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Effect of melatonin on reproductive function in propylthiouracil induced hypothyroidism in adult male rats

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Abstract. – OBJECTIVE: Thyroid hormones are essential for regulating metabolism, reproduction, and growth. Hypothyroidism is connected with lower sperm count and motility, leading to male infertility. Oxidative stress is likely to be linked to this interaction. Melatonin, being known as an oxidative scavenger, may offer a feasible treatment method for reproductive dysfunction accompanying hypothyroidism in adult male rats. The purpose of this investigation was to determine the mechanism by which melatonin treatment affected spermatogenic and steroidogenic function in an experimental model-induced hypothyroidism in adult male rats.

MATERIALS AND METHODS: Twenty-one male albino adult rats weighing between 150 and 210 g were used in this experiment. Rats were split into three groups and studied for 11 weeks. The control euthyroid group, in which rats received 0.9% Sodium Chloride (NaCl) solution by intraperitoneal injection [solvent for 6-propyl 2-thouracil (PTU)], 6 days/week for 8 weeks; the PTU-induced hypothyroid group, in which chemical thyroidectomy was induced by intraperitoneal injection of PTU at a dose of 10 mg/kg body weight, 6 days/week for 8 weeks; and the melatonin-treated hypothyroid group, which received 3 mg/kg melatonin intraperitoneally daily for 21 days plasma free Triiodothyronine (T3), free Thyroxin (T4), thyroid stimulating hormone (TSH), free testosterone, Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and prolactin were measured. Also, semen analysis, testicular tissue malondialdehyde (MDA), and tumor necrosis factor-α (TNF-α) were accessed.

RESULTS: The results indicated that melatonin significantly increased sperm viability and motility compared to the untreated PTU-induced hypothyroid group ($p<0.001$). Testicular MDA and TNF-α showed a significant decrease in the melatonin-treated hypothyroid group compared with the PTU-induced hypothyroid group ($p<0.05$). In addition, plasma testosterone levels were significantly increased, accompanied by a significant reduction of plasma prolactin levels compared to the untreated hypothyroid group ($p<0.05$ for both).

CONCLUSIONS: Based on the study findings, melatonin could mitigate gonadal dysfunction induced by hypothyroidism by improving several components of semen analysis, such as sperm motility and sperm viability, as well as by enhancing testosterone production focusing on oxidative and inflammatory stress as the underlying mechanisms.

Key Words:
Hypothyroidism, Melatonin, Propylthiouracil, Male infertility.

Introduction

Sperm quality[1-3] and, therefore, male fertility[4,5] are negatively impacted by hypothyroidism, a significant endocrinopathy. Studies[5,6] suggest a worldwide decline in semen quality, which is concerning since semen analysis is a key

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predictor of male fertility. Hypothyroidism has been associated with hypogonadism, decreased serum testosterone, and altered sperm morphology and motility, all of which contribute to this reduction[7].

Propylthiouracil (PTU) is a commonly used antithyroid drug that can be used to induce hypothyroidism in rats. PTU works by blocking the thyroid peroxidase enzyme, which is essential for the synthesis of thyroid hormones. PTU has been shown to produce a significant decrease in serum thyroid hormone levels and to induce a range of biochemical and physiological changes characteristic of hypothyroidism[8,9].

The exact mechanism by which hypothyroidism causes infertility is not fully understood, but it is thought to be related to the effects of thyroid hormones on the reproductive system[10,11]. Thyroid hormones are essential for spermatogenesis and steroidogenesis. They are well-established regulators of Sertoli cell proliferation and functional maturation, as well as in Leydig cell differentiation[12,13].

Thyroid hormones are suspected to have a role in hypothyroidism-related infertility, although the specific mechanism is not well known[10,11]. Spermatogenesis and steroidogenesis cannot occur without thyroid hormones. They are known to have a role in Leydig maturation and in the development and proliferation of Sertoli cells[12,13].

In addition, it is important to note that thyroid hormones have a significant physiological function in mitigating the effects of oxidative stress induced by reactive oxygen species. Consequently, a lack of thyroid hormones leads to a state of hypothyroidism and oxidative stress, which is often linked to impaired testicular function and infertility[14].

Pineal gland melatonin is thought to have antioxidant and anti-inflammatory effects. Melatonin has been found to be far more effective than traditional antioxidants at protecting molecules from damage caused by free radicals[15]. Melatonin could be a promising treatment for reproductive dysfunction in hypothyroidism[16].

Melatonin's influence on semen parameters in male rats with PTU-induced hypothyroidism has not precisely been studied before. Therefore, our study examined the effect of melatonin administration on spermatogenic and steroidogenic function in a model of experimentally induced hypothyroidism in adult male rats, hypothesizing that melatonin could have beneficial effects on those rats.

Materials and Methods

Experimental Animals

The current experimental study was conducted on a sample of 21 adult male albino rats with body weights ranging from 150 to 210 grams. The rats used in this study were obtained from the Egyptian Organization for Biological Products and Vaccines (VACSERA) and housed at the Animal House, Physiology Department, Faculty of Medicine, Ain Shams University. The rats were maintained under standard boarding circumstances and subjected to a regular light/dark cycle. The availability of food and water was unrestricted. Ethical approval for the study was given by the Standing Committee of Bioethics Research, Deanship of Scientific Research at Prince Sattam bin Abdulaziz University (SCBR/144-2023).

Rats were divided into three groups of identical size and kept in the lab for 11 weeks. Euthyroid control rats were injected intraperitoneally with 0.9% Sodium Chloride (NaCl) solution [the solvent for 6-propyl 2-thouracil (PTU)] on a daily basis for 8 weeks (group I). Group II received intraperitoneal injections of PTU at a dosage of 10 mg/kg body weight, 6 days a week for 8 weeks, in order to induce a chemical thyroidectomy. After inducing hypothyroidism, similarly, in group II, group III administered 3 mg/kg melatonin intraperitoneally daily for 21 days.

PTU (Sigma Chemicals Co., St. Louis, MO, USA) with a dose of 10 mg/kg body weight[17] given intraperitoneally once daily for 8 weeks was used to induce experimental hypothyroidism in rats[18]. After inducing hypothyroidism, rats in group III received 3 mg/kg intraperitoneal melatonin (Co: Puritan's Pride, UAE) daily for 21 days[19].

Experimental Procedure

The overnight fasted rats were weighed and anesthetized with i.p. injection of thiopental sodium (EIPICO, Egypt), in a dose of 40 mg/kg B.W., then the abdominal aorta was exposed and cannulated with polyethylene catheter. The obtained blood samples were immediately centrifuged at 3,000 rpm for 15 minutes to separate sera. The separated sera were then pipetted and stored at -80°C for later measurement of serum urea and creatinine levels as well as serum catalase, malondialdehyde (MDA), and tumor necrosis factor-alpha (TNF-α) levels.

At the end of the study period (11 weeks), the overnight fasted rats were weighed. Rectal temperature was recorded by inserting an ordinary mercury thermometer into the anus for 2

minutes. Subsequently, the rats were anesthetized with an intraperitoneal injection of thiopental sodium (Sandoz, Austria) at a dosage of 40 mg/kg body weight. Blood samples were obtained from the abdominal aorta and placed in a plastic tube containing heparin to prevent coagulation. Subsequently, the tube was subjected to centrifugation at a speed of 4,000 rpm for a duration of 10 minutes in order to separate the plasma. Subsequently, the plasma was transferred into sterile storage tubes using a pipette and preserved at a temperature of -20°C. This was done in order to facilitate the subsequent analysis of plasma free Triiodothyronine (T3), free Thyroxin (T4), Thyroid Stimulating Hormone (TSH), free testosterone, Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), and prolactin.

The retroperitoneal fat was dissected out and weighed using 5-Digit-Metler balance (AE 163). Then, the left testis was exposed; epididymis was carefully separated from it and minced in 1 ml of phosphate buffered saline (PBS) to be used for semen analysis. The right testis was removed, dried by filter paper, weighed and then preserved at -80°C for further measurement of testicular malondialdehyde (MDA) and tumor necrosis factor alpha (TNF-α).

Semen Analysis

Epididymal sperm count

After being carefully separated from testis, the epididymis was minced in 1 ml of phosphate buffered saline (PBS), pH 7.2, and the suspension was filtered through a nylon mesh. The determination of sperm count was conducted using a hemocytometer in the following manner: a volume of 0.05 ml was extracted from the filtered sperm suspension and then diluted at a ratio of 1:40 with PBS. The resulting mixture was properly mixed, and a portion of the diluted sperm suspension was then put into a counting chamber located inside a hemocytometer. The researchers conducted an assessment of the cumulative sperm count inside eight individual squares, each measuring 1 mm^2. This count was then multiplied by a factor of 5×10^4 in order to ascertain the overall quantity of sperm present within the epididymis[20].

Epididymal sperm viability

100 µl of the same sperm suspension was combined with 100 µl of 0.5% eosin solution on a microscope slide and inspected under a 200x objective lens after thirty minutes to determine sperm viability, as outlined in the WHO Laparotomy Manual[21]. The dead sperms were stained with eosin solution, appearing as red spots, while the viable sperms were unstained. By counting the number of viable sperms (unstained) and the number of dead sperms (eosin-stained) in the examined field, the percentage ratio of viable sperms was calculated as:

$$\frac{\text{Number of viable sperms}}{\text{Total number of sperms}} \times 100$$

Epididymal sperm motility

Sperm motility is an important factor in male infertility. The motility of sperm produced in the epididymis was evaluated using a microscope (BX51 Phase, Olympus, Tokyo, Japan). To facilitate spermatozoa motility and tubule escape, sperm from both caudal epididymides were minced into 2 ml of 0.9% physiological solution and incubated at 37°C for 20 minutes. To calculate sperm motility, the number of sperm that were moving progressively was divided by the total number of sperm and the result was multiplied by 100 to get a percentage[21,22].

Biochemical Analysis

Electrochemiluminescence immunoassay "ECLIA" methods were used to measure free T3, free T4, and TSH in plasma, using kits provided by Roche Diagnostics Switzerland[23]. Testing for follicle-stimulating hormone, luteinizing hormone, and prolactin in plasma was performed using enzyme-linked immunosorbent assay ("ELISA") methods and kits from (DRG diagnostics, Marburg, Germany)[24]. Enzyme-linked immune sorbent assay "ELISA" methods employing kits provided by DRG diagnostics were used to measure plasma-free testosterone levels[25]. Malondialdehyde (MDA) levels in testicular tissue were determined using a method similar to that developed by Ohkawa et al[26] (1979). This was done with the use of kits provided by Bio-diagnostic, Egypt. Rat quantitative ELISA kit (R&D systems Co., USA) was used to measure tumor necrosis factor-alpha (TNF-α) in testicular tissue.

Statistical Analysis

Data was compiled and shown as mean SEM. Student's *t*-test for unpaired data was performed at a significance level of $p<0.05$ to establish statistical significance. The SPSS software (V. 25, IBM Corp., Armonk, NY, USA) was used to conduct an ANOVA on all the statistical data to determine its significance.

Table I. Body weight gain (%), weights of retroperitoneal fat (g), testicular weight (g) and body temperature (°C) in the three studied groups.

Parameters	Control group n = 7	Hypothyroid group n = 7	Melatonin treated hypothyroid group n = 7
Body weight gain (%)	20 ± 3.18	31 ± 3.91[a]	23 ± 4.06
Retroperitoneal fat weight (g)	2.54 ± 0.29	3.66 ± 0.33[a]	2.73 ± 0.34
Testicular weight (g)	0.99 ± 0.05	1.13 ± 0.13	0.93 ± 0.09
Body temperature (°C)	37.9 ± 0.12	36.81 ± 0.13[a]	37.05 ± 0.27[a]

All values are expressed as mean ± SEM. [a]: Significance from the control group, calculated by Student's "*t*" test for unpaired data.

Results

The body weight gain was significantly higher in the untreated hypothyroid group compared to the control group (31±3.91 *vs.* 20±3.18, $p<0.05$). Moreover, retroperitoneal fat weight significantly increased in the untreated hypothyroid group compared to the control group (3.66±0.33 *vs.* 2.54±0.29, $p<0.05$). On the other hand, testicular weight did not show any significant difference among all rat groups. Body temperature significantly decreased in both hypothyroid groups (36.81±0.13 and 37.05±0.27, respectively) compared to the control group (37.9±0.12, $p<0.01$ for both). The melatonin-treated hypothyroid group did not reveal a significant change in these parameters compared to the untreated hypothyroid group (Table I).

When comparing the hypothyroid groups to the control group, plasma-free T3 levels were significantly lowered in the hypothyroid groups (0.97±0.18 and 1.05±0.13, respectively) *vs.* (1.83±0.12) with $p<0.001$ and $p<0.01$, respectively. In addition, plasma-free T4 levels were found to be significantly lowered (1.92±0.28 and 2.03±0.43, respectively) compared to the control group (3.57±0.38, $p<0.01$), and plasma TSH levels were found to be significantly higher (0.28±0.05 and 0.24±0.06 *vs.* 0.09±0.02, $p<0.01$ and $p<0.05$, respectively) than in the control group. These results verified that the hypothyroid condition had been successfully induced in these cohorts, as detailed in Table II.

The current study elucidated that both testicular tissue MDA and TNF-α were significantly superior in the untreated hypothyroid group than the control group (31.82±1.23 *vs.* 22.36±1.06 and 8.18±0.92 *vs.* 5.26±0.82, $p<0.001$ and $p<0.05$, respectively). In contrast, the melatonin-treated hypothyroid group had significant decreases in both measures (18.77±1.35 and 4.23±0.04) compared to the untreated hypothyroid group (31.82±1.23 and 8.18±0.92, respectively, $p<0.001$ and $p<0.01$, respectively) and being non significantly different from the control group (Figure 1A-B).

Epididymal sperm count showed a significant decrease in hypothyroid rats (7.15±0.66 and

Table II. Plasma levels of free T_3 (ng/dl), free T_4 (ng/ml), TSH (μIU/ml), testicular tissue levels of MDA (μmol/gm) and TNF-α (pg/mg) in the three studied groups.

Parameters	Control group n = 7	Hypothyroid group n = 7	Melatonin treated hypothyroid group n = 7
T3 (ng/dl)	1.83 ± 0.12	0.97 ± 0.18[a]	1.05 ± 0.13[a]
T4 (ng/dl)	3.57 ± 0.38	1.92 ± 0.28[a]	2.03 ± 0.43[a]
TSH (μIU/ml)	0.09 ± 0.02	0.28 ± 0.05[a]	0.24 ± 0.06[a]
MDA (μmol/gm)	22.36 ± 1.06	31.82 ± 1.23[a]	18.77 ± 1.35[b]
TNF-α (pg/mg)	5.26 ± 0.82	8.18 ± 0.92[a]	4.23 ± 0.40[b]

T3: Triiodothyronine, T4: Thyroxine, TSH: Thyroid stimulating hormone, MDA: Malondialdehyde, TNF-α: Tumor necrosis factor. All values are expressed as mean ± SEM. [a]: Significance from the control group, calculated by Student's "*t*" test for unpaired data. [b]: Significance from the hypothyroid group, calculated by Student's "*t*" test for unpaired data.

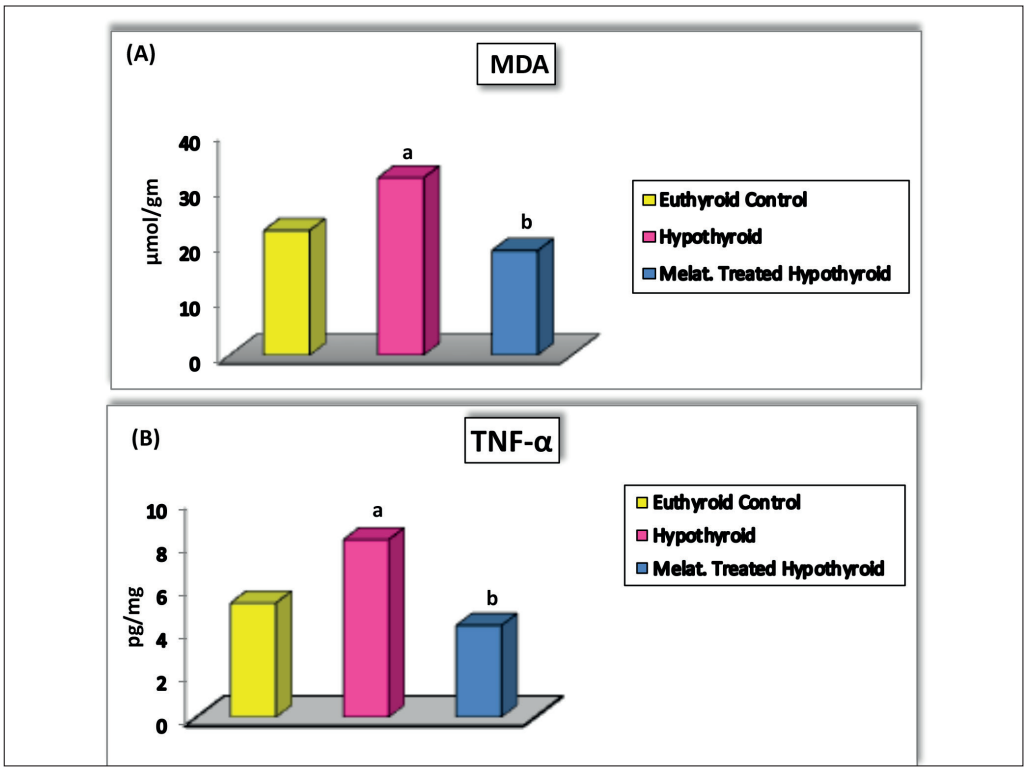

Figure 1. Changes of (**A**) testicular tissue MDA and (**B**) TNF-α in the three studied groups. a: significance from the control group, calculated by Student's *t*-test for unpaired data. b: significance from the hypothyroid group, calculated by Student's *t*-test for unpaired data.

8.86±0.77, respectively) compared to control rats (14.61±1.13, $p<0.001$ for both). Sperm viability was significantly reduced in the untreated hypothyroid group compared to the control group (30.46±1.11 *vs.* 56.33±3.3, $p<0.001$). Meanwhile, melatonin-treated hypothyroid rats revealed a significant increase in sperm viability compared to untreated hypothyroid rats (41.25±2.74 *vs.* 30.46±1.11 $p<0.01$) and was still significant as compared to the control rats (41.25±2.74 *vs.* 56.33±3.3, $p<0.01$). In addition, sperm motility was significantly reduced in the untreated hypothyroid group compared to the control group (29.25±1.14 *vs.* 52.15±2.8, $p<0.001$). The melatonin-treated hypothyroid rats revealed a significant increase in sperm motility compared to untreated hypothyroid rats (39.41±2.52 *vs.* 29.25±1.14, $p<0.01$) and were still significant as compared to the control rats (39.41±2.52 *vs.* 52.15±2.8, $p<0.01$) as detailed in Table III.

Moreover, the untreated hypothyroid rats showed significant elevation in plasma FSH and LH (8.25±0.16 *vs.* 6.09±0.63 and 10.98±0.85 *vs.* 8.65±0.42, $p<0.05$ for both, respectively) associated with a significant decrease in plasma free testosterone levels (1.75±0.17 *vs.* 3.22±0.41, $p<0.01$), in addition to a significant increase in plasma prolactin levels (0.88±0.16 *vs.* 0.65±0.05, $p<0.05$) when compared to the control rats. Melatonin administration to hypothyroid rats resulted in a significant decrease in plasma FSH and LH (5.08±0.75 *vs.* 8.25±0.16 and 8.47±0.41 *vs.* 10.98±0.85, $p<0.01$ for both, respectively) compared to the untreated hypothyroid group, being insignificantly different compared to the control group. Also, a significant increase in plasma free testosterone levels (2.86±0.34 *vs.* 1.75±0.17, $p<0.05$) and a significant decrease in plasma prolactin levels (0.52±0.07 *vs.* 0.88±0.16, $p<0.05$) were observed in melatonin treated hypothyroid group compared to the untreated hypothyroid group, both were insignificantly different from the control group as shown in Table IV.

Discussion

To the best of our knowledge, the current study is the first to examine the effect of melatonin on both spermatogenic and steroidogenic

Table III. Sperm count per epididymis (×10⁶), epididymal sperm viability (%), epididymal sperm motility (%) in the three studied groups.

Parameters	Control group n = 7	Hypothyroid group n = 7	Melatonin treated hypothyroid group n = 7
Epididymal sperm count (×10⁶)	14.61 ± 1.13	7.15 ± 0.66[a]	8.86 ± 0.77[a]
Epididymal sperm viability (%)	56.33 ± 3.3	30.46 ± 1.11[a]	41.25 ± 2.74[a,b]
Epididymal sperm motility (%)	52.15 ± 2.8	29.25 ± 1.14[a]	39.41 ± 2.52[a,b]

All values are expressed as mean ± SEM. [a]: Significance from the control group, calculated by Student's *t*-test for unpaired data. [b]: Significance from the hypothyroid group, calculated by Student's *t*-test for unpaired data.

testicular dysfunction in adult male hypothyroid rats, hypothesizing that melatonin could have beneficial effects on those rats. The study findings confirmed our hypothesis that the melatonin treated PTU- induced hypothyroid rats showed a significant increase in epididymal sperm viability, motility, and serum testosterone levels with significant decreases in MDA and TNF-α when compared with the untreated PTU-induced hypothyroid rats.

PTU is an anti-thyroid medication that works by inhibiting the thyroid peroxidase enzyme, hence preventing the production of thyroid hormones. To confirm the development of hypothyroidism, it was reported that plasma-free T3 and T4 levels dropped significantly after PTU injection, while TSH levels rose[27]. This hypothyroid profile could be explained by a loss of negative feedback effects of free T3 on the hypothalamic-pituitary-thyroid axis[28,29].

Along with the significant decrease in both plasma-free T3 and T4 and an increase in TSH, an increase in body weight gain, retroperitoneal fat weight, as well as a decrease in body temperature was observed after PTU administration, which further confirmed the hypothyroid condition. Thyroid hormones have a role in lipid production, metabolism, and mobilization. Because thyroid hormones are usually lipolytic in nature and accelerate metabolism, the increased weight gain and decreased body temperature seen in PTU-induced hypothyroid rats was expected in the current study. Thereby, a lack of thyroid hormones will result in weight gain and low body temperature. This finding agrees with a previously reported finding in hypothyroid rats[30].

Deficiency of thyroid hormones is associated with decreased mitochondrial biogenesis, impaired mitochondrial function, and slowed mitochondrial turnover that can lead to a decrease in ATP production[31].

Our results also indicated that testicular MDA level was significantly higher in PTU-induced hypothyroid rats than euthyroid control rats, suggesting increased oxidative stress in hypothyroidism conditions. This could be explained by the increased TSH levels as elevated TSH levels *per se* could affect oxidative stress progressions[32] and are directly associated with oxidative stress[33].

Reactive oxygen species (ROS) are associated with hypothyroidism because more free radicals are being produced, and antioxidant defense against them is weakening. The accelerated pro-

Table IV. Plasma levels of FSH (ng/ml) and LH (ng/ml), prolactin (pg/ml) and free testosterone (pg/ml) in the three studied groups.

Parameters	Control group n = 7	Hypothyroid group n = 7	Melatonin treated hypothyroid group n = 7
FSH (ng/ml)	6.09 ± 0.63	8.25 ± 0.16[a]	5.08 ± 0.75[b]
LH (ng/ml)	8.65 ± 0.42	10.98 ± 0.85[a]	8.47 ± 0.41[b]
Prolactin (pg/ml)	0.65 ± 0.05	0.88 ± 0.16[a]	0.52 ± 0.07[b]
Testosterone (pg/dl)	3.22 ± 0.41	1.75 ± 0.17[a]	2.86 ± 0.34[b]

FSH: Follicle-stimulating hormone, LH: Luteinizing hormone. All values are expressed as mean ± SEM. [a]: Significance from the control group, calculated by Student's *t*-test for unpaired data. [b]: Significance from the hypothyroid group, calculated by Student's *t*-test for unpaired data.

duction of free radicals could be attributed to the mitochondrial respiratory chain dysfunction. In addition, hyperlipidemia, which is a coherent biochemical characteristic in hypothyroidism, leads to increased lipid peroxidation, which is implicated in hypothyroidism-induced oxidative stress[34].

In this study, TNF-α was considerably higher in PTU-induced hypothyroid rats, indicating an inflammatory state in the testes that may have been brought on by an increase in oxidative stress. Nuclear factor-κB (NF-κB) is suggested to be the essential connection between oxidative stress and inflammation. When oxidative stress is present, NF-κB is activated, which in turn causes an increase in nitric oxide production, which in turn activates NF-κB. The latter sets off a cascade of inflammation-promoting signaling molecules, including the inflammatory cytokine as TNF-α[35].

In addition, the current study declared a significant reduction in free plasma testosterone levels in hypothyroid rats, which is in accordance with previous study findings[36]. This work provides more evidence that the oxidative stress associated with a hypothyroid condition may be responsible for the damage to Leydig cells that results in low testosterone levels. It was documented that oxidative stress in the testes may lead to a decrease in testosterone synthesis due to Leydig cell damage[37].

In addition, PTU hypothyroid rats of the current study exhibited a significant increase in plasma prolactin levels, which is a well-known effect of this disorder[38,39]. This could be attributed to the excitatory influence of the heightened TRH on the secretion of prolactin with hypothyroidism[40]. Moreover, elevated pituitary VIP may play a role in hypothyroidism-induced hyperprolactinemia by functioning as a paracrine or autocrine regulation of prolactin release[41]. In fact, the increased prolactin levels could explain the reduced plasma testosterone observed in the hypothyroid rats in the current study, which was consistent with recent study findings[42].

It is to be noted that the decline in sperm count, viability, and impaired sperm motility in hypothyroid rats of this study could be linked to aforementioned oxidative, inflammatory states, and reduced testosterone production. Our findings are supported by those stated in various experimental models-induced hypothyroidism[43-45].

Interactions between ROS and membrane lipids, proteins, and nuclear and mitochondrial DNA may disrupt normal sperm production as well as quality (motility, viability, and function)[46,47]. Peroxidative damage to the DNA in sperm has also been linked to oxidative stress in the testis[48,49], making this a hot topic in the study of male infertility. DNA damage in sperm cells has been linked to poor sperm quality and failed fertilization since spermatozoa rely on genomic integrity to reproduce[50,51].

Because sperm membrane polyunsaturated fatty acids (PUFA) are essential for spermatozoa to carry out their normal physiological functions (such as the acrosome reaction and sperm-egg fusion during fertilization), the impairment of sperm motility in PTU-hypothyroid rats may be linked to loss of membrane fluidity induced by reactive oxygen species[52]. This, in turn, disrupts the cellular ionic balance and compromises cellular Ca^{2+} homeostasis, both of which have been linked to a decrease in sperm motility[53].

Hypothyroidism is associated with gonadal dysfunction, but the mechanisms for these changes are not clear. Hypothyroidism causes oxidative damage to the testis with a subsequent decrease in testosterone and impairment in semen quality. Therefore, we could assume that increased plasma LH and FSH is a consequence of the primary hypogonadism encountered in hypothyroid rats[41].

Our results clearly suggest that the elevated plasma FSH and LH are the consequence of a decreased negative feedback effect of free testosterone and that hypothyroidism is the direct cause of gonadal dysfunction. However, a previous publication by Aiceles et al[54] contradicted our findings by showing that hypothyroidism reduces FSH and LH. The current research suggests that elevated levels of plasma FSH, a sensitive indicator of germinal cell dysfunction, in hypothyroid rats may contribute to testicular germ cell damage, as seen by decreased sperm count, viability, and motility. Also, it was ascertained that hypothyroidism is associated with a decrease in testosterone production by Leydig cells, indicating impaired both spermatogenesis and steroidogenesis as well[55].

Interestingly, melatonin administration to PTU-induced hypothyroid rats decreased plasma MDA levels, suggesting its antioxidative role. This study's observation that melatonin has antioxidative potential is in line with prior research[56], showing that melatonin may increase the expression of a number of endogenous antioxidant enzymes, including glutathione peroxidase and superoxide dismutase.

Likewise, melatonin has been found to protect the testis from the oxidative stress brought on by hypothyroidism by increasing the tes-

ticular production of nuclear factor erythroid 2-related factor 2 (Nrf2), a key transcription factor for many antioxidant genes[57]. Therefore, by mitigation of oxidative stress, melatonin can provide protection against harm inflicted upon reproductive cells[58].

Melatonin dramatically reduced the response to inflammation and lowered the inflammatory cytokines, including TNF-α, in the testicular tissue of PTU-induced hypothyroid rats, which had already been shown to reduce oxidative stress. In the current study, melatonin administration significantly increased sperm motility and viability but did not affect the number of spermatozoa. In agreement with our study results, previous studies[59] have demonstrated that melatonin exhibits positive effects on both sperm quality and fertility outcomes in males. It has the potential to enhance the quantity, mobility, and structural characteristics of sperm. Melatonin has the potential to provide protective effects against free radical-induced damage to sperm.

Various processes might be proposed to explain the enhanced sperm quality seen in hypothyroid mice as a result of melatonin administration. The first observation pertains to the enhancement of the testicular microenvironment, which creates a favorable setting for the process of spermatogenesis. This improvement is attributed to the effects of melatonin, which has been shown to boost spermatogenesis and promote the survivability of germ cells. These effects are achieved *via* the attenuation of oxidative stress, inflammatory response, and apoptosis, as well as the facilitation of meiosis[60]. Additionally, it is worth noting that melatonin has a direct impact on sperm due to the presence of melatonin in seminal fluid and the existence of melatonin receptors on spermatozoa[61].

The process of spermatogenesis is dependent on the intricate interplay between germ cells and Sertoli cells inside the seminiferous tubules[62]. Hence, the preservation of the typical arrangement of seminiferous tubules by melatonin may possibly provide the necessary conditions for the process of spermatogenesis.

Moreover, melatonin reduced hyperprolactinemia observed in hypothyroid rats. This finding agrees with earlier studies[63] suggesting that melatonin has a direct effect on the lactotroph in the regulation of prolactin production and melatonin caused inhibition of prolactin secretion as it reduced both TRH and vasoactive intestinal peptide (VIP), which are known stimulators of prolactin secretion.

Furthermore, the increased plasma testosterone levels in melatonin-treated hypothyroid rats of our study could explain the observed decrease in plasma FSH and LH levels as melatonin has the potential to counteract the aberrant changes seen in the hypothalamic-pituitary-gonadal (HPG) axis, as well as in spermatogenesis and sperm function[64]. Additionally, this effect might be ascribed to its capacity to downregulate the neuropeptides neurokinin B and kisspeptin, which are known to stimulate the production of Gonadotrophin Releasing Hormone (GnRH)[65,66]. In contrast to the data shown in our study, previous publications[64] have indicated that melatonin could activate the HPG axis, leading to a rise in the blood levels of GnRH, LH, and FSH.

Accordingly, it is plausible to speculate that the reestablishment of normal testicular sperm viability and motility in semen analysis following melatonin administration resulted not only from the reduction of oxidative stress and inflammatory state but also from the increase in testosterone levels. Being a metabolic regulator of Leydig cells[67,68], melatonin, as a metabolic regulator of Leydig cells, could inhibit apoptosis of Leydig cells and enhance the expression of genes associated with testosterone synthesis, including steroidogenic factor-1, transcription factor GATA-4, and steroidogenic acute regulatory protein in Leydig cells[69]. Consequently, melatonin facilitates the restoration of testosterone production by Leydig cells.

There is a need for additional research to completely investigate the effects of melatonin on reproductive function; nevertheless, the current evidence suggests that melatonin has a potentially therapeutic role as an antioxidant, anti-inflammatory, and endocrine modulating agent on reproductive dysfunction associating hypothyroidism in adult male rats.

Conclusions

Our findings indicated that melatonin could mitigate gonadal dysfunction induced by hypothyroidism by improving several components of semen analysis, such as sperm motility and sperm viability, as well as by enhancing testosterone production, focusing on oxidative and inflammatory stress as the underlying mechanisms. These results provide strong support for the need for future clinical trials to evaluate the safety and effectiveness of melatonin as a treatment for male infertility-associated hypothyroidism.

Conflict of Interest
The authors declare no conflicts of interest.

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Ethics Approval
The study was approved by the Standing Committee of Bioethics Research, Deanship of Scientific Research, Prince Sattam bin Abdulaziz University (SCBR/144-2023).

Data Availability
The authors declare that all relevant data supporting the findings of this study are available within the article.

Authors' Contribution
WE, WKA, RSH, ARA, AS, and SY were involved in the planning and design of the study. WE, WKA, RSH, ARA, AS, and SY were involved in the literature search. WE and SY completed the study procedures, collected, and analyzed the data. WE, WKA, RSH, ARA, AS, and SY developed the manuscript, and all authors agreed to its final submission. All authors guarantee the integrity of the content and the study. All authors read and accepted the final manuscript.

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