Original Article

The Effects of Melatonin Alone or in Combination with Zinc on Gonadotropin and Thyroid Hormones in Female Rats

Rahimi, F[1], Zendehdel, M[2*], Rezaee, MJ[3], Vazir, B[1], Fakour, Sh[4]

1. Department of Basic Science, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
3. Department of Anatomy, Faculty of Medicine, Kurdistan university of Medical Science, Sanandaj, Iran
4. Department of Clinical Science, Faculty of Veterinary Medicine, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran

How to cite this article: **Rahimi F, Zendehdel M, Rezaee MJ, Vazir B, Fakour Sh. The Effects of Melatonin Alone or in Combination with Zinc on Gonadotropin and Thyroid Hormones in Female Rats. Archives of Razi Institute. 2023;78(6):1698-708.**

DOI: 10.32592/ARI.2023.78.6.1698

Article Info:
Received: 17 April 2023
Accepted: 06 May 2023
Published: 30 December 2023

Corresponding Author's E-Mail:
zendedel@ut.ac.ir

ABSTRACT

Thyroid and gonadotropin hormones play an essential role in the regulation of regulating various physiological functions. The effects of melatonin and zinc (Zn) on these hormones have already been investigated. The aim of the present study was to investigate the effect of melatonin with and without zinc on the levels of gonadotropin hormones and thyroid hormones (triiodothyronine (T_3), thyroxine (T_4) and thyroid-stimulating hormone (TSH)) in female rats. In general, 35 sexually mature female rats were randomly divided into five treatment groups, with each group comprising 7 rats, in a completely randomized design (CRD) during the research. The rats were treated daily with Zn and melatonin via gavage as follows: T1 (control 1, basal diet), T2 (control 2, treatment with normal saline) and the other experimental groups, including T3, T4 and T5, were treated with Zn (40 ppm), melatonin (5 mg/kg) or a combination of Zn and melatonin at the same dose. The administration of the drugs was continued for 20 days (daily) . Plasma samples were then taken for the determination of LH, FFH, LH/FSH, estrogen, progesterone, T3, T4 and TSH levels. The results showed no significant differences in FSH and LH levels between treatments. Estrogen, progesterone and TSH levels were higher in the rats receiving 5 mg melatonin per day than in the other groups, but not statistically significant (P>0.05). However, T3 levels decreased significantly in the group receiving 40 mg/kg Zn compared to the other experiments. (P<0.05). The results showed no significant difference between the treatments in terms of T_4 levels (P>0.05). In conclusion, no remarkable changes in other variables were observed in female rats receiving melatonin, Zn or a combination of melatonin and Zn, with the exception of T3.

Keywords: Estrogen, Female rat, Melatonin, T3, T4, TSH, Progesterone, Zinc

1. Introduction

Zinc is one of the necessary elements for the proper functioning of thyroid hormones, reproductive performance, the immune system and cell division in animals and humans. Although this element is needed in the milligram level, it plays a crucial role in living organisms, for example as a cofactor in the structure of about 250 to 300 enzymes (1). Melatonin (N-acetyl-5-methoxy-tryptamine), which is primarily known for its contribution to the regulation of reproductive cycles, also plays an important role in stress reduction by influencing both the cardiovascular system and heat loss through evaporation (2). In addition, it has been found to alter the genomic representation of antioxidant enzymes in the organism (3), and its function in controlling many sexual processes , such as puberty, ovarian activity and gestation has been demonstrated (4, 5). The increase in reactive oxygen species (ROS) production during in vitro fertilization (IVF) has been shown to adversely affect the percentage of IVF outcomes achieved. (6-8). Recently, researchers have reported on the antioxidant properties of melatonin. For this reason, they have attempted to harness the benefits of melatonin by prescribing or ingesting it to improve reproductive rate and efficiency in the population (9, 10). In humans, melatonin was found to have reduced oxidative damage to sperm mitochondria caused by ROS . In all living organisms, melatonin is synthesized during the night by the pineal gland, retina, digestive system and various other organs (11). The amount of melatonin synthesis is influenced by external factors (such as light) and physiological factors (such as age). Research has shown that reproductive capacity and the production of sex cells and related hormones decline with age. This situation has a negative effect on the fertility rate and the quality of ovum (9, 12-15).

In adult women, the quality of the oocytes gradually decreases and degenerative and destructive changes in the oocyte increase, so that this condition can be a negative factor leading to infertility and/or reduced fertility efficiency in women . With increasing age, the probability of fertility success decreases. Therefore, research on laboratory animals that leads to and desired results can be generalized to humans, solving part of the problems associated with infertility. Nowadays, hormone therapy and the use of drugs such as estrogen and progesterone, as well as various assisted reproductive techniques (ART), intrauterine insemination (IUI), microinjection (ICSI) and IVF are common and are performed (16). However, it is noteworthy that the quantity and quality of oocytes is influenced by parameters such as cultural and economic status, aging, poor nutrition and genetic factors, which ultimately leads to a decrease in reproductive efficiency.

Since 1960, numerous drugs have been used to stimulate ovulation and treat all types of infertility in humans, and now the use of these drugs has increased. Although much research has been done in this field, there are still inconsistencies and contradictory results in scientific reports. Therefore, in order to make progress in the treatment of infertility, the use of new products may be useful. To our current knowledge, there is no scientific research on the effects of zinc and its combination with melatonin on thyroid and sex hormones in female rats. Therefore, attention to this issue may justify the need to conduct this research.

2. Materials and Methods

2.1. Animals and Experimental Design

The present study was conducted in the laboratory animal center of Islamic Azad University, Sanandaj branch. The moral committee of the centre evaluated and approved each one of our experiments. In general, 70 female Wistar rats with an average weight of 225±24 g were provided from the animal house of University of Medical Sciences, Sanandaj. Two animals per cage (50 x 50 cm) were kept in a light-controlled room (12L: 12D h, light switched on at 6 h). Humidity and temperature were controlled at

50±5% and 21±1◦C, respectively. All rats received uniform amounts of normal ration and tap water. The study period was 20 days (17). Rats were divided into five exploratory groups in a completely randomized design (CRD) with seven duplicates (n=7) and two rats in each cage. Rats were treated daily with gavage as follows: T1: (control group 1) received standard feed only, T2: (control group 2) received normal saline, and the other groups, including groups 3, 4 and 5, were treated with Zn (40 ppm), melatonin (5 mg/kg) and the mixture of Zn and melatonin at the same doses by gavage. Zn and melatonin were purchased from Sigma Chemical Co. (St. Louis, MO). Melatonin was dissolved in saline and administered daily at 12:00 h at a dose of 5 mg/kg to the rats in the experimental group.

2.2. Blood Collection

Twenty-four hours after the last shot, the rats were selected between 8 and 12 o'clock in the morning. Blood was collected and plasma was separated by centrifugation (3000 g, 15 minutes) at 4°C and stored at −20 ◦C until analysis of the concentration of various parameters. The plasma samples were stored in a 1 ml plastic tube without graduation and without metal.

2.3. Measurement of the hormones

Follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels were determined by radioimmunoassay (RIA) kits (NIADDK, USA) using an automated microplate reader (State Fax® 2100, Awareness, USA); in addition, triiodothyronine (T_3) was determined using a commercial kit (Thyro-kit, Germany) with a sensitivity of 0.1 ng/ml. Thyroxine (T_4) was determined using a commercial kit (Thyro-kit, Germany) with a sensitivity of 10 nmol/l. TSH was measured by a chemiluminescent enzyme immunometric assay (Immulite, Deerfield, IL). The hormones estrogen and progesterone were measured using an Elisa kit (Glory Company, London, England) (18-20).

2.4. Data analysis

The completely randomized design (CRD) was used for the present study. The statistical analysis of the results was carried out using 'SPSS 22 software. Means and standard deviations (SD) were determined for each variable. ANOVA was used to assess the differences between treatments. Least significant difference (LSD) was used to compare the means of the treatments that were deemed statistically notable by the variance assessment. The level of statistical significance was set at $P< 0.05$.

3. Results

The results of this study are shown in Figures 1-8. According to Figure 1, the mean FSH concentration

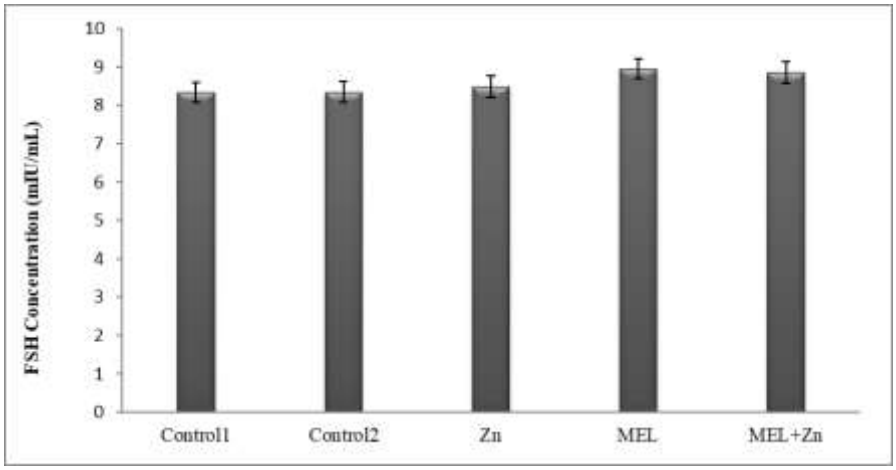

Figure 1. The effect of Zn (40 ppm), melatonin (5 mg/kg), and Zn (40 ppm) + melatonin (5 mg/kg) on the mean FSH concentration in female rats (n=7). Information are expressed in the form of an average ± the standard error of the mean.

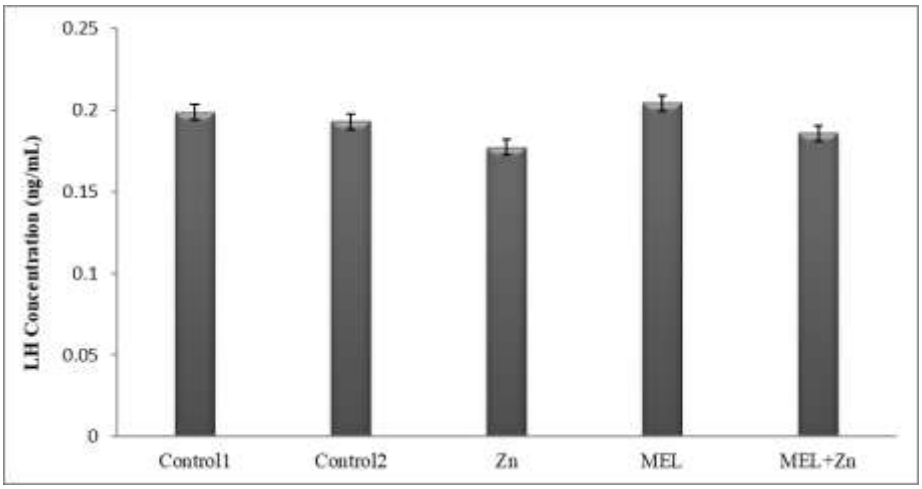

Figure 2. The effect of Zn (40 ppm), melatonin (5 mg/kg), and Zn (40 ppm) + melatonin (5 mg/kg) on the mean LH concentration in female rats (n=7). Information are expressed in the form of an average ± the standard error of the mean.

was 8.3 (mIU/ml) in control group 1, 8.36 (mIU/ml) in control group 2, 8.4 (mIU/ml) in the zinc group, 8.96 (mIU/ml) in the melatonin group and 8.86 (mIU/ml) in the zinc and melatonin group. According to the results, there was no significant difference between the treatments in the FSH concentration (P>0.05).

Figure 2 shows that the average LH index was 0.2 (mIU/ml) in control group 1, 0.19 (mIU/ml) in control group 2, 0.18 (mIU/ml) in the zinc group, 0.2 (mIU/ml) in the melatonin group and 0.19 (mIU/ml) in the zinc and melatonin groups. No significant difference in LH levels was found between the test groups (P>0.05).

Figure 3 shows that the LH/FSH ratio was 0.024 in control group 1, 0.022in control group, 2 0.021 in the zinc group, 0.022in the melatonin group and 0.021 in the zinc and melatonin group. Based on the results, no significant difference in the LH/FSH ratio was found in the rats (P>0.05).

Figure 4 shows that the average estrogen index was 9.66 (ng/ml) in the control group1, 9.61 (ng/ml) in the control group 2, 10.47 (ng/ml) in the zinc group, 10.91 (ng/ml) in the melatonin group, and 9.67 (ng/ml) in the zinc and melatonin group. The results showed no significant difference in estrogen levels

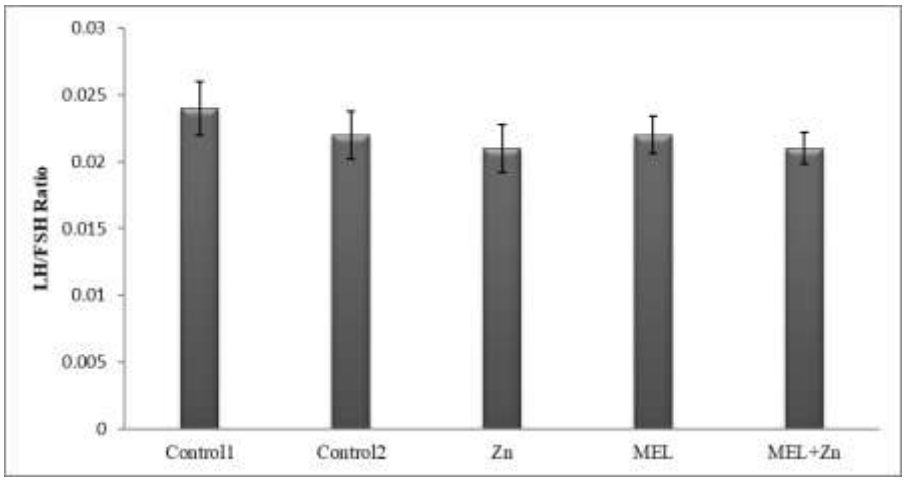

Figure 3. The effect of Zn (40 ppm), melatonin (5 mg/kg), and Zn (40 ppm) + melatonin (5 mg/kg) on the LH/FSH ratio in female rats (n=7). Information are expressed in the form of an average ± the standard error of the mean.

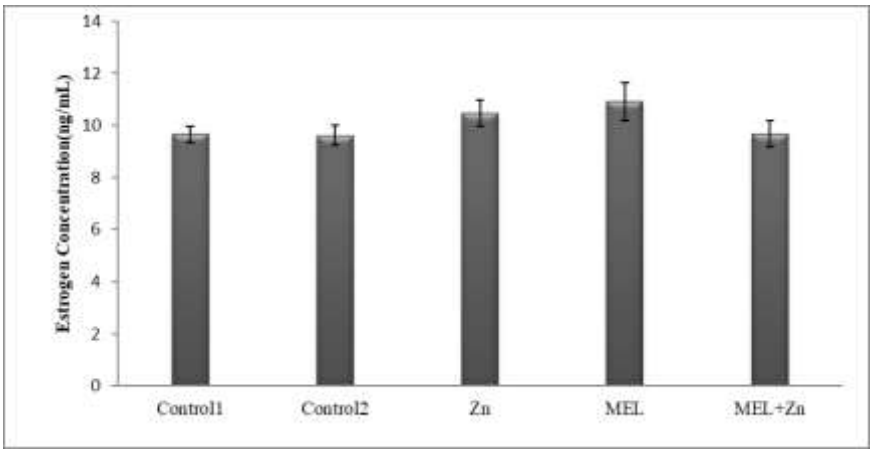

Figure 4. The effect of Zn (40 ppm), melatonin (5 mg/kg), and Zn (40 ppm) + melatonin (5 mg/kg) on the mean estrogen concentration in female rats (n=7). Information are expressed in the form of an average ± the standard error of the mean.

between the treatments (P>0.05). The average estrogen was higher in rats receiving 5 mg melatonin per day than in other rats, but this difference could not be considered statistically significant (P>0.05).

Figure 5 shows that the average progesterone was 43.54 (ng/ml) in control group1, 43.23 (ng/ml) in control group 2, 44.97 (ng/ml) in the zinc group, 47.43 (ng/ml) in the melatonin group, and 45.99 (ng/ml) in the zinc and melatonin group. The results showed no significant difference in progesterone levels between the treatments (P>0.05). In the rats receiving 5 mg melatonin per day , the average progesterone level was higher than in the other rats, but statistical analysis showed that this difference was not significant (P>0.05).

Figure 6 shows that the average TSH level was 4.43 (ng/ml) in control group 1, 4.23 (ng/ml) in control group 2, 4.20 (ng/ml) in the zinc group, 4.10 (ng/ml) in the melatonin group, and 4.21 (ng/ml) in the zinc and melatonin group (ng/ml). The results showed no significant difference in TSH levels between the treatments (P>0.05). The average TSH level was higher in the rats of control group 1 than in the other rats, but this difference from the other treatments was not statistically remarkable (P>0.05).

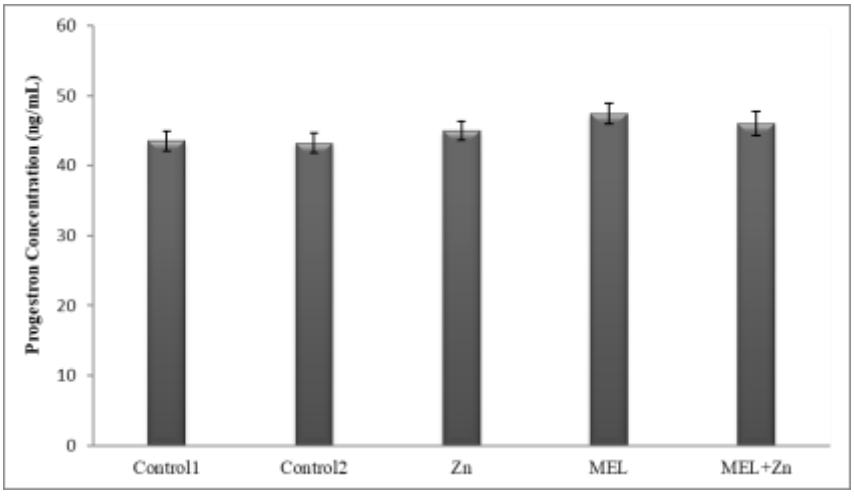

Figure 5. The effect of Zn (40 ppm), melatonin (5 mg/kg), and Zn (40 ppm) + melatonin (5 mg/kg) on the mean progesterone concentration in female rats (n=7). Information are expressed in the form of an average ± the standard error of the mean.

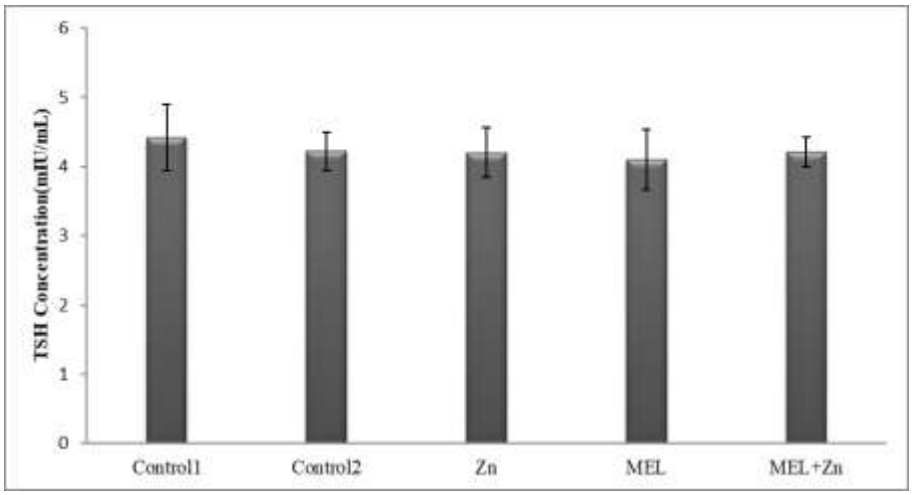

Figure 6. The effect of Zn (40 ppm), melatonin (5 mg/kg), and Zn (40 ppm) + melatonin (5 mg/kg) on the mean TSH concentration in female rats (n=7). Information are expressed in the form of an average ± the standard error of the mean.

Figure 7 shows that the average T_3 was 5.67 (pmol/ml) in control group 1, 5.67 (pmol/ml) in control group 2, 5.06 (pmol/ml) in the zinc group, 5.36 (pmol/ml) in the melatonin group, and 5.36 (pmol/ml) in the zinc and melatonin group. The results showed a significant difference between the treatments in the amount of T_3 ($P< 0.05$). The results also showed that there was a notable difference between the rats receiving 40 mg zinc daily and control groups 1 and 2 ($P< 0.05$). From the difference in mean values, it can be concluded that the T3 level decreased significantly in the group that received zinc ($P< 0.05$).

Figure 8 shows that the T_4 level in control group 1 was 2.19 (pmol/ml), in control group 2 2.04 (pmol/ml), in the zinc group 2.30 (pmol/ml), in the melatonin group 1.90 (pmol/ml) and in the zinc and melatonin group 1.91 (pmol/ml). The results showed no significant changes in T_4 levels in the test groups ($P>0.05$).

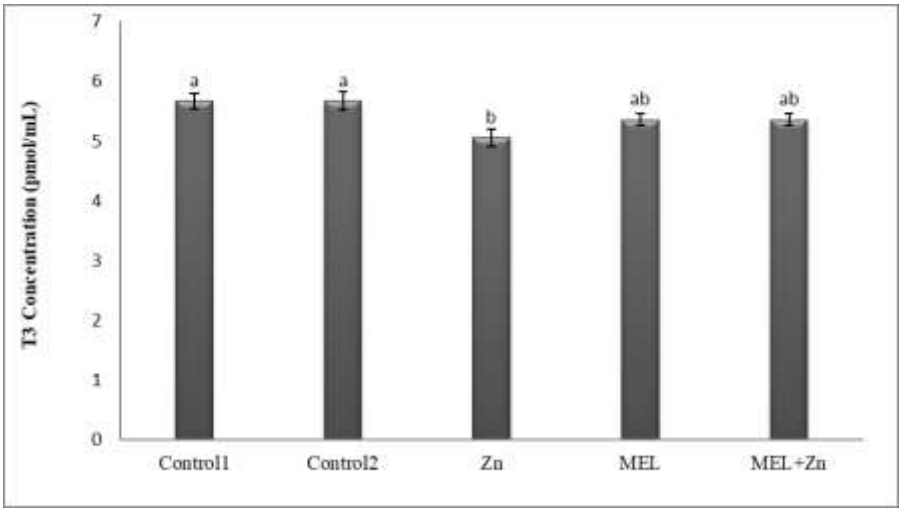

Figure 7. The effect of Zn (40 ppm), melatonin (5 mg/kg), and Zn (40 ppm) + melatonin (5 mg/kg) on the mean T_3 concentration in female rats (n=7). Information are expressed in the form of an average ± the standard error of the mean. Distinct letters (a and b) are used to denote significant variations between the different trials ($P< 0.05$).

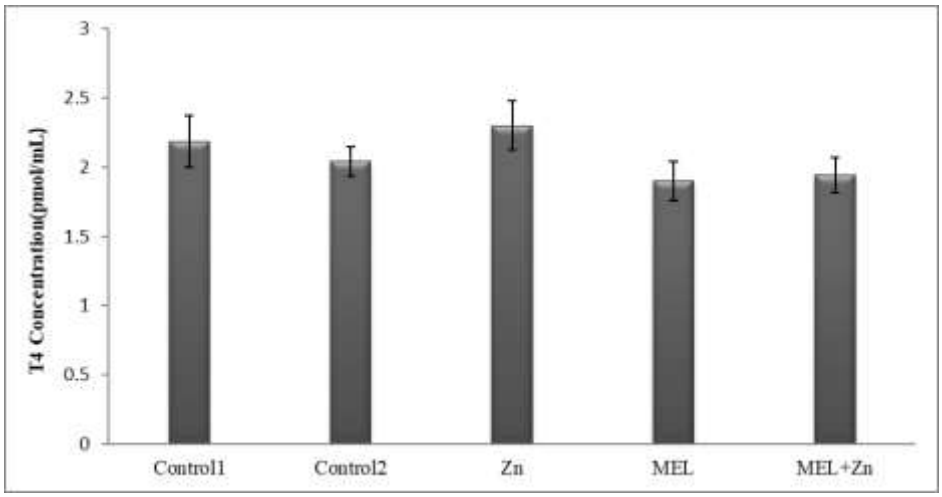

Figure 8. The effect of Zn (40 ppm), melatonin (5 mg/kg), and Zn (40 ppm) + melatonin (5 mg/kg) on the mean T_4 concentration in female rats (n=7). Information are expressed in the form of an average ± the standard error of the mean.

4. Discussion

Melatonin is known for its contribution to animal reproduction. Zinc is also an essential microelement that plays an important role in the body, including in the reproductive system (21). Each of the aforementioned elements is critical for reproductive function (22, 23); however, no report has been found that examines the effects of the combination of zinc and melatonin on thyroid and gonadotropin hormones in animals or humans. In the present study, we investigated this question to determine the association between these supplements and the above hormones. According to our results, no significant differences were found between the experimental treatments in FSH and LH concentrations. The estrogen and progesterone levels of the rats receiving 5 mg melatonin per day were higher than in the other groups, but the difference was not statistically remarkable. In addition, the TSH level in control group 1 was higher than in the other groups, although not statistically. In the group receiving 40 mg zinc, however, the T_3 level decreased significantly. No significant difference was found in T_4 levels between the treatments . In agreement with the results of our study, a significant decrease in T3 levels and no change in serum T4 levels was observed in an experiment in rats receiving ZnCl2 (24). Another study also showed that serum T3 levels were significantly reduced during treatment with zinc acetate, while no significant changes were observed in T4 and TSH levels compared to the control group (25). It is possible that the decrease in serum T3 levels is related to a disturbance in the conversion of T4 to T3 (24, 26). Other researchers have reported that thyroid secretion at the endocrine level impairs animal productivity (27). The secretion of thyroid hormones decreased in the rats in our study as a result of zinc supplementation. Since , as previous studies have shown , thyroid hormones are crucial for the proper functioning of the reproductive system, especially in female animals, e.g. for the metabolism and growth of ovarian, uterine and placental tissue (28), this zinc-depleting effect on thyroid hormones could have a negative impact on the reproductive activity of female rats.

Melatonin was also found to have a positive effect as it has free radical scavenging properties in addition to stimulating various other antioxidant enzymes (29-32). Regarding the effects of melatonin on female sexual processes, it should be emphasized that melatonin receptors have been identified in different cell types of the reproductive system in female animals. The documentation also points to dual

expression of MT1 and MT2 receptors as potential targets for melatonin action (33-37). In addition, melatonin affects several pathways that modulate Sertoli cells, leading to sperm production as well as estrogen and progesterone production (38). Melatonin also has an enormous antioxidant effect to eliminate harmful substances, such as ROS. The unmediated scavenging of free radicals is not dependent on a receptor. Reactive oxygen species play an important role in sexual functions, including ovulation. However, an excess of reactive oxygen species can also have a detrimental effect on the egg due to oxidative stress and thus cause sterility. Melatonin has been shown to be present in the follicular fluid of the ovary and in the oocyte itself, protecting it from oxidative stress while having beneficial effects on oocyte maturation, fertilization and embryonic development. Human experiments have investigated the improved outcomes of reproductive technologies, such as artificial insemination and embryo transfer (IVF-ET) by prescribing melatonin to sterile clients (39). In another study, researchers investigated the relationship between melatonin and hyperthyroidism (40). Hyperthyroidism is caused by excessive secretion of thyroid hormones with varying clinical and biochemical results (41). Hyperthyroidism has negative effects on the cardiovascular, genitourinary and endocrine systems, electrophysiological functions and oxidative metabolism in various organs. The main function of melatonin, which is secreted by the pineal gland, is to control the circadian rhythm (42, 43). Numerous experiments have indicated the inhibitory effect of melatonin on the thyroid gland and its function. Various methods have been used under laboratory conditions (test tubes or test containers) and on living organisms, including laboratory animals. Studies have been conducted on the short and long-term administration of melatonin, on the impairment of pineal gland activity by light and on the removal of the pineal gland . Melatonin may have a direct or indirect effect on the thyroid gland and its functions; however, the basic mechanisms are not yet fully understood. Considering the inhibitory effects of melatonin on the thyroid gland and hormone secretion as well as its antioxidant effects, it could represent a new possibility for the treatment of hyperthyroidism (44). Laskar et al. (45) investigated the effects of exogenous melatonin on thyroid hormone concentrations and the expression patterns of melatonin receptor proteins (MT1, MT2) on the thyroid gland in male and female rats of different age groups. The pineal melatonin hormone controls various endocrine processes, such as the regulation of thyroid hormones under different physiological conditions of the body. Melatonin exerts many of its functions via membrane-bound receptors (MT1 and MT2). In the above study, the effects of melatonin on the levels of thyroid hormones and the expression patterns of MT1 and MT2 on the thyroid gland were to be investigated at different ages (two, four and eight months) in albino Swiss rats. In the group of male rats, the administration of melatonin can inhibit the T_4 hormone without changing the TSH level in the age group of two and four months , but in the age group of eight months it caused an increase in the T_4 hormone as well as the TSH concentration. In the group of female rats, melatonin increased the T4 level, while the TSH concentration remained unchanged in all age groups of rats. However, melatonin signifcantly reduced the expression of thyroid MT1 receptor proteins in female and male rats. In contrast, thyroid MT2 receptor proteins were differentially expressed by melatonin supplementation in the different age groups of male and female rats. These results showed that melatonin, gender and age have different effects on T_4 levels. This research also showed that melatonin may favor MT2 receptors on the thyroid gland to regulate the release of the hormone T4, which is dependent on age and gender in albino rats (45, 46). Chowdhury and his group (46) investigated the role of melatonin in the release of gonadotropin-inhibiting hormone (GnIH) in the birds' hypothalamus. GnIH, a hormone that inhibits the formation and release of gonadotropin, was first discovered in the

hypothalamus of quails. The gonadotropin -inhibiting hormone acts on the pituitary gland, and the GnIH neurons in the hypothalamus are responsible for the growth and maintenance of the gonads. In addition, gonadotropin-inhibiting hormone expresses melatonin receptors and melatonin expresses GnIH receptors in the quail brain. Therefore, melatonin appears to be an essential element of GnIH activity. The results show that melatonin not only plays a role in increasing GnIH expression, but also in the secretion of GnIH, thereby inhibiting the plasma concentration of LH in quail (47).

In one study, the influence of Zn on the metabolic function of the thyroid gland was investigated. The thyroid gland is the most important gland of the endocrine system in the body, which fulfills numerous tasks. Thyroid hormones, such as T4 and T3 are crucial for metabolic processes and are also required for the optimal functioning of tissues. These hormones influence oxygen uptake and metabolism. The key element of thyroid hormones is iodine. In regions where the amount of iodine is reduced, thyroid-related disorders and ailments are extremely common. Thyroid disorders are the most widespread functional disorder of the endocrine system worldwide. Thyroid dysfunction is caused by either excessive or insufficient secretion of thyroid hormones. In addition, Zn deficiency is associated with increased expression of hepatic thyroxine-5-deiodinase, an enzyme that deactivates thyroid hormones (48).

Overall, the present study shows that except for T_3, none of the parameters in this study, including T_4, TSH, FSH and LH as well as estrogen and progesterone hormones, were affected by the administration of zinc and melatonin to female rats. In addition, the combination of these supplements in the current study had no adverse effects on the measured parameters, demonstrating the safety of the use of these supplements.

Acknowledgment

This research is part of the Ph.D. thesis of the first author and was supported by the Veterinary Medicine of Science and Research Branch, Islamic Azad University, Tehran, Iran. Also, we thank the central laboratory (Dr. Rastegar Lab.) of Faculty of veterinary medicine, university of Tehran, Tehran, Iran.

Authors' Contribution

Study concept and design: F. R. and M.J.R

Acquisition of data: F. R.

Analysis and interpretation of data: M.J.R.

Drafting of the manuscript: F. R., M.Z. and S.F.

Critical revision of the manuscript for important intellectual content: F. R., M.Z., M.J.R and S.F.

Ethics

All trials were performed according to the Guide for the Care and Use of Laboratory Animals and were approved by the institutional animal ethics committee.

Conflict of Interest

The writers declare to the fact that they have no conflict of interest.

Funding

This study was supported by Science and Research Branch, Islamic Azad University, Tehran, Iran.

References

1. Li Y, Yin Z, Zhang Y, Liu J, Cheng Y, Wang J, Pi F, Zhang Y, Sun X. Perspective of Microbe-based Minerals Fortification in Nutrition Security. Food Rev. Int. 2022;38(3):268-81.
2. Harlow HJ. Influence of the pineal gland and melatonin on blood flow and evaporative water loss during heat stress in rats. J Pineal Res. 1987;4(2):147-59.
3. Bonior J, Jaworek J, Konturek SJ, Pawlik WW. Increase of heat shock protein gene expression by melatonin in AR42J cells. J Physiol Pharmacol. 2005;56(3):471.
4. Vittorio U, Emanuela R, Piero R, Silvia B. Effect of a supplementation with myo-inositol plus melatonin on oocyte quality in women who failed to conceive in previousin vitrofertilization cycles for poor oocyte quality: a prospective, longitudinal, cohort study. Gynecol Endocrinol. 2011;27(11):857-61.

5. Lampiao F, Du Plessis SS. New developments of the effect of melatonin on reproduction. World J Obstet Gynecol. 2013;10;2(2):8-15.
6. Eryilmaz OG, Devran A, Sarikaya E, Aksakal FN, Mollamahmutoğlu L, Cicek N. Melatonin improves the oocyte and the embryo in IVF patients with sleep disturbances, but does not improve the sleeping problems. J Assist Reprod Genet. 2011;28:815-820.
7. Tamura H, Takasaki A, Taketani T, Tanabe M, Kizuka F, Lee L, Tamura I, Maekawa R, Asada H, Yamagata Y. Melatonin as a free radical scavenger in the ovarian follicle. Endocr J. 2013; 60:1-13.
8. Kim MK, Park EA, Kim HJ, Choi WY, Cho JH, Lee WS, Cha KY, Kim YS, Lee DR, Yoon TK. Does supplementation of in-vitro culture medium with melatonin improve IVF outcome in PCOS. Reprod Biomed Online. 2013;26:22-29.
9. Lipovac MG. Antioxidative effect of melatonin on human spermatozoa. Arch Androl. 2000;44(1):23-7.
10. Hapon MB, Simoncini M, Via G, Jahn GA. Effect of hypothyroidism on hormone profiles in virgin, pregnant and lactating rats, and on lactation. Reprod. 2003;126(3):371-82.
11. Tordjman S, Chokron S, Delorme R, Charrier A, Bellissant E, Jaafari N, Fougerou C. Melatonin: pharmacology, functions and therapeutic benefits. Curr Neuropharmacol. 2017;15(3):434-43.
12. Adah Sylvanus A, Adah Arimie D, Nwonuma Charles O, Oyekunle T, Olaosebikan B. Melatonin modulates haematological and water quality parameters following a 100 km transportation of Clarias gariepinus by road. *Iran* J Vet Med. *2022*. doi: 10.22059/ijvm.2022. 348436.1005310
13. Zawilska JB, Skene DJ, Arendt J. Physiology and pharmacology of melatonin in relation to biological rhythms. Pharmacol Rep. 2009;61(3):383-410.
14. Maciel LM, Magalhães PK. Thyroid and pregnancy. Arq Bras Endocrinol Metabol. 2008;52:1084-95.
15. Maganhin CC, Fuchs LF, Simões RS, Oliveira-Filho RM, de Jesus Simões M, Baracat EC, Soares Jr JM. Effects of melatonin on ovarian follicles. Eur J Obstet Gynecol. 2013;166(2):178-84.
16. Tamura H, Takasaki A, Miwa I, Taniguchi K, Maekawa R, Asada H, Taketani T, Matsuoka A, Yamagata Y, Shimamura K, Morioka H. Oxidative stress impairs oocyte quality and melatonin protects oocytes from free radical damage and improves fertilization rate. J Pineal Res. 2008;44(3):280-7.
17. Yilmaz M, Mogulkoc R, Baltaci AK. Effect of three-week zinc and melatonin supplementation on the oxidant-antioxidant system in experimental renal ischemia-reperfusion in rats. Acta Clin Croat. 2015;54(4).
18. Veldhuis JD, Liem AY, South S, Weltman A, Weltman J, Clemmons DA, Abbott R, Mulligan T, Johnson ML, Pincus S. Differential impact of age, sex steroid hormones, and obesity on basal versus pulsatile growth hormone secretion in men as assessed in an ultrasensitive chemiluminescence assay. J Clin Endocrinol Metab. 1995;80(11):3209-22.
19. Shah N, Evans WS, Veldhuis JD. Actions of estrogen on pulsatile, nyctohemeral, and entropic modes of growth hormone secretion. Am J Physiol Regul Integr Comp Physiol. 1999;276(5):R1351-8.
20. Caufriez A, Leproult R, L'Hermite-Balériaux M, Kerkhofs M, Copinschi G. Progesterone prevents sleep disturbances and modulates GH, TSH, and melatonin secretion in postmenopausal women. J Clin Endocrinol Metab. 2011;96(4):E614-23.
21. Nasiadek M, Stragierowicz J, Klimczak M, Kilanowicz A. The role of zinc in selected female reproductive system disorders. Nutrients. 2020;12(8):2464.
22. Garner TB, Hester JM, Carothers A, Diaz FJ. Role of zinc in female reproduction. Biol Reprod. 2021;104(5):976-994.
23. Yong W, Ma H, Na M, Gao T, Zhang Y, Hao L, Yu H, Yang H, Deng X. Roles of melatonin in the field of reproductive medicine. Biomed Pharmacother. 2021;144:112001.
24. Moustafa SA. Effect of glutathione (GSH) depletion on the serum levels of triiodothyronine (T3), thyroxine (T4), and T3/T4 ratio in allyl alcohol-treated male rats and possible protection with zinc. Int J Toxicol. 2001;20(1):15-20.
25. Piao F, Yokoyama K, Ma N, Yamauchi T. Subacute toxic effects of zinc on various tissues and organs of rats. Toxicol Lett. 2003;145(1):28-35.
26. Chaurasia SS, Gupta P, Kar A, Maiti PK. Lead induced thyroid dysfunction and lipid peroxidation in the fish Clarias batrachus with special reference to hepatic type I-5′-monodeiodinase activity. Bull Environ Contam Toxicol. 1996;56:649-654.
27. Todini L. Thyroid hormones in small ruminants: effects of endogenous, environmental and nutritional factors. Animal. 2007;1(7):997-1008.
28. Silva JF, Ocarino NM, Serakides R. Thyroid hormones

and female reproduction. Biol Reprod. 2018;99(5):907-921.
29. Webb SM, Puig-Domingo M. Role of melatonin in health and disease. Clin Endocrinol. 1995;42(3):221-34.
30. Zang LY, Cosma G, Gardner H, Vallyathan V. Scavenging of reactive oxygen species by melatonin. Biochim Biophys Acta Gen Subj. 1998;1425(3):469-77.
31. Reiter RJ, Tan DX, Osuna C, Gitto E. Actions of melatonin in the reduction of oxidative stress. J Biomed Sci. 2000;7(6):444-58.
32. Escames G, Ozturk G, Baño-Otálora B, Pozo MJ, Madrid JA, Reiter RJ, Serrano E, Concepción M, Acuña-Castroviejo D. Exercise and melatonin in humans: reciprocal benefits. J pineal res. 2012;52(1):1-1.
33. Webley GE, Luck MR. Melatonin directly stimulates the secretion of progesterone by human and bovine granulosa cells in vitro. Reprod. 1986;78(2):711-7.
34. Webley GE, Luck MR, Hearn JP. Stimulation of progesterone secretion by cultured human granulosa cells with melatonin and catecholamines. Reprod. 1988;84(2):669-77.
35. Niles LP, Wang J, Shen L, Lobb DK, Younglai EV. Melatonin receptor mRNA expression in human granulosa cells. Mol Cell Endocrinol. 1999;156(1-2):107-10.
36. Woo MM, Tai CJ, Kang SK, Nathwani PS, Pang SF, Leung PC. Direct action of melatonin in human granulosa-luteal cells. J Clin Endocrinol Metab. 2001;86(10):4789-97.
37. Taketani T, Tamura H, Takasaki A, Lee L, Kizuka F, Tamura I, Taniguchi K, Maekawa R, Asada H, Shimamura K, Reiter RJ. Protective role of melatonin in progesterone production by human luteal cells. J pineal res. 2011;51(2):207-13.
38. Cipolla-Neto J, Amaral FG, Soares Jr JM, Gallo CC, Furtado A, Cavaco JE, Gonçalves I, Santos CR, Quintela T. The crosstalk between melatonin and sex steroid hormones. Neuroendocrinol. 2022;112(2):115-29.
39. Tamura H, Jozaki M, Tanabe M, Shirafuta Y, Mihara Y, Shinagawa M, Tamura I, Maekawa R, Sato S, Taketani T, Takasaki A. Importance of melatonin in assisted reproductive technology and ovarian aging. Int J Mol Sci. 2020;21(3):1135.
40. D'Angelo G, Marseglia L, Manti S, Colavita L, Cuppari C, Impellizzeri P, Arena S, Arrigo T, Salpietro C, Gitto E. Atopy and autoimmune thyroid diseases: melatonin can be useful? Ital J Pediatr. 2016;*42*:1-6.
41. De Leo S, Lee SY, Braverman LE. Hyperthyroidism. The Lancet. 2016;388(10047):906-918.
42. Díez JJ, Iglesias P. Spontaneous subclinical hypothyroidism in patients older than 55 years: an analysis of natural course and risk factors for the development of overt thyroid failure. J Clin Endocrinol Metab. 2004;89(10):4890-7.
43. Kaklamanos I, Zarokosta M, Flessas I, Zoulamoglou M, Katsoulas T, Birbas K, Troupis T, Mariolis-Sapsakos T. Surgical anatomy of double pyramidal lobe on total thyroidectomy: a rare case report. J Surg Case Rep. 2017;2017(3):rjx035.
44. Üstündağ H, Şentürk E, Gül M. Melatonin and hyperthyroidism. Arch Basic Clin Res. 2020;2(2):59-64.
45. Laskar P, Acharjee S, Singh SS. Effect of Exogenous Melatonin on Thyroxine (T 4), Thyrotropin (TSH) Hormone Levels and Expression patterns of Melatonin Receptor (MT1 and MT2) Proteins on Thyroid gland during Different age groups of Male and Female Swiss albino Mice. Adv biores. 2015;6(1).
46. Badi N, Fazelipour S, Naji T, Babaei M, Kalantari Hesari A. Histomorphometric and Biochemical Study of Liver and Thyroid Hormones Following Administration of MoO3 Nanoparticles in Female Rats. Iran J Vet Med. 2022; 16(2): 188-201. doi: 10.22059/ijvm.2021.330872.1005196
47. Chowdhury VS, Yamamoto K, Ubuka T, Bentley GE, Hattori A, Tsutsui K. Melatonin stimulates the release of gonadotropin-inhibitory hormone by the avian hypothalamus. Endocrinol. 2010;151(1):271-80.
48. Khanam S. Impact of zinc on thyroid metabolism. J Diabetes Metab Disord Control. 2018;5(1):27-8.