REVIEW ARTICLE

A Study of the Impact of Serum Melatonin Level on Males Infertility and Reproductive Hormones and its Correlation to Semen Parameters

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Abstract

Background: Infertility is the inability of couples to achieve pregnancy after one year of regular unprotected coitus. Infertility is the problem of 15% of young couples in different countries and male factor infertility accounts for 50% of cases. Melatonin or 5-methoxy-N-acetyltryptamine was discovered by Aaron Lerner in 1958 while studying bovine pineal glands. Significant advancements have been made in the research on melatonin, which has revealed its vital roles in regulating sleep-wake cycle rhythms, decelerating the aging process, and functioning as an antioxidant or anti-inflammatory agent. Additionally, melatonin, a critical component in reproductive functions, can eliminate free radicals such as (ROS).

Objective: The present study aims to evaluate the impact of Melatonin on male infertility.

Materials and methods: Serum melatonin was measured by using the Elabscience ELISA kit. A case-control study included the fertile group as the control (n=40) and the infertile group (n=50). All the subjects were included in this study between October 2022 and June 2023. The diagnosis of the participants was executed by a medical senior based on clinical characteristics, the history of patients, and biochemical tests. Age, family history, and BMI were measured. Blood samples were provided by each participant for the biochemical estimation of serum melatonin, FSH, LH, and testosterone. While semen samples were collected from each participant for the seminal fluid analysis.

Results: The results of this study showed a decrease in the level of melatonin in infertile men as compared with fertile men. Moreover, there was no significant correlation between melatonin and FSH, LH, testosterone, and some semen parameters.

Conclusions: The study indicated that decreased melatonin levels can be associated with male infertility, but it did not indicate a direct relationship between melatonin and hormone levels or specific semen parameters.

Key words: Male infertility; Melatonin; FSH, LH; Testosterone; Semen parameters

Introduction:

The World Health Organization (WHO) defines infertility as the failure to achieve pregnancy after one year of regular unprotected coitus. There are many potential causes of male infertility, including anatomical abnormalities, sexual dysfunction, varicocele, obesity, oligospermia, smoking, and heavy metal toxicity (1). Infertility is the problem of 15% of young couples in different countries and male factor infertility accounts for 50% of cases. Thus, any support for this issue would develop the health status of the human being (2). Male infertility has been linked to genetic and pathological factors such as abnormal hormonal levels, varicocele, cystic fibrosis gene mutations, Y chromosome abnormalities, testicular cancer, epigenetic errors, pituitary tumors, and even idiopathic factors (3). Moreover, it has been indicated that environmental, occupational, and lifestyle factors can affect male infertility, compromising sperm quality. Indeed, studies have demonstrated that elements like alcohol consumption, cigarette smoking, obesity, radiation, genital heat stress, dietary practices, and illicit drug use contribute to a decline in human sperm quality (4). Treatment for male infertility depends on the underlying cause. Options include medication to improve sperm production, surgery to correct

obstructions or other problems in the reproductive tract, and assisted reproductive technologies (ART), such as in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI). It is important for men experiencing fertility issues to seek medical evaluation and treatment (5). Melatonin or 5-methoxy-N-acetyltryptamine was discovered by Aaron Lerner in 1958 while studying bovine pineal glands (6). Melatonin has been present in organisms for a very long time and has been conserved throughout evolution (7). Tryptophan undergoes hydroxylation to form 5-hydroxytryptophan in animals. This compound is then decarboxylated to produce serotonin. Serotonin can either be acetylated to give N-acetylserotonin or methylated to form 5-methoxytryptamine. These compounds are further methylated or acetylated to form melatonin (8). In mammals, including humans, the neurohormone melatonin mainly acts on two high-affinity G protein-coupled receptors, the melatonin MT1 and MT2 receptors (9). It is primarily produced during the dark phase of the circadian cycle. This hormone is critical in the regulation of circadian changes in a variety of physiological and neuroendocrine functions (10). Significant advancements have been made in the research on melatonin, which has revealed its vital roles in regulating sleep-wake cycle rhythms, decelerating the aging process, and functioning as an antioxidant or anti-inflammatory agent. Additionally, melatonin, a critical component in reproductive functions, has the ability to eliminate free radicals such as (ROS) (11). Melatonin was discovered in 1993 to be a highly effective and efficient endogenous radical scavenger (12). Melatonin and its metabolites have a wide range of anti-oxidative properties, such as the capability to neutralize superoxide anion, hydroxyl radical, single oxygen, hydrogen peroxide, hypochlorous acid, nitric oxide, and peroxy-nitrite anion (13). It enhances human spermatozoa motility by protecting them from apoptosis and DNA fragmentation caused by ROS, thereby preserving sperm viability in the reproductive tract during transportation (14). It also has the capacity to impact male reproductive processes through the regulation of hypothalamic gonadotropin-releasing hormone and pituitary luteinizing hormone, which are crucial hormones involved in male reproductive regulation. In addition, the testicular cell functions can be directly affected by Melatonin. A notable benefit of melatonin in terms of testicular functions is its ability to eliminate free radicals, thus protecting the testes from oxidative damage (15). Melatonin has been shown to affect the anterior pituitary gonadotropins, gonadal steroids, and testicular activities in humans through particular receptors that are highly expressed in the reproductive organs and the central nervous system (16). Testes exposed to endocrine toxicants or genital infectious diseases can exhibit spermatogenic disruption (17). It is well known for its ability to preserve the testicles from these reproductive disruptions and the ability to protect sperm cells from ischemia and enhance sperm abnormalities as a result of its antioxidant activities (18). High-melatonin sperm maturation medium has been shown to boost sperm mitochondrial activity, elevate the number of motile sperms, and accelerate sperm progressive motility while lowering endogenous nitric oxide levels (19). Male fertility is primarily regulated by the endocrine system, which is responsible for producing and regulating the hormones necessary for the development and maintenance of the male reproductive system. The endocrine system is a complicated network of glands that produce and release hormones into the bloodstream, which then travel to target organs and tissues, where they exert their effects (20). Moreover, it is regulated by several key hormones, namely GnRH, LH, and FSH. The hypothalamus produces GnRH, which prompts the pituitary gland to secrete both LH and FSH. LH, on the other hand, triggers the Leydig cells located in the testes to manufacture testosterone. Meanwhile, FSH stimulates the Sertoli cells present in the testes to support the production of sperm (21).

The Methods

The Study Design

ase-control comparative study was used to find out the effect of the serum level of Melatonin on infertile men when being compared with fertile ones, in the private medical clinics in Babylon Governorate in Iraq.

The Setting of the Study

This study had been conducted on patients with infertility, taking all the details of the patient's condition such as (family history, age, weight, and height). All the tests were conducted in the laboratories of the Department of Medicinal Chemistry in the College of Medicine at the University of Al-Qadisiyah.

The Patients

50 patients (infertile) with an average age of 20-45 years old were involved in this study as well as 40 healthy subjects (fertile) as controls with the same age, having at least one child. All the subjects were included in this study between October 2022 and June 2023, they signed a written informed consent form by themselves. All the study methods were approved by the Ethical Committee of the College of Medicine University at Al-Qadisiyah in Al-Diwaniyah, Iraq. The diagnosis of the participants was executed by medical seniors based on the clinical characteristics, history of patients, and biochemical tests. Finally, age, family history, and BMI were measured.

The Inclusion Criteria

All the males who were diagnosed with primary infertility by specialist doctors were included in the study.

The Exclusion Criteria

All the males with diabetes mellitus, autoimmune diseases, and chronic conditions were excluded from the study. The males with secondary infertility and azoospermia were also excluded, in addition to several other males who had not abstained from sexual intercourse for at least 2 days.

The Collection of Blood Samples

In this study, each participant provided a blood sample of 5 ml through vein puncture. The collected blood was transferred into sterile gel tubes and left to clot for 30 minutes. Afterward, the serum was separated by centrifugation at 4000 rpm for 15 to 20 minutes at room temperature (24-25°C). The resulting serum was then preserved for biochemical analysis using Eppendorf tubes, which were kept at a temperature of -40 °C.

The Collection of Semen Samples

The male participants were asked to masturbate to obtain semen samples, which were then immediately collected in a sterile, wide-mouth container. To improve the quality and quantity of the seminal fluid, both the patients and normal individuals were advised to abstain from sexual intercourse for three days before the sample collection.

The Assay Procedure

The Seminal Fluid Analysis

The semen samples were subjected to a 30-minute liquefaction process before undergoing basic semen analyses. The WHO 2010 guidelines were used to assess oligozoospermia, asthenozoospermia, and teratozoospermia based on the percentage of sperm motility, sperm count (1x106 cells/ml), and abnormal head or tail forms of sperm, which were observed under a light microscope at a magnification of x100 using a hemocytometer. The deformity rate (%) was determined by using an auto-analyzer microscope.

The Melatonin Level

The melatonin level was measured using E-lab science ELISA kits as it is illustrated in the following steps:

1. 50 µL of standard or sample was added to the wells, and immediately after, 50 µL of Biotinylated detection Ab working solution was added to each well. The plate was then incubated

for 45 minutes at 37□.
2. The plate was aspirated and washed three times.
3. 100 µL HRP conjugate working solution was added, and the plate was incubated for 30 minutes at 37□. Then, it was aspirated and washed five times.
4. 90 µL substrate reagent was added and incubated for 15 min at 37□.
5. 50 µL stop solution was added.
6. The plate was read at 450 nm immediately.

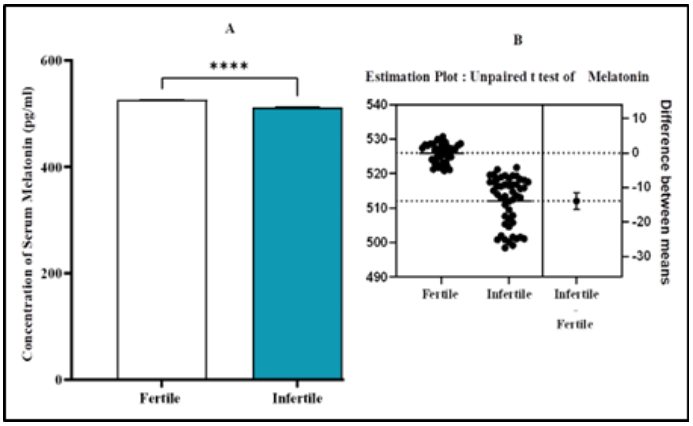
FSH, LH, and Testosterone

The levels of FSH, LH, and Testosterone hormones were measured using DRG ELISA kits in the following steps:
1. The desired number of Microtiterwells were placed into the holder securely.
2. New disposable tips were used and 25 µL of each Standard, control, and sample were dispensed into the appropriate wells.
3. 100 µL of Enzyme Conjugate was dispensed into each well and mixed thoroughly for 10 seconds to ensure complete mixing.
4. The plate was incubated at room temperature for 30 minutes.
5. The contents of the wells were vigorously shaken out and each well was rinsed five times with 400 µL of aqua dest. After the rinsing process, the wells were struck sharply on absorbent paper to remove any residual droplets.
6. 100 µL of Substrate Solution was added to each well.
7. The plate was incubated at room temperature for 10 minutes.
8. 50 µL of Stop Solution was added to each well to stop the enzymatic reaction.
9. A microtiter plate reader was used to determine the absorbance (OD) of each well at 450±10 nm.

The Results

The Measurement of Melatonin

The measurement of melatonin concentration (pg/mL) in serum was found to be significantly lower in the infertile group as compared with the fertile group (512.0 ± 7.077),(526.0 ± 2.699) (pg/mL) respectively; the significant difference (p-value <0.0001) as in Figure (1).

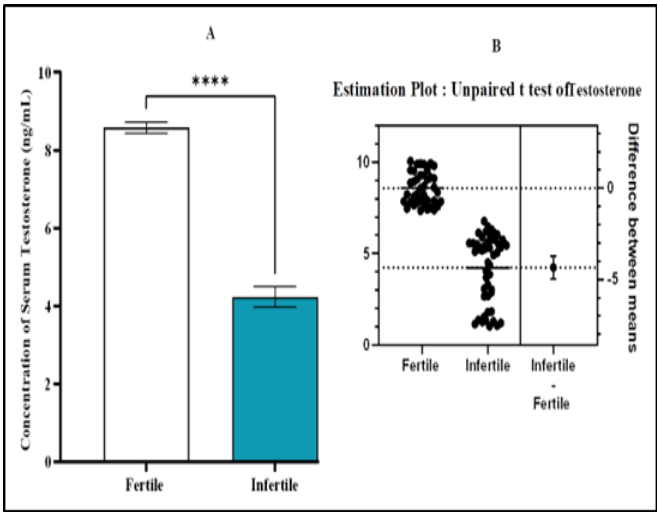


Figure(1) Estimation of serum Melatonin (pg/mL). (A) a comparison between the fertile and infertile groups, (B) an estimation plot that illustrates the presence of a significant decrease in the level of melatonin in the infertile group as compared to the infertile, the significant difference (p-value <0.0001). Data are expressed as means ± SD. indicaMelatonins *significant differences compared to the control, P≤0.05.

The Measurement of FSH, LH, and Testosterone

The Serum Testosterone Measurement

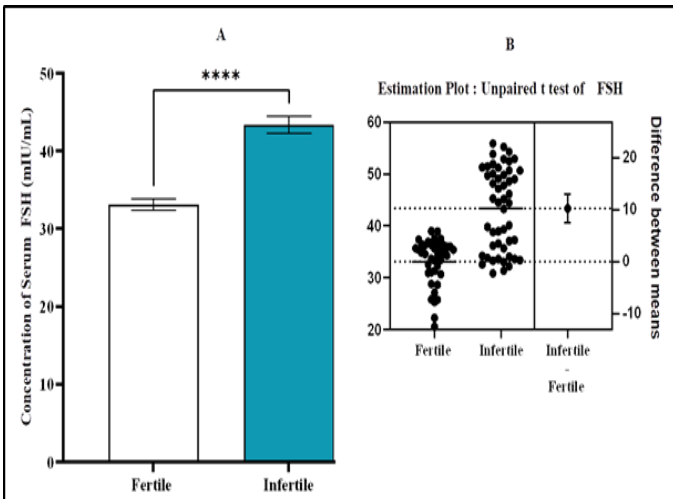
The results showed decreased levels of testosterone concentration (TE) (ng/mL) in the infertile as compared with the fertile group (4.233 ± 1.846),(8.582 ± 0.869) (ng/mL) respectively; the significant difference (p-value <0.0001) as in Figure (2).



Figure(2) Estimation of serum Testosterone ((ng/mL). (A) a comparison between the fertile and infertile groups, (B) an estimation plot that illustrates the presence of a significant decrease in the level of TE in the infertile group as compared to the infertile, the significant difference (p-value <0.0001). Data are expressed as means ± SD. indicates *significant differences compared to the control, P≤0.05.

The Follicle Stimulating Hormone (FSH) Measurement

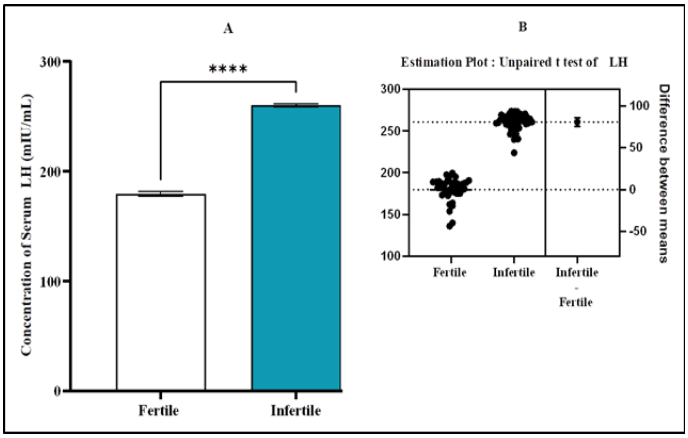
The results showed increased levels of follicle-stimulating hormone (FSH) concentration (mIU/mL) in the infertile group as compared with the fertile group (43.37± 7.906),(33.12± 4.480) (mIU/mL) respectively; the significant difference was (p-value <0.0001) as in Figure (3).



Figure(3) Estimation of serum Follicle stimulating hormone (FSH) (mIU/mL). (A) a comparison between the fertile and infertile groups, (B) an estimation plot that illustrates the presence of a significant decrease in the level of FSH in the infertile group as compared to the infertile, the significant difference (p-value <0.0001). Data are expressed as means ± SD. indicates *significant differences compared to the control, P≤0.05.

The Luteinizing Hormone (LH) Measurement

The results showed increased levels of luteinizing hormone concentration (LH) (mIU/mL) in the infertile participants as compared with the fertile group (260.4± 10.07), (179.6± 13.85) (mIU/mL) respectively; the significant difference (p-value <0.0001) as in Figure (5).



Figure(5) Estimation of serum Luteinizing hormone (LH) (mIU/mL). (A) a comparison between the fertile and infertile groups, (B) an estimation plot that illustrates the presence of a significant decrease in the level of LH in the infertile group as compared to the infertile, the significant difference (p-value <0.0001). Data are expressed as means ± SD. indicates *significant differences compared to the control, P≤0.05.

Table(1) Comparison of mean values of the studied biomarkers among the fertile and infertile groups.

Characteristic	Fertile	Infertile	P-value
	n= 40	*n*=50	
Melatonin (pg/mL)			
Range	520.8 – 530.7	498.4 – 521.7	<0.0001 ****
Mean ± SD	526.0 ± 2.699	512.0 ± 7.077	
Testosterone (ng/mL)			
Range	7.353 – 10.06	1.024 – 6.774	<0.0001 ****
Mean ± SD	8.582 ± 0.869	4.233 ± 1.846	
Luteinizing hormone (mIU/mL)			
Range	135.9 - 199.4	223.7 - 273.3	<0.0001 ****
Mean ± SD	179.6 ± 13.85	260.4 ± 10.07	
Follicle stimulating hormone (mIU/mL)			
Range	20.61 – 38.98	30.85 – 50.88	<0.0001 ****
Mean ± SD	33.12 ± 4.480	43.37 ± 7.906	

Table(2) Comparison of semen parameters between fertile and infertile

Characteristic	Fertile	Infertile	P value
Volume			
Range	2 - 6	1- 9	0.2468 ns
Mean ± SD	3.9 ± 1.111	3.51 ± 1.867	
Total count			
Range	100 - 276	10 --90	<0.0001 ****
Mean ± SD	169.5 ± 46.27	43.47 ± 20.98	
Total motility			
Range	0.45 - 0.7	0 - 65	<0.0001 ****
Mean ± SD	0.563 ± 0.06014	32.36 ± 18.9	
Immotile			
Range	0.3 - 0.55	35 - 100	<0.0001 ****
Mean ± SD	0.4373 ± 0.06017	65.18 ± 18.58	
Deformity rate			
Range	0.03 - 0.13	13 - 45	<0.0001 ****
Mean ± SD	0.0581 ± 0.02702	20.84 ± 6.635	

Table(3) Correlations among biomarkers in infertile

Characteristic		Testosterone	FSH	LH	volume	Total count	Total motility	Immotile
Melatonin	Pearson r	0.063	0.049	0.010	0.187	0.071	0.005	-0.050
	P value	0.664	0.735	0.946	0.193	0.626	0.973	0.729

Table(4) Correlations among biomarkers in fertile

Characteristic		Testosterone	FSH	LH	volume	Total count	Total motility	Immotile	Deformity rate
Melatonin	Pearson r	0.005	-0.011	0.006	0.298	0.167	-0.176	0.181	-0.083
	P value	0.978	0.945	0.969	0.062	0.303	0.277	0.263	0.612

The Discussion

Melatonin is a hormone that plays a crucial role in regulating the sleep-wake cycle, but it also has other functions in the body, including antioxidant and anti-inflammatory effects. Previous studies indicated that melatonin has a protective effect on male reproductive function by reducing oxidative stress and improving sperm motility and morphology. (٢٢). In various species, seasonal reproductive cycles are influenced by melatonin role in reproduction. In humans, it has been shown that alterations in melatonin secretion from the pineal gland can affect the functioning of the reproductive neuroendocrine axis. (٢٣). The current study showed decreased levels of Melatonin concentration (pg/mL) in the infertile individuals as compared with the fertile group (٢,٦٩٩ ± ٥٢٦,٠) ,(٧,٠٧٧ ± ٥١٢,٠) respectively; the significant difference was (p-value <٠,٠٠٠١) as in Figure (١). In the present results, the serum melatonin levels in all the infertile men were reduced significantly compared to their levels in the fertile group this is compatible with studies performed by (٢٤,٢٥). While these results are inconsistent with (٢٦) which found that there was not any significant difference in the melatonin levels between the fertile and infertile groups. The present study presents a possible explanation for the low levels of melatonin that were found in infertile men, as individual melatonin levels can vary widely based on mean values. However, it should be noted that various factors can significantly influence melatonin levels in human blood, including light exposure, stress, body position, physical activity, and time of day (٢٧). Based on the results of the present study, it appeared that there was no significant correlation between the serum melatonin levels and the semen parameters among fertile and infertile men in terms of total count, total motility, volume, immotile sperm count, and deformity rate, as in Tables (٣,٤). These results are consistent with (٢٥,٢٨). Moreover, the present study found that there was no significant correlation

between melatonin level and FSH, LH, and testosterone among fertile and infertile men, as in Tables(٣,٤). These results corresponded with (٢٨) who reported no significant correlation.

Conclusions

The study indicated that decreased melatonin levels can be associated with male infertility, but it did not indicate a direct relationship between melatonin and hormone levels or specific semen parameters.

Acknowledgments: The University of Al-Qadisiyah/College of Medicine provided facilities, which aided in enhancing the quality of this work, are greatly appreciated by the authors.

Conflict of Interest

The authors have no conflicts of interest regarding the publication of this article.

Funding

The study was funded by the researchers themselves.

Adherence to Ethical Standards

The study was approved by the ethical committee at the University of Al-Qadisiyah (registration code CMUQ3544 on 15.12.2020).

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