscle in rats can mimic ovarian conditions due to its low vascularization. Melatonin absorption occurs throughout the gastrointestinal tract, with the greatest absorption being in the rectum and ileum and the least in the stomach [13]. The bioavailability of orally administered melatonin is about 3% [14], so the dosage in the drinking water had to be quite high.

Melatonin is generally considered to be an antiangiogenic factor, and it acts by VEGF expression downregulation and by direct inhibition of endothelial cells proliferation. A number of studies over the years have confirmed its positive effect in cancer treatment by these mechanisms of action [15]. On the other hand, melatonin exerts its physiological and pathological functions through its antioxidant, anti-inflammatory, antiapoptotic, and anti-ageing properties, and it is used in therapy of neurodegenerative diseases, liver cirrhosis, wound healing, myocardial infarction, kidney ischemia injury, osteoporosis, etc. [16]. In the present study, the authors confirmed a slight increase in angiogenesis after 14 days in the group treated with melatonin, but after 28 days, this increase was even statistically significant. Recent studies

have demonstrated the beneficial effects of melatonin on healing of gastric and duodenal ulcers, as well as burn injuries [17]. In the study on a rat model published by Soybir *et al.*, the authors documented at all stages a significant increase ($p < 0.05$) in the number of vessels of the study group treated by melatonin but not in the control group, and they concluded that melatonin may have a positive effect on both angiogenesis and wound healing [18]. The present authors agree with this conclusion, although whether this is really neoangiogenesis potentiation or it is only a positive effect of melatonin based on other mechanisms of action is questionable.

One of the most important problems that must be solved after ovarian tissue transplantation is the reduction of ischemia follicle damage. This follicular loss is caused by free radicals originated by the tissue transplantation procedure after ischemia-reperfusion syndrome [19]. Unlike other antioxidants, melatonin has both hydrophilic and lipophilic affinities, so its diffusion through intracellular compartments is very simple and broad. Melatonin receptors are present in the ovaries of many species, including humans [20]. In rat ovaries, there are two types of melatonin receptors – MT1 and MT2 [21]. These receptors can affect steroidogenesis through the cAMP mediated pathway.

A systematic review from 2016 showed that thus far five acceptable studies have been published to analyze the melatonin effect on rodent ovary after ovarian tissue transplantation, in total on 503 animals [7, 22, 23, 24, 25]. In these studies, melatonin reduced the number of apoptosis and atretic follicles, enhanced follicle quality, quantity, and graft size in low dosages, diminished Th1/Th2 immunological reaction and longer graft lifespan with high dosages, enhanced corpora lutea, secondary and antral follicles, improved mean graft survival, ovarian size, and revascularization, diminished ovarian and plasmatic malondialdehyde and ovarian necrosis, and enhanced glutathione peroxidase and superoxidismutase. Thus, the effect of melatonin is caused not only by enhancing free-radical scavengers, but also by activating other enzymes linked to oxidative damage decrease. Recent studies of diabetic wound healing showed that melatonin promotes wound healing by several mechanisms leading to inflammation and apoptosis reduction [26]. The same mechanism seems to be a potential benefit factor of melatonin after ovarian transplantation.

Unlike with neoangiogenesis, in the present study the authors did not record a positive effect of melatonin on the percentage of necrosis in the transplanted graft. Melatonin has thus far been tested after transplantation of liver, lung, pancreas, and kidney, and in most studies with documented positive effects and in previously published ovarian graft studies, the positive effect of melatonin on the graft function has been confirmed without depending on the route of administration [6]. The present authors also observed that,

although the evaluation of necrosis of the tissue graft did not record a statistically significant improvement in the treated group, in combination with the rise of angiogenesis, melatonin seems to be an interesting substance capable positively affecting ovarian tissue transplant survival. In the future, it will be necessary to take into account endogenous melatonin; some authors recommend the use of pinealectomized animals or melatonin receptor blockers. In any case, based on the present results, the impact of melatonin must be evaluated as positive, but requires more thorough analysis and more extensive research.

Today, it is well-documented that melatonin can also reduce oxidative stress and improve ovarian function in cases that can lead to ovarian insufficiency, but the concrete molecular mechanisms remain unclear [27]. Melatonin in ovarian follicles contributes to ovarian maturation, oocyte development, and luteinization of granulosa cells [28]. In studies concerning premature ovarian insufficiency, it was found that melatonin has a protective effect on primary ovarian insufficiency by improving the estrous phase, ovarian and uterus mass and index, increasing ovarian follicles and the corpus luteum, decreasing atresia follicles, and ameliorating ovarian endocrine and reverse functions with a decreased FSH level and an increased AMH level [27]. These effects occur through the Silent information regulator 1 (SIRT 1) upon its activation. Aside from its direct free-radical scavenger actions, which are receptor independent, its indirect antioxidant effects can be mediated by membrane or nucleus receptors [29]. Blockade of the melatonin receptor significantly downregulates SIRT1 signaling expression, thus aggravating oxidative stress and apoptosis [27]. Also the present findings suggest that melatonin can also protect ovarian tissue by activation of the molecular pathway in a melatonin-receptor dependent manner.

Conclusions

Within the limits of this experimental study, the administration of supplemental melatonin in rats after frozen-thawed ovarian tissue transplantation demonstrated positive histologic effects on the ovarian transplant, especially in the sense of neoangiogenesis. Melatonin is known as a powerful free-radical scavenger and a broad-spectrum antioxidant and current studies indicate that it can be used, not only in the case of ovarian transplantation, but also in the treatment of premature ovarian insufficiency. Further studies on both of these melatonin effects are still needed.

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References

- [1] Coccia P.F., Altman J., Bhatia S., Borinstein S.C., Flynn J., George S., *et al.*: "Adolescent and young adult oncology. Clinical practice guidelines in oncology". *J. Natl. Compr. Canc. Netw.*, 2012, 10, 1112.
- [2] Imbert R., Moffa F., Tsepelidis S., Simon P., Delbaere A., Devreker F., *et al.*: "Safety and usefulness of cryopreservation of ovarian tissue to preserve fertility: a 12-year retrospective analysis". *Hum. Reprod.*, 2014, 29, 1931.
- [3] Lee S.J., Schover L.R., Partridge A.H., Patrizio P., Wallace W.H., Hagerty K., *et al.*: "American Society of Clinical Oncology recommendations on fertility preservation in cancer patients". *J. Clin. Oncol.*, 2006, 24, 2917.
- [4] Jensen A.K., Macklon K.T., Fedder J., Ernst E., Humaidan P., and Andersen C.Y.: "86 successful births and 9 ongoing pregnancies worldwide in women transplanted with frozen-thawed ovarian tissue: focus on birth and perinatal outcome in 40 of these children". *J. Assist. Reprod. Genet.*, 2017, 34, 325.
- [5] Hernandez D., Muriel A., and Abreira V.: "Current state of clinical end-points assessment in transplant: Key points". *Transplant. Rev. (Orlando)*, 2016, 30, 92.
- [6] Shiroma M.E., Botelho N.M., Damous L.L., Baracat E.C. and Soares-Jr J.M.: "Melatonin influence in ovary transplantation: systematic review". *J. Ovarian. Res.*, 2016, 9, 33.
- [7] Friedman O., Orvieto R., Fisch B., Felz C., Freud E., Ben-Haroush A. and Abir R.: "Possible improvements in human ovarian grafting by various host and graft treatments". *Hum. Reprod.*, 2012, 27, 474.
- [8] Reiter R.J., Mayo J.C., Tan D.X., Sainz R.M., Alatorre-Jimenez M. and Qin L.: "Melatonin as an antioxidant: under promises but over delivers". *J. Pineal. Res.*, 2016, 61, 253.
- [9] Tan D.X., Manchester L.C., Esteban-Zubero E., Zhou Z. and Reiter R.J.: "Melatonin as a Potent and Inducible Endogenous Antioxidant: Synthesis and Metabolism". *Molecules*, 2015, 20, 18886.
- [10] Gal P., Toporcer T., Vidinsky B., Mokry M., Grendel T., Novotny M., *et al.*: "Postsurgical administration of estradiol benzoate decreases tensile strength of healing skin wounds in ovariectomized rats". *J. Surg. Res.*, 2008, 147, 117.
- [11] Krinke G.: "The Laboratory Rat". 1st ed. Academic Press, 2000, 756
- [12] Mardesic T., Jelinkova L. and Melicharova L.: "Successful ovarian tissue transplantation in woman with premature ovarian failure after gonadotoxic treatment". *Ceska Gynekol.*, 2017, 82, 38.
- [13] Tran H.T., Tran P.H. and Lee B.J.: "New findings on melatonin absorption and alterations by pharmaceutical excipients using the Ussing chamber technique with mounted rat gastrointestinal segments". *Int. J. Pharm.*, 2009, 378, 9.
- [14] Andersen L.P., Werner M.U., Rosenkilde M.M., Harpsoe N.G., Fuglsang H., Rosenberg J. and Gogenur I.: "Pharmacokinetics of oral and intravenous melatonin in healthy volunteers". *BMC Pharmacol. Toxicol.*, 2016, 17, 8.
- [15] Cerezo A.B., Hornedo-Ortega R., Alvarez-Fernandez M.A., Troncoso A.M. and Garcia-Parrilla, M.C.: "Inhibition of VEGF-Induced VEGFR-2 Activation and HUVEC Migration by Melatonin and Other Bioactive Indolic Compounds". *Nutrients*, 2017, 9, ii: E249. doi: 10.3390/nu9030249.
- [16] Zhang S., Chen S., Li Y. and Liu Y.: "Melatonin as a promising agent of regulating stem cell biology and its application in disease therapy". *Pharmacol. Res.*, 2017, 117, 252.
- [17] Ersoy O.F., Ozkan N., Ozsoy Z., Kayaoglu H.A., Yenidogan E., Celik A., *et al.*: "Effects of melatonin on cytokine release and healing of colonic anastomoses in an experimental sepsis model". *Ulus. Travma. Acil. Cerrahi. Derg.*, 2016, 22, 315.
- [18] Soybir G., Topuzlu C., Odabas O., Dolay K., Bilir A. and Koksoy, F.: "The effects of melatonin on angiogenesis and wound healing". *Surg. Today*, 2003, 33, 896.
- [19] Demeestere I., Simon P., Emiliani S., Delbaere A. and Englert Y.: "Orthotopic and heterotopic ovarian tissue transplantation". *Hum. Reprod. Update*, 2009, 15, 649.
- [20] Yie S.M., Niles L.P. and Younglai E.V.: "Melatonin receptors on

- human granulosa cell membranes". *J. Clin. Endocrinol. Metab.*, 1995, 80, 1747.
- [21] Soares J.M.Jr., Masana M.I., Ersahin C. and Dubocovich M.L.: "Functional melatonin receptors in rat ovaries at various stages of the estrous cycle". *J. Pharmacol. Exp. Ther.*, 2003, 306, 694.
- [22] Sapmaz E., Ayar A., Celik H., Sapmaz T., Kilic N. and Yasar M.A.: "Effects of melatonin and oxytetracycline in autologous intraperitoneal ovary transplantation in rats". *Neuro Endocrinol. Lett.*, 2003, 24, 350.
- [23] Hemadi M., Abolhassani F., Akbari M., Sobhani A., Pasbakhsh P., Ahrlund-Richter L., *et al.*: "Melatonin promotes the cumulus-oocyte complexes quality of vitrified-thawed murine ovaries; with increased mean number of follicles survival and ovary size following heterotopic transplantation". *Eur. J. Pharmacol.*, 2009, 618, 84.
- [24] Hemadi M., Saki G., Shokri S. and Ghasemi F.M.: "Follicular dynamics in neonate vitrified ovarian grafts after host treatment with melatonin". *Folia Morphol. (Warsz.)*, 2011, 70, 18.
- [25] Hemadi M., Shokri S., Pourmatroud E., Moramezi F. and Khodadai A.: "Follicular dynamic and immunoreactions of the vitrified ovarian graft after host treatment with variable regimens of melatonin". *Am. J. Reprod. Immunol.*, 2012, 67, 401.
- [26] Song R., Ren L., Ma H., Hu R., Gao H., Wang L., *et al.*: "Melatonin promotes diabetic wound healing in vitro by regulating keratinocyte activity". *Am. J. Transl. Res.*, 2016, 8, 4682.
- [27] Ma M., Chen X.Y., Li B. and Li X.T.: "Melatonin protects premature ovarian insufficiency induced by tripterygium glycosides: role of SIRT1". *Am. J. Transl. Res.*, 2017, 9, 1580.
- [28] Tamura H., Takasaki A., Taketani T., Tanabe M., Lee L., Tamura I., *et al.*: "Melatonin and female reproduction". *J. Obstet. Gynaecol. Res.*, 2014, 40, 1.
- [29] Yu L., Sun Y., Cheng L., Jin Z., Yang Y., Zhai M., *et al.*: "Melatonin receptor-mediated protection against myocardial ischemia/reperfusion injury: role of SIRT1". *J. Pineal. Res.*, 2014, 57, 228.

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