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# Determination of the Effect of Coenzyme Q-10 on Spermatogenic Stem Cells by Immunohistochemical Techniques in Male Rats with Experimental Hypothyroidism

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## Abstract

**BACKGROUND/AIMS:** Testicular dysfunction has been reported in hypothyroidism. However, the effect of hypothyroidism on spermatogonial stem cells (SSCs) is not understood. This study aimed to investigate the effect of hypothyroidism on SSCs in comparison with coenzyme Q10 (CoQ10), which is widely used in the treatment of male infertility.

**MATERIALS AND METHODS:** Histomorphologic examinations of rat testes, immunohistochemical analyses to detect SSCs expressions, and Enzyme-Linked Immunosorbent Assay tests for serum hormone levels were performed.

**RESULTS:** Hypothyroidism caused an increase in the body weight of the rats, but there was no difference between the groups when the testicular weights of the animals were evaluated. In thyroid function test evaluation, CoQ10 supplementation provided a therapeutic effect by showing positive results in thyroid stimulating hormone, triiodothyronine, and tetraiodothyronine levels. Histomorphologic evaluation showed no difference in seminiferous tubule diameters between the groups, although irregularities in the spermatogenesis process were observed. Hypothyroidism prevents SSCs from undergoing cell differentiation and thus reduces sperm production in the seminiferous tubules. CoQ10 supplementation does not affect spermatogenic stem cells, but has a beneficial effect on other cells (primary spermatocytes, secondary spermatocytes, spermatids, and spermatozoa).

**CONCLUSION:** Although CoQ10 supplementation does not directly affect the differentiation of SSCs, it may alleviate some of the deleterious effects of hypothyroidism in the later stages of spermatogenesis, providing a potential therapeutic benefit in maintaining sperm production.

**Keywords:** Coenzyme, hypothyroidism, stem cells

## INTRODUCTION

Hypothyroidism is an endocrine disorder that occurs when the thyroid gland does not function properly.[1] Hypothyroidism can lead to sexual dysfunction in men and cause infertility.[2] The thyroid gland hormones triiodothyronine (T3) and tetraiodothyronine (T4) regulate testicular function through genomic and non-genomic effects.[3] Genomic effects result from the binding of T3 to its corresponding receptor thyroid hormone receptor (TR), in the nucleus of Sertoli and Leydig cells, where, after binding to thyroid hormone response elements, the hormone-receptor complex activates gene transcription and protein synthesis.[4] The non-genomic effects of thyroid hormones result from their binding to non-nuclear receptors in the cytoplasmic membrane, cytoplasm, cytoskeleton and mitochondria of the spermatozoon, increasing cyclic

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adenosine monophosphate synthesis, $Ca^{2+}$ release, and ultimately sperm motility.[3-6] Although it is known that Sertoli and Leydig cells contain TRs and thus these cells are directly or indirectly affected by changes in thyroid hormone levels, the impact of hypothyroidism on the mechanisms affecting spermatogonia (SPG) in both fetal and adult life are not fully understood. In addition to this information, it is known that thyroid hormones regulate the redox state of the testis, which is mediated by various antioxidant systems.[3]

Coenzyme Q10 [(CoQ10), ubiquinone], an antioxidant, is a vitamin-like molecule found in every cell membrane. It is a normal part of our diet but is also synthesized endogenously. CoQ10 is one of the electron carriers in the mitochondrial respiratory chain and adenosine triphosphate production process.[7] In addition to this information, CoQ10 has been reported to play a role in membrane stability, cell signaling, gene expression, apoptosis control, and cell growth.[8] Studies have reported that CoQ10 has beneficial effects on male infertility by improving sperm function. A meta-analysis conducted in 2020 demonstrated that CoQ10 supplementation significantly improved sperm concentration, motility, and morphology.[9] The antioxidant properties of CoQ10 help reduce sperm DNA damage and prevent oxidative stress, which is a crucial factor in enhancing sperm quality, particularly in infertile men.[10] Human studies also support the notion that CoQ10 can protect testicular functions and enhance sperm production.[11]

Spermatogonial stem cells (SSCs) are undifferentiated germ cells responsible for spermatogenesis.[12] Throughout most of the male lifespan, SSCs provide the basis for continuous sperm production.[13] In recent years, many studies have succeeded in understanding the biology of SSCs and have drawn attention to their great potential in the field of reproductive/regenerative medicine. Recent studies on the treatment of male infertility are promising.[14,15] Recent studies have provided a wealth of information on phenotypic biomarkers of human and mouse SSCs: such as thymocyte differentiation antigen-1 (THY-1), promyelocytic leukemia zinc finger (PLZF), SRY-Box3 (SOX-3), glial cell line-derived neurotrophic factor family receptor alpha 1 (GFRα1) have been identified as biomarkers observed in human and mouse SSCs.[16,17] The identification and evaluation of these biomarkers is crucial for advancing our understanding of SSC biology and their role in male infertility. These biomarkers not only help in isolating and characterizing SSCs but also provide insights into the molecular mechanisms underlying spermatogenesis and its dysregulation in infertile men. For instance, PLZF is essential for maintaining the undifferentiated state of SSCs, and its dysregulation has been linked to impaired spermatogenesis and infertility.[18] Similarly, THY-1 and SOX-3 play critical roles in SSC self-renewal and differentiation, and their expression patterns are often altered in cases of male infertility.[19] Therefore, the evaluation of these biomarkers is crucial for the development of targeted therapies for male infertility, as it provides a better understanding of the molecular pathways involved in SSC function and spermatogenesis. Using these biomarkers, they may discover new therapies to restore fertility in men with impaired spermatogenesis, such as SSC transplantation or *in vitro* spermatogenesis.

In light of this information, we aimed to investigate the beneficial effects of CoQ10, a powerful antioxidant found in all cell membranes, vital for cellular energy, inhibiting free radicals, and protecting the cell membrane from lipid peroxidation, on testicular function, especially at the SSC level, given that its bioavailability is very high. Based on this information, the effects of CoQ10 supplementation on testicular histopathology in hypothyroidism were investigated. Additionally, the study will evaluate specific biomarkers involved in spermatogenesis, such as THY-1, PLZF, SOX-3 and GFRα1, providing greater insights into the molecular mechanisms of CoQ10's impact on male fertility, and contributing to the development of targeted therapies for male infertility.

## MATERIALS AND METHODS

### Study Design and Ethical Approval

In this study, 28 male *Wistar albino* rats weighing 255-304 g were divided into four groups to evaluate the effects of hypothyroidism and CoQ10 supplementation on testicular function: 1) Control, 2) hypothyroidism with PTU, 3) CoQ10 treatment, 4) hypothyroidism + CoQ10. Induction of hypothyroidism was achieved in the second and fourth groups with drinking water containing 0.05% PTU (6-n-propyl-2-thiouracil).[20] CoQ10 was administered intraperitoneally to the third and fourth groups at a dose of 10 mg/kg daily. At the end of the experiment, blood samples were taken for thyroid stimulating hormone (TSH), free triiodothyronine (fT3), and free thyroxine (fT4) levels. The testes were removed, weighed, and prepared for histological examination; immunohistochemical (IHC) analysis was performed on the right testis, and microscopic analysis with hematoxylin-eosin was performed on the left testis.

This study was carried out with the approval of the Necmettin Erbakan University Ethics Committee for Experimental Animal Research (approval number: 2022-036, date: 06.07.2022). The research was supported by the Scientific Research Projects Coordination Office (project no: 221418001).

### Hematoxylin-Eosin Staining

For deparaffinization, sections were placed in an oven at 37 °C overnight and 60 °C for 1-hour, the next day. They were exposed to xylene (Tekkim, catalog no: 190822134001) twice for 15 minutes each. Tissues were dehydrated through 100%, 96%, and 80% ethyl alcohol (Isolab, catalog no: LR0090611AL0), for 5 minutes each, and they were washed in distilled water twice. After staining with hematoxylin (Sigma-Aldrich, catalog no: HX17558974) for 1 minute, tissues were immersed in acid-alcohol, washed in tap water, and stained with eosin (Sigma-Aldrich, catalog no: HX17224144) for 2 minutes. They were then washed in water for 1 minute and passed through increasing concentrations of ethyl alcohol (70%, 80%, 96%, 100%) for 5 minutes each, followed by dehydration in xylene (Tekkim, catalog no: 190822134001) twice for 15 minutes. Stained tissues were examined under a light microscope, and photographs were taken. Twenty seminiferous tubules per group were selected for spermatogenesis evaluation using modified Johnsen scoring, and SPG were counted in the same tubules.[21]

### Morphometric Study

The diameter of 20 selected round or nearly round seminiferous tubules was measured using an Olympus BX53 light microscope with the Image Pro program and Olympus SC50 camera attachment at x20 magnification, and subsequently, the mean value was calculated.

### Immunohistochemical Study

Dehydrated testis tissues were washed twice with distilled water to remove alcohol. Slides were placed in a microwave-proof dish with

10% citrate buffer (Thermo Scientific, catalog no: AX201007) at 500 °C for 5 minutes a process repeated three times. After washing with PBS (BioShop, catalog no: 5B37414) for 5 minutes, hydrogen peroxide (1%, Merck, 64271) was applied for 10 minutes, followed by Super Block (ScyTek, catalog no: AAA125) for 10 minutes. Slides were incubated with primary antibodies for THY1 (CD90, Abcam, catalog no: AB203022), SOX-3 (Abcam, catalog no: AB183606), PLZF (Santa Cruz, catalog no: SC-28319), and GFRα1 (Abcam, catalog no: AB216667) (1:200 dilution) for 60 minutes. After washing with PBS, slides were incubated with secondary antibody (Santa Cruz, catalog no: SC-516102) for 20 minutes, followed by streptavidin peroxidase (Thermo Scientific, catalog no: TS-125-HR) for 20 minutes. AEC chromogen (ScyTek, catalog no: 25768) was applied for 15 minutes, and Mayer's hematoxylin stain (Sigma-Aldrich, catalog no: HX17558974) was used for 5 minutes. After washing in tap water for 3 minutes, immunoscoring was performed by three independent investigators using a scale of "-", "+", "++", and "+++". Differences between the groups, and severity, were recorded.

### Biochemical Study

TSH (Elabscience, catalog no: E-EL-R0976), fT3 (Elabscience, catalog no: E-EL-0079), and fT4 (Elabscience, catalog no: E-EL-0122) levels in sera collected at the end of the experiment were measured using Enzyme-Linked Immunosorbent Assay kits on an Allsheng AMR-100 reader. TSH was calculated in ng/mL, and fT3 and fT4 in pg/mL.

### Statistical Analysis

Statistical significance was determined using SPSS software (version 22; SPSS Inc., Chicago, IL). Seminiferous tubule areas, SPG cell counts, Johnsen scores, animal, and testicular weights, and biochemical analyses were compared using the Duncan test. Immunoscoring for THY-1, GFRα1, PLZF, and SOX-3 expression in spermatogonium was performed. Group comparisons were made using a one-way analysis of variance, with significance tested at $p<0.05$.

## RESULTS

### Weight Results

Animal weights at the beginning ($p=0.307$) and end ($p<0.001$) of the experiment were compared and the results are shown in Table 1. Weight gains were seen in groups 2 and 4 treated with PTU; there were significant differences between the initial and final weights in group 2 ($p<0.004$) and group 4 ($p=0.002$). No significant difference was found in the testicular weights (right + left), as shown in Table 1 ($p=0.0604$).

### Hematoxylin-Eosin Staining Results

Germ cells were present at all stages of spermiogenesis within regular seminiferous tubule borders in groups 1 and 3. Group 2 showed irregular seminiferous tubule borders and arrested spermatogenesis. Group 4 exhibited smooth tubule borders and better germ cell arrangement than group 2. Differences between groups were observed in Johnsen scoring ($p=0.00$) (Table 1 and Figure 1).

### Spermatogonial Cell Count Results

Statistical differences in mean SPG cell counts were observed between all experimental groups ($p<0.002$) (Table 1). The highest SPG cell count was in group 2, followed by group 4, group 1, and group 3.

### Seminiferous Tubule Diameter Results

There was no statistical difference between the experimental groups ($p<0.186$) (Table 1).

### Biochemical Results

Thyroid function test results (TSH, fT3, fT4) are shown in Table 2. Significant differences were observed between the groups in TSH ($p=0.001$), fT3 ($p=0.003$) and fT4 ($p=0.001$) levels. TSH: Normal in group 1, decreased in group 3; increased in group 2 and significantly decreased in group 4. fT3: Normal in group 1, increased in group 3, decreased in group 2, and significantly increased in group 4. fT4: Normal in group 1, increased in group 3; decreased in group 2 and significantly higher in group 4.

### Immunohistochemical Results

No differences were observed in the expression of THY-1, GFRα1, and PLZF proteins between the groups ($p>0.05$). Expressions in SSCs were strong (+++) in all groups. In contrast, SOX-3 immunopositivity was strong (+++) in group 1, moderate (++) in group 2, and strong (+++) in group 3. Group 4 showed a decrease in immunopositivity similar to group 2, with moderate (++) expression, which was statistically significant ($p<0.05$). Overall, no significant difference was found in protein expression among all biomarkers (Figure 2).

## DISCUSSION

Hypothyroidism negatively affects testicular function in animal models, with agents like carbimazole, methimazole, and propylthiouracil causing reduced testicular weight, impaired sperm motility and count, and disrupting spermatogenesis and steroidogenesis. Hypothyroidism also disrupts the thyroid hormone-regulated antioxidant balance, leading to increased oxidative stress and testicular damage. Antioxidants such as alpha-lipoic acid and L-carnitine have shown protective effects, reducing oxidative stress, improving sperm quality, and potentially restoring testicular function. These findings suggest antioxidant supplementation could mitigate hypothyroidism-induced testicular toxicity.[22-24]

**Table 1. Histomorphological results**

| Parameter | Group 1 | Group 2 | Group 3 | Group 4 | p-value |
|---|---|---|---|---|---|
| Start of experiment AW (g) ± SD | $288.42^{a}\pm17.84$ | $255.14^{b}\pm14.79$ | $304.00^{a}\pm14.71$ | $266.80^{b}\pm17.18$ | p=0.307 |
| End of experiment AW (g) ± SD | $310.57^{a}\pm21.4$ | 297.85±26.11 | 322.42±20.15 | 300.14±15.38 | p<0.00 |
| Testicular weight (g) ± SD | 3.18±0.51 | 3.13±0.40 | 3.27±0.27 | 3.00±0.26 | p=0.0604 |
| SPG (cells/field) ± SD | $65^{b}\pm7.04$ | $71.4^{a}\pm1.98$ | $62.42^{b}\pm1.7$ | $68^{a,b}\pm1.91$ | p<0.002 |
| ST (µm) ± SD | 273.42±14.04 | 250.54±49.22 | 280.64±7.65 | 272.65±7.45 | p=0.186 |
| HE (µm) ± SD | 11.3±0.5 | 8.4±1.4 | 10.5±1.2 | 9.4±1.4 | p=0.00 |

AW: Animal weight, SD: Standard deviation, SPG: Spermatogonium, ST: Seminiferous tubule, H&E: Hematoxylin and eosin staining, [a]: Similar to each other, different from [b] and [c]. [b]: Different from [a], similar within itself. [c]: Different from both [a] and [b]. [ab]: Similar to both [a] and [b].

Thyroid hormones, T4 and T3, are crucial for the growth, development, and metabolism of mammalian tissues, and their imbalance can affect various organs. However, the impact of iodothyronines on human male reproduction remains controversial, as clinical signs related to male gonadal function during hypo- and hypersecretion are not clear, and controlled studies are limited. Additionally, thyroid diseases are more common in women. Recent studies have confirmed the expression of TRs in the testis at both the mRNA (reverse transcription polymerase chain reaction) and protein levels.[4,5] A study in mouse Leydig cells found that long-term T3 hormone treatment enhanced steroidogenesis in the cells in a coordinated manner.[25] Mendeluk and Rosales[6] showed that the T4 hormone added to semen increased sperm motility. In our study, experimental hypothyroidism was confirmed by analyses of TSH, fT3, and fT4. In addition, a decrease in TSH hormone levels and an increase in T3 and T4 hormone levels were observed in animals receiving CoQ10 (group 3, 4) compared to the hypothyroid group (group 2). Given that these hormones affect the process of spermatogenesis, it is logical to suggest that CoQ10 may affect the differentiation of germ cells.

Thyroid hormones are critical regulators of energy expenditure and body weight, and their deficiency usually results in decreased energy expenditure and increased adiposity.[26] Consistent with this, we observed in our study significant weight gain in animals with induced hypothyroidism. However, CoQ10 supplementation effectively attenuated this hypothyroidism-induced weight gain, suggesting its potential role in regulating thyroid hormone-related metabolic disorders. Consistent with previous studies, Yousofvand et al.[27] reported a significant decrease in testicular weight in PTU-induced hypothyroid rats. Although a decrease in testicular weight was also observed in the hypothyroid group in our study, statistical analysis did not reveal a significant difference in testicular weight between the experimental groups. This suggests that although CoQ10 supplementation attenuated the metabolic effects of hypothyroidism, it did not completely eliminate the effect on testicular weight, and, therefore, the mechanisms involved should be further investigated.

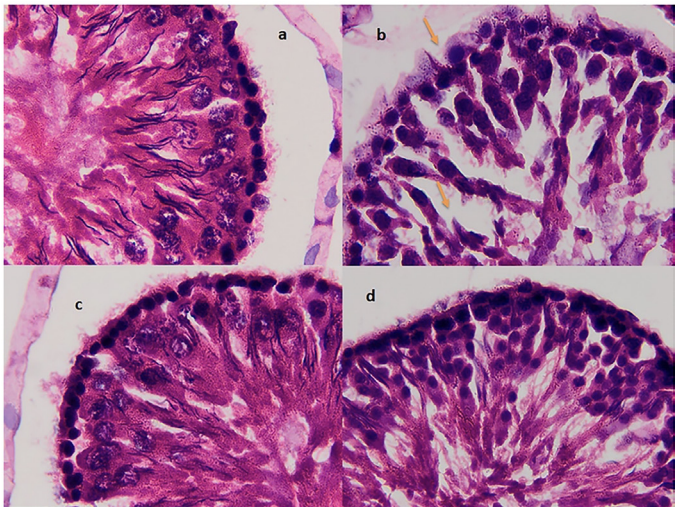


**Figure 1.** Hematoxylin and eosin (H&E) staining of seminiferous tubules: (a) group 1 shows regular seminiferous tubule wall and regular arrangement of spermatogenic cells. (b) group 2 shows irregular seminiferous tubule wall and disruption of spermatogenic process (orange arrow). (c) group 3 shows regular seminiferous tubule and presence of spermatogenic cell types. (d) group 4 shows a more regular seminiferous tubule structure (compared to group 2). Sections were stained using the H&E staining method, where hematoxylin stains cell nuclei blue-purple and eosin stains cytoplasm and extracellular matrix pink. Objective magnification: x100, scale bar: 20 µm.

H&E: Hematoxylin and eosin.

Lara et al.[28] reported no significant difference in tubular diameter between experimental groups in their study using PTU. In line with their findings, our study also observed a decrease in tubular diameter in the hypothyroid group. However, statistical analysis did not reveal any significant differences between the groups, suggesting that while hypothyroidism may influence tubular structure, the effect on diameter may not be substantial enough to reach statistical significance in our experimental setup.

De la Balze et al.[29] reported that testicular biopsies from adult male hypothyroid patients exhibited abnormal histological structures. Similarly, long-term hypothyroidism in adult mice resulted in a significant reduction in germ cell numbers in the epididymis, and a decreased percentage of live spermatozoa.[30] In line with these findings, our study observed in the PTU-induced hypothyroid groups a decrease in the number of seminiferous germ cells and structural defects, such as gaps between spermatogenic series cells. Interestingly, while the number of seminiferous germ cells decreased, the total number of SSCs increased. This suggests that hypothyroidism may impair the differentiation process of SSCs rather than affecting their self-renewal capacity. Notably, CoQ10 supplementation resulted in improvements in these parameters, suggesting a positive effect of CoQ10 on SSC differentiation and spermatogenesis. Consequently, our findings support the idea that hypothyroidism inhibits SSC differentiation and sperm production in the seminiferous tubules, while CoQ10 supplementation may mitigate some of these adverse effects, promoting spermatogenesis.

THY-1/CD90 (Differentiation Cluster 90) is a well-established cell surface marker of SSCs. In humans, a highly enriched population of undifferentiated SPG has been identified as β-2 microglobulin (β-2M)-SPα-6+THY1+, which further highlights the role of THY-1 in SSCs.[31] In mice, the gene expression profile of SSCs (mSSCs) also demonstrates high levels of THY-1 expression.[32] These findings indicate that THY-1 is a characteristic marker of SSCs in both humans and mice and may be involved in maintaining SSC self-renewal and differentiation. Moreover, THY-1 has been identified as a key regulator of cellular differentiation in various cell types, influencing signaling pathways and controlling cellular functions, largely through its role as an adhesion molecule on the cell membrane, rather than via intracellular pathways. Several studies have

**Table 2. Biochemical results**

| Parameter | Group 1 | Group 2 | Group 3 | Group 4 | p-value |
|---|---|---|---|---|---|
| TSH ± SD | 2.03±1.06 | 7.46$^{a}$±1.94 | 1.32±1.06 | 2.19±0.71 | p=0.001 |
| fT3 ± SD | 10.05$^{a}$±3.57 | 6.85$^{a}$±2.21 | 18.32$^{b}$±6.66 | 8.44±2.91 | p=0.003 |
| fT4 ± SD | 5.74$^{a}$±1.33 | 1.42$^{b}$±0.4 | 8.15$^{c}$±1.63 | 3.27±0.27 | p=0.001 |

TSH: Thyroid-stimulating hormone, fT3: Free triiodothyronine, fT4: Freethyroxine, SD: Standard deviation, $^{a}$: Similar to each other, different from $^{b}$ and $^{c}$. $^{b}$: Different from $^{a}$, similar within itself. $^{c}$: Different from both $^{a}$ and $^{b}$. $^{ab}$: Similar to both $^{a}$ and $^{b}$.

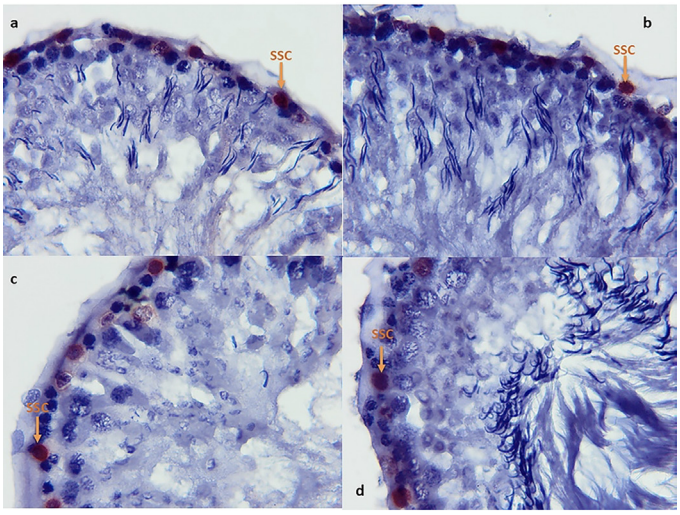


**Figure 2.** Immunohistochemical staining, Results: figures (a-d) show positive SOX3 staining (orange arrow). Immunohistochemical stain used: anti-SOX3. Seminiferous tubule, transverse section. Objective magnification: x100, scale bar: 20 µm.

SSC: Spermatogonial stem cell

confirmed high expression of THY-1 in undifferentiated SPG.[16,33,34] In our study, we observed strong THY-1 expression in SSCs, consistent with previous reports. To date, however, there are no studies exploring the potential effects of hypothyroidism on THY-1 expression in the male reproductive system. In contrast, Oltulu et al.[35] reported significantly decreased THY-1 expression in rat ovarian tissue in an experimental thyrotoxicosis model, suggesting a role for THY-1 in apoptosis regulation under conditions of thyroid dysfunction. In our study, THY-1 expression was strong in all experimental groups, indicating that hypothyroidism did not suppress THY-1 expression in SSCs. This suggests that the THY-1 marker may not be directly involved in apoptotic processes in response to hypothyroidism, possibly due to its role as an adhesion molecule that facilitates protein binding rather than as a direct mediator of apoptosis. Additionally, no significant differences were observed in THY-1 expression in the CoQ10-treated groups. Given that CoQ10 did not alter THY-1 expression and considering the importance of THY-1 in cellular differentiation and apoptosis, our findings suggest that CoQ10 supplementation does not significantly modulate the pathways involved in apoptosis or the cell cycle in testicular cells. Therefore, it seems unlikely that CoQ10 plays a critical role in regulating these processes under hypothyroid conditions.

SOX-3 is a key member of the SRY-related high mobility group box family of transcription factors and plays a pivotal role in testicular development and spermatogenesis.[36] It is highly expressed in SSCs, where it regulates the transition from a self-renewing state to a differentiating amplification compartment.[37] Studies have shown that SOX-3 directly targets neurogenin-3 in SPG cells, further implicating SOX-3 in the regulation of Spg differentiation. In teleost species, SOX-3 expression is upregulated during testis development, indicating its involvement in testicular function. Additionally, in black rockfish, SOX-3 plays a role in oogenesis and ovarian differentiation, exhibiting sexually dimorphic expression patterns in adult gonads.[38,39] Together, these findings suggest that SOX-3 is a critical transcription factor influencing various aspects of male reproductive development. In our study, SOX-3 protein was highly expressed in the nuclei of SSCs in groups 1 and 3, consistent with its known role in spermatogenesis. However, in groups 2 and 4 (hypothyroid and CoQ10-supplemented hypothyroid groups), a downregulation of SOX-3 protein expression was observed. This reduction in SOX-3 levels in the hypothyroid group may reflect impaired SPG differentiation, which could be a consequence of the negative effects of hypothyroidism on the regulation of the SSC differentiation process. Specifically, the decreased expression of SOX-3 in the hypothyroid group may be linked to a disruption in the differentiation of other spermatogenic lineage cells, rather than a direct effect on SSC self-renewal. Interestingly, no significant difference in SOX-3 expression was observed between the CoQ10-supplemented groups (groups 3 and 4) and the hypothyroid group (group 2). This suggests that CoQ10 supplementation did not affect SOX-3 immunoreactivity in the context of hypothyroidism. Given SOX-3's role in spermatogenesis, the lack of an effect by CoQ10 on its expression may imply CoQ10 does not significantly modulate the differentiation of SPG in hypothyroid conditions. This finding warrants further investigation into the potential mechanisms by which CoQ10 influences spermatogenesis under thyroid dysfunction.

PLZF is a SPG-specific transcription factor that plays a crucial role in regulating the self-renewal of SSCs and maintaining the SSC pool in the testis.[40] It is thought to function as a cell-autonomous factor that ensures the stability and self-renewal of SSCs.[41] PLZF also regulates SSC self-renewal by inhibiting the mammalian target of rapamycin complex 1 (mTORC1), a critical regulator of cellular processes that balances self-renewal and differentiation. Activated mTORC1 can downregulate the expression of GDNF receptors, and PLZF promotes the expression of Redd1, a negative regulator of the mTORC1 pathway, thus contributing to the maintenance of SSCs.[42]

In our study, we observed PLZF expression in SSCs, which is consistent with findings from previous studies. To our knowledge, this is the first study to report on PLZF expression in the context of hypothyroidism. We found that PLZF expression was confined to SPG, with no significant differences observed between the experimental groups. This suggests that hypothyroidism does not significantly affect PLZF expression in testicular SSCs. Similarly, CoQ10 supplementation did not influence PLZF immunoreactivity in our study. These findings suggest that PLZF may not play a direct role in DNA replication or stem cell division in the context of hypothyroidism or CoQ10 supplementation. The lack of change in PLZF expression in both the hypothyroid and CoQ10-treated groups may indicate that PLZF's regulatory role in SSCs is not impacted by these conditions.

GFRα1 is a well-known receptor for GDNF, regulating SSC self-renewal and differentiation by signaling through GFRα1, thus maintaining a balanced SSC population.[17] Meng et al.[43] who showed that GFRα1-deficient mice had a depleted SSC population, indicated that overexpression of GFRα1 led to the accumulation of undifferentiated SPG. Furthermore, the study by Meng et al.[43] highlighted the importance of the GDNF/FSH signaling axis in controlling SSC population size and maintaining the balance between self-renewal and differentiation. These findings highlight the fundamental importance of GFRα1 in regulating spermatogenesis and its potential as a therapeutic target in male infertility. Our study found that GFRα1 expression was detectable in both the hypothyroid and CoQ10-supplemented groups, which is consistent with previous reports suggesting its presence in the testis. However, no significant difference in GFRα1 expression was observed between the hypothyroid and CoQ10-treated groups. This suggests that CoQ10 supplementation may have a broader protective effect on testicular histopathology, but

does not directly affect GFRα1 expression in the testes. This result is consistent with the hypothesis that CoQ10 may attenuate metabolic and oxidative stress associated with hypothyroidism, but its effect on molecular pathways governing spermatogenesis, such as the GDNF/GFRα1 signaling pathway, may be more limited. Additionally, we observed an association between hypothyroidism and GFRα1 expression that deserves further attention. Kamyshna et al[44], previously reported a significant decrease in GFRα1 expression, in patients with primary hypothyroidism, raising the possibility that hypothyroid states may lead to decreased GFRα1 expression and thus impair SSC function and spermatogenesis. This is consistent with the findings in our study, where hypothyroidism appears to affect GFRα1 expression, potentially contributing to the observed impairments in testicular function. The relationship between thyroid dysfunction and GFRα1 expression is also supported by the study of Bilous et al.[45], who reported decreased GFRα1 levels in thyroid patients and observed GFRα1 expression in thyroid follicular cells. These findings point to a possible link between thyroid function and the regulation of spermatogenesis via GFRα1. However, in our study, CoQ10 supplementation did not significantly alter GFRα1 expression, suggesting that, although CoQ10 may exert its effects through different mechanisms, it may not directly affect the GDNF/GFRα1 signaling pathway. In conclusion, our findings reinforce the importance of GFRα1 in maintaining spermatogenesis and highlight its potential as a therapeutic target for male infertility. While hypothyroidism-induced decreases in GFRα1 expression may contribute to spermatogenic dysfunction, CoQ10 supplementation appears to offer some protective benefits, although it does not directly affect GFRα1 expression. Further research is needed to better understand the precise molecular mechanisms underlying these observations and to explore additional therapeutic strategies, including vitamin D supplementation, to restore GFRα1 expression and improve testicular function in hypothyroid individuals.

### Study Limitations

Very few articles similar to the subject of this study were found in the literature review, which can be considered a limitation of this study. Although there are studies on SSC status in hypothyroid patients in the literature, they are few, and this study is one of the pioneering studies examining the relationship with antioxidants. Furthermore, the effects of vitamin D and other antioxidants were not investigated in this study, leaving room for future research.

## CONCLUSION

In this study, the effects of CoQ10 supplementation on testicular tissue in adult Wistar albino rats with experimental hypothyroidism were investigated using IHC, biochemical and morphometric methods. CoQ10 given after hypothyroidism, was shown to have a beneficial effect on spermatogenesis in testicular tissue. This effect affected other SPG lineage cells rather than SSC cells. CoQ10 supplementation showed a therapeutic benefit by improving serum TSH, fT3, and fT4 levels. Since this study is a scientific study investigating SSC differentiation in hypothyroidism and also a study SSC differentiation in hypothyroidism and CoQ10, which is widely used in male infertility, on this process, we are determined to carry our research to further research topics. Low expression of the SOX-3 marker was observed after hypothyroidism. In conclusion, after this study, we plan to investigate the mechanism of SOX-3 during differentiation of SSCs with molecular and genetic studies.

## MAIN POINTS

- Although there were no significant differences in testicular weight or seminiferous tubule diameter between the groups, hypothyroidism was found to inhibit differentiation of SSCs and lead to decreased sperm production in the seminiferous tubules.
- While CoQ10 supplementation does not directly affect SSCs, it has a positive effect on other stages of spermatogenesis, including primary and secondary spermatocytes, spermatids, and spermatozoa.
- CoQ10 has shown therapeutic effects on thyroid function as demonstrated by improvements in TSH, T3, and T4 levels.

## ETHICS

**Ethics Committee Approval:** This study was carried out with the approval of the Necmettin Erbakan University Ethics Committee for Experimental Animal Research (approval number: 2022-036, date: 06.07.2022).

**Informed Consent:** Patient approval has not been obtained as it is performed on animals.

### Footnotes

### Authorship Contributions

Surgical and Medical Practices: H.N.Ş., F.Z.E., G.C., Concept: H.N.Ş., S.K., Design: H.N.Ş., G.C., Data Collection and/or Processing: H.N.Ş., F.Z.E., Analysis and/or Interpretation: H.N.Ş., F.Z.E., G.C., Literature Search: H.N.Ş., F.Z.E., G.C., S.K., Writing: H.N.Ş., F.Z.E.

## DISCLOSURES

**Conflict of Interest:** No conflict of interest was declared by the authors.

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